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RESEARCH ARTICLE

Comparative Study on Phytochemical Screening of Root and Bark with Leaf of *Cardiospermum Halicacabum*

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ABSTRACT

Aim: The Present study was designed to compare the phytochemical screening of root and bark with leaf of Cardiospermum halicacabum. **Materials and Methods:** Samples were taken leaf (N=24) root and bark (N=24) based on the total sample size using clinical.com. The leaf, root and bark extract were collected. The phytochemicals were extracted by sequential extraction using three solvents methanol, ethanol and acetone. The quantification of flavonoids and phenols was performed by using Folin-Ciocalteu and quercetin as standard. Quantification of tannins was determined by using an insoluble polyvinyl-polypyrrolidone (PVPP) as standard. **Results:** Statistical analysis showed that methanol extract of root (0.49mg/ml) has highest phenolic content and acetone extract of root has highest tannin (0.64mg/ml) and flavonoid (1.18mg/ml) content when compared with leaf and bark. There appears to be a statistically significant difference in the mean of root when compared with leaf and bark ($p<0.01$, independent samples). **Conclusion:** In this study root appears to have better phytochemical and phenol content when compared with the content in leaf and bark.

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Introduction

This research is about the screening of phytochemicals, quantification of phenols from bark and root of *Cardiospermum halicacabum (Muhammad et al. 2014)*. This research is important as evaluation of phytochemicals is viewed as viable in finding bioactive compounds of plants of medical significance (Masih and Singh 2012). This work can be implemented in clinical practice to study antidiabetic activity, anti-inflammatory activity, antibacterial activity (Stalin, Vivekanandan, and Bhavya 2014); (Babu and Krishnakumari 2005).

Methanol extract of leaf (0.43mg/ml) of *Cardiospermum halicacabum* revealed higher phytochemical content (Stalin, Vivekanandan, and Bhavya 2014).

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Acetone (0.60mg/ml) and chloroform (0.59mg/ml), concentrates of leaf had higher phytochemical content ("Website" n.d.) whereas the present work revealed that acetone extract of root (0.63mg/ml) had higher phytochemical content. *C.halicacabum* showed the existence of bioactive phytochemical compounds which is considered to be more effective work compared to other research works (Stalin, Vivekanandan, and Bhavya 2014).

Previously our team has a rich experience in working on various research projects across multiple disciplines (Sathish and Karthick 2020; Varghese, Ramesh, and Veeraiyan 2019; S. R. Samuel, Acharya, and Rao 2020; Venu, Raju, and Subramani 2019; M. S. Samuel et al. 2019; Venu, Subramani, and Raju 2019; Mehta et al. 2019; Sharma et al. 2019; Malli Sureshbabu et al. 2019; Krishnaswamy et al. 2020; Muthukrishnan et al. 2020; Gheena and Ezhilarasan 2019; Vignesh et al. 2019; Ke et al. 2019; Vijayakumar Jain et al.

2019; Jose, Ajitha, and Subbaiyan 2020). Now the growing trend in this area motivated us to pursue this project.

This work was carried out for the phytochemical screening of root and bark of *Cardiospermum halicacabum* using a novel solvent extraction method. The authors were expertised in the field of Microbiology and able to conduct studies of comparison of root bark and leaf of Cardiospermum halicacabum in biomedical aspect. The aim of this study is comparison of screening of phytochemicals of bark and root with leaf of *Cardiospermum halicacabum*.

Materials and Method

This study was carried out in the Biochemistry lab at Saveetha school of Engineering located in chennai. The sample calculation was calculated by using previous study results ("Website" n.d.) using clinicalc.com, by keeping alpha error-threshold by 0.05, enrollment ratio as 0:1, 95% confidence interval, power 80%. There are two groups, Control group (Leaf extract) and Study group (Root and Bark extract), each group with sample size of 24. Fresh *Cardiospermum halicacabum* plant was collected from nearby village, Chettipedu.

The testing setup used in this study were Calorimeter (CC01/M3), Weighing scale (Omron HN-286), Water Bath (PURA 4), Centrifuge (Remi r303). For the control group, the samples were prepared from the leaves of *Cardiospermum halicacabum* plant (0.1-0.4%) using solvent extraction method. For the study group the samples were prepared from the bark and root of *Cardiospermum halicacabum* using solvent extraction method (0.5%-1%). The quantification of flavonoids and phenols was performed by using Folin-Ciocalteu and quercetin as standard (Rami and Patel 2015). Quantification of tannins was determined by using an insoluble polyvinyl-polypyrrolidone (PVPP) as standard (Pulipati, Srinivasa Babu, and Lakshmi Narasu 2014).

Statistical Analysis

Statistical analysis of comparison for leaf, root and bark extract in *Cardiospermum halicacabum* using one way ANOVA was done using IBM SPSS 27.0.1 software. Independent variables in this study are phytochemical contents (mg/ml), phenolic contents (mg/ml).

Results

In this study, comparing leaf, root and bark using methanol, ethanol and acetone extract of *Cardiospermum halicacabum,* there is a higher phenol content in root (0.49 mg/ml) using methanol concentration compared to ethanol and acetone as depicted in Table 1. There appears to be a statistically significant difference ($p<0.01$, Independent samples) using One Way Anova in the phenol extraction of root and bark compared with leaf as shown in Table 2. Methanol extraction showed higher phenol content (0.49mg/ml) in root as shown in Fig.1. The leaf and bark extracts showed only less amount of phenol content due to the solvent extraction method.

Table 1. Comparison of mean between leaf, root and bark of methanol, ethanol and acetone extract of *Cardiospermum halicacabum*.

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
PMleaf	Control Group	24	0.351	0.031	0.006	0.338	0.364	0.310	0.400
	Study Group	24	0.438	0.030	0.006	0.425	0.451	0.340	0.470
	Total	48	0.395	0.053	0.007	0.379	0.410	0.310	0.470
PMRoo	Control Group	24	0.353	0.022	0.004	0.343	0.362	0.330	0.400
	Study Group	24	0.437	0.030	0.006	0.425	0.450	0.400	0.490
	Total	48	0.395	0.050	0.007	0.381	0.410	0.330	0.490
PMBark	Control Group	24	0.341	0.027	0.005	0.330	0.353	0.310	0.420
	Study Group	24	0.413	0.016	0.003	0.406	0.420	0.390	0.440
	Total	48	0.377	0.042	0.006	0.365	0.390	0.310	0.440
PEleaf	Control Group	24	0.325	0.000	0.000	0.325	0.325	0.325	0.326
	Study Group	24	0.326	0.000	0.000	0.326	0.326	0.325	0.327
	Total	48	0.326	0.000	0.000	0.325	0.326	0.325	0.327
PERoot	Control Group	24	0.335	0.000	0.000	0.335	0.336	0.335	0.337
	Study Group	24	0.336	0.000	0.000	0.336	0.336	0.336	0.337
	Total	48	0.336	0.000	0.000	0.336	0.336	0.335	0.337
PEBark	Control Group	24	0.315	0.000	0.000	0.315	0.315	0.315	0.316
	Study Group	24	0.316	0.000	0.000	0.316	0.317	0.316	0.318
	Total	48	0.316	0.000	0.000	0.316	0.316	0.315	0.318
PAlea	Control Group	24	0.093	0.003	0.000	0.091	0.094	0.089	0.098
	Study Group	24	0.126	0.020	0.004	0.118	0.135	0.098	0.147
	Total	48	0.110	0.022	0.003	0.103	0.116	0.089	0.147
PARoo	Control Group	24	0.100	0.009	0.001	0.096	0.104	0.090	0.117
	Study Group	24	0.142	0.020	0.004	0.133	0.150	0.118	0.165
	Total	48	0.121	0.026	0.003	0.113	0.129	0.090	0.165
PABark	Control Group	24	0.087	0.008	0.001	0.084	0.091	0.079	0.100
	Study Group	24	0.113	0.013	0.002	0.108	0.119	0.100	0.138
	Total	48	0.100	0.017	0.002	0.095	0.105	0.079	0.138

Table 2: There appears to be a statistically significant difference (p<0.01, Independent samples) using One Way Anova in the phenol extraction of root and bark compared with leaf.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
PMea	Between Groups	0.091	1	0.091	95.582	<0.01
	Within Groups	0.044	46	0.001		
	Total	0.135	47			
PMRoot	Between Groups	0.086	1	0.086	121.405	<0.01
	Within Groups	0.033	46	0.001		
	Total	0.118	47			
PMBar	Between Groups	0.062	1	0.062	125.272	<0.01
	Within Groups	0.023	46	0.000		
	Total	0.085	47			
PElea	Between Groups	0.000	1	0.000	38.923	<0.01
	Within Groups	0.000	46	0.000		
	Total	0.000	47			
PERoot	Between Groups	0.000	1	0.000	86.001	<0.01
	Within Groups	0.000	46	0.000		
	Total	0.000	47			
PEBark	Between Groups	0.000	1	0.000	41.013	<0.01
	Within Groups	0.000	46	0.000		
	Total	0.000	47			
PAleaf	Between Groups	0.014	1	0.014	61.534	<0.01
	Within Groups	0.010	46	0.000		
	Total	0.024	47			
PARoot	Between Groups	0.020	1	0.020	78.520	<0.01
	Within Groups	0.012	46	0.000		
	Total	0.032	47			
PABark	Between Groups	0.008	1	0.008	66.355	<0.01
	Within Groups	0.006	46	0.000		
	Total	0.014	47			

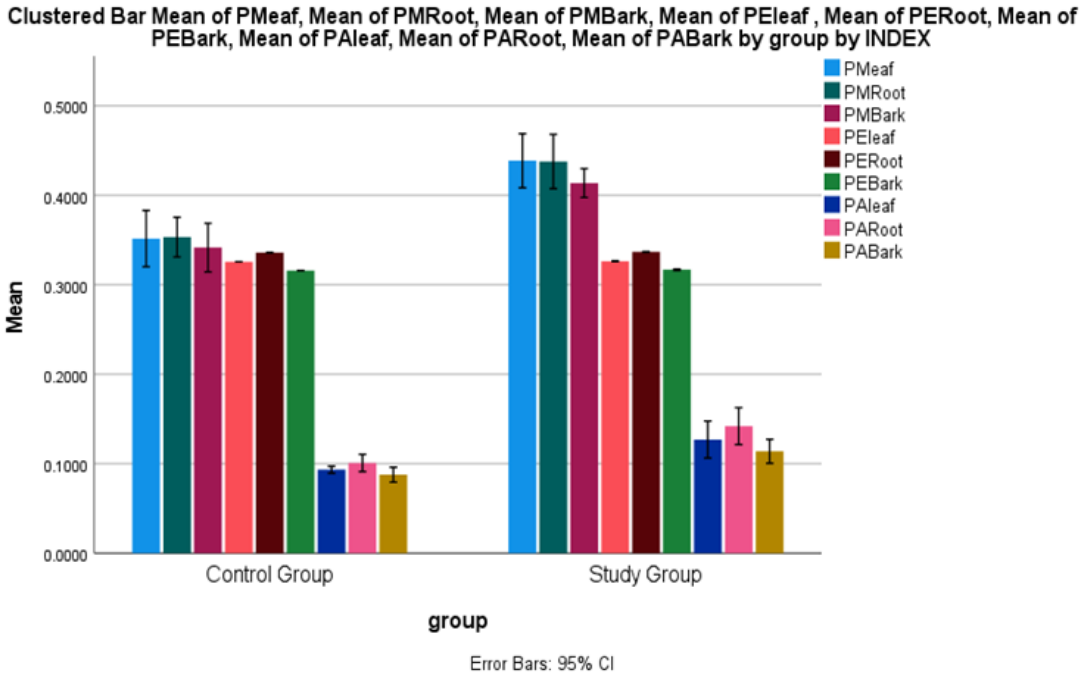

Fig. 1 Comparison of root and bark with leaf of *Cardiospermum halicacabum* in terms of mean. The mean of phenol content in root using methanol concentration appears to be better than in leaf and bark and standard deviation of phenol content in root is slightly better than the phenol content in leaf and bark. X Axis: Phenol content in leaf Vs Phenol content in root and bark. Y Axis: Mean with SD of ±1. (PM-Phenols in Methanol, PE-Phenols in ethanol PAbark-Phenols in acetone).

There is a higher tannin content in root, 0.63 mg/ml and leaf, 0.61 mg/ml using acetone concentration. Acetone concentration showed higher tannin content when compared with other extracts observed in Table 3. Root (0.63mg/ml) and leaf (0.61mg/ml) appears to produce most consistent results with higher tannin content using acetone

concentration. There appears to be a statistically significant difference ($p<0.01$, Independent samples) using One Way Anova in the tannin extraction of root and bark compared with leaf as given in Table 4. Acetone extraction showed higher tannin content (0.63mg/ml) in root as shown in Fig.2

Table 3. Comparison of the leaf, root and bark of methanol, ethanol and acetone extract of *Cardiospermum halicacabum*.

Descriptives									
		N			Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
TMleaf	Control group	24	0.460	0.018	0.003	0.453	0.468	0.446	0.484
	Study group	24	0.486	0.001	0.000	0.486	0.487	0.484	0.487
	Total	48	0.473	0.018	0.002	0.468	0.479	0.446	0.487
TMroot	Control group	24	0.466	0.016	0.003	0.459	0.473	0.457	0.495
	Study group	24	0.496	0.001	0.000	0.496	0.497	0.495	0.498
	Total	48	0.481	0.019	0.002	0.476	0.487	0.457	0.498
TMbark	Control group	24	0.443	0.009	0.001	0.439	0.447	0.435	0.465
	Study group	24	0.476	0.001	0.000	0.475	0.476	0.4700	0.477
	Total	48	0.459	0.017	0.002	0.454	0.464	0.435	0.477
TEleaf	Control group	24	0.467	0.005	0.001	0.465	0.470	0.460	0.471
	Study group	24	0.498	0.008	0.001	0.495	0.502	0.471	0.501
	Total	48	0.483	0.017	0.002	0.478	0.488	0.460	0.501
TEroot	Control group	24	0.476	0.005	0.001	0.474	0.479	0.471	0.490
	Study group	24	0.507	0.007	0.001	0.504	0.510	0.491	0.512
	Total	48	0.492	0.016	0.002	0.487	0.497	0.471	0.512
TEbark	Control group	24	0.456	0.004	0.000	0.454	0.457	0.450	0.461
	Study group	24	0.483	0.011	0.002	0.478	0.488	0.461	0.491
	Total	48	0.469	0.016	0.002	0.464	0.474	0.450	0.491
TAleaf	Control group	24	0.613	0.005	0.001	0.611	0.616	0.606	0.620
	Study group	24	0.624	0.002	0.000	0.623	0.625	0.620	0.627
	Total	48	0.619	0.007	0.001	0.616	0.621	0.606	0.627
TAroot	Control group	24	0.625	0.004	0.000	0.623	0.627	0.618	0.630
	Study group	24	0.634	0.003	0.000	0.633	0.636	0.630	0.639
	Total	48	0.630	0.006	0.000	0.628	0.631	0.618	0.639
TAbark	Control group	24	0.602	0.003	0.000	0.601	0.604	0.596	0.608
	Study group	24	0.614	0.003	0.000	0.612	0.615	0.609	0.617
	Total	48	0.608	0.006	0.000	0.606	0.610	0.596	0.617

Table 4. There appears to be a statistically significant difference ($p<0.01$, Independent samples) using One Way Anova in the tannin extraction of root and bark compared with leaf.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
TMleaf	Between Groups	0.008	1	0.008	45.140	<0.01
	Within Groups	0.008	46	0.000		
	Total	0.016	47			
TMroot	Between Groups	0.011	1	0.011	77.781	<0.01
	Within Groups	0.006	46	0.000		
	Total	0.017	47			
TMbark	Between Groups	0.013	1	0.013	286.033	<0.01
	Within Groups	0.002	46	0.000		
	Total	0.015	47			
TEleaf	Between Groups	0.011	1	0.011	236.665	<0.01
	Within Groups	0.002	46	0.000		
	Total	0.014	47			
TEroot	Between Groups	0.011	1	0.011	270.315	<0.01
	Within Groups	0.002	46	0.000		
	Total	0.013	47			
TEbark	Between Groups	0.009	1	0.009	119.734	<0.01
	Within Groups	0.003	46	0.000		
	Total	0.012	47			
TAleaf	Between Groups	0.001	1	0.001	63.766	<0.01
	Within Groups	0.001	46	0.000		
	Total	0.002	47			
TAroot	Between Groups	0.001	1	0.001	58.166	<0.01
	Within Groups	0.001	46	0.000		
	Total	0.002	47			
TAbark	Between Groups	0.002	1	0.002	113.587	<0.01
	Within Groups	0.001	46	0.000		
	Total	0.002	47			

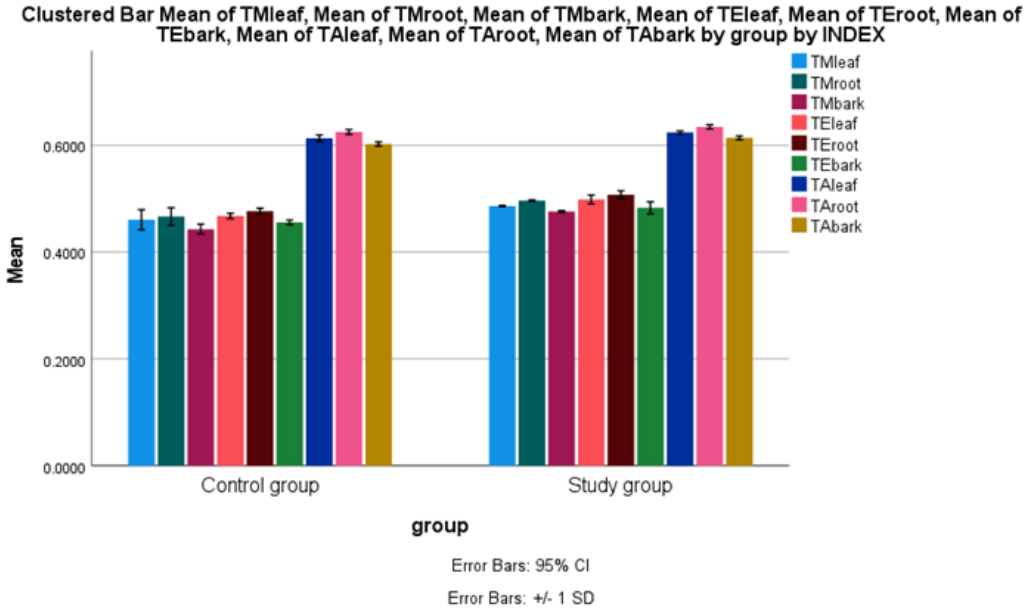

Fig. 2. Comparison of tannins in root and bark with leaf of *Cardiospermum halicacabum* in terms of mean. The mean of tannin content in root using acetone concentration appears to be better than in leaf and bark and standard deviation of tannin content in root is slightly better than the tannin content in leaf and bark.X Axis: tannin content in leaf Vs tannin content in root and bark. Y Axis:Mean with SD of ±1. (TM-Tannins in Methanol, TE-Tannins in ethanol extract, TA-Tannins in acetone).

Acetone concentration showed higher flavonoid content in root (1.18mg/ml) when compared with leaf (1.16mg/ml) and bark reported in Table 5. There appears to be a statistically significant difference ($p<0.01$, Independent samples), Oneway Anova in the flavonoid extraction of leaf and root compared with bark represented in Table 6. Root has higher flavonoid content (1.18mg/ml) as shown in Fig.3. The above results showed that concentration of solvent extraction affects the extraction of phytochemicals.

Table 5. Comparison of the leaf, root and bark of methanol, ethanol and acetone extract of *Cardiospermum halicacabum*.

Descriptives									
		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
FMleaf	.00	24	0.240	0.015	0.000	0.234	0.247	0.217	0.267
	1.00	24	0.280	0.003	0.000	0.278	0.281	0.274	0.285
	Total	48	0.260	0.022	0.003	0.253	0.267	0.217	0.285
FMroot	.00	24	0.250	0.014	0.002	0.244	0.257	0.228	0.275
	1.00	24	0.289	0.002	0.000	0.288	0.290	0.285	0.296
	Total	48	0.270	0.022	0.003	0.263	0.276	0.228	0.296
FMbark	.00	24	0.230	0.014	0.002	0.224	0.236	0.207	0.255
	1.00	24	0.267	0.002	0.0005	0.266	0.268	0.264	0.275
	Total	48	0.249	0.021	0.003	0.242	0.255	0.207	0.275
FEleaf	.00	24	0.177	0.003	0.0006	0.175	0.178	0.171	0.182
	1.00	24	0.195	0.007	0.001	0.192	0.198	0.184	0.211
	Total	48	0.186	0.010	0.001	0.183	0.189	0.171	0.211
FEroot	.00	24	0.219	0.026	0.005	0.208	0.231	0.181	0.242
	1.00	24	0.205	0.007	0.001	0.202	0.208	0.194	0.221
	Total	48	0.212	0.020	0.002	0.206	0.218	0.181	0.242
FEbark	.00	24	0.167	0.003	0.0007	0.165	0.168	0.161	0.174
	1.00	24	0.188	0.008	0.001	0.185	0.192	0.175	0.201
	Total	48	0.178	0.012	0.0018	0.174	0.181	0.161	0.201
FAleaf	.00	24	0.924	0.001	0.0003	0.923	0.925	0.922	0.932
	1.00	24	1.033	0.062	0.012	1.007	1.060	0.940	1.177
	Total	48	0.979	0.070	0.010	0.958	0.999	0.922	1.177
FAroot	.00	24	0.935	0.001	0.0003	0.934	0.935	0.933	0.942
	1.00	24	1.057	0.068	0.013	1.028	1.086	0.9511	1.189
	Total	48	0.996	0.078	0.011	0.973	1.018	0.933	1.189
FAbark	.00	24	0.370	0.285	0.058	0.249	0.490	0.226	0.922
	1.00	24	1.034	0.067	0.013	1.006	1.063	0.930	1.167
	Total	48	0.702	0.393	0.056	0.588	0.816	0.226	1.167

Table 6. There appears to be a statistically significant difference (p<0.01, Independent samples) Oneway Anova in the flavonoid extraction of leaf and root compared with bark

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
FMleaf	Between Groups	0.019	1	0.019	142.683	<0.01
	Within Groups	0.006	46	0.000		
	Total	0.025	47			
FMroot	Between Groups	0.018	1	0.018	161.131	<0.01
	Within Groups	0.005	46	0.000		
	Total	0.023	47			
FMbark	Between Groups	0.016	1	0.016	148.081	<0.001
	Within Groups	0.005	46	0.000		
	Total	0.021	47			
FEleaf	Between Groups	0.004	1	0.004	130.372	<0.01
	Within Groups	0.001	46	0.000		
	Total	0.006	47			
FEroot	Between Groups	0.003	1	0.003	6.711	0.013
	Within Groups	0.017	46	0.000		
	Total	0.020	47			
FEbark	Between Groups	0.006	1	0.006	135.577	0.000
	Within Groups	0.002	46	0.000		
	Total	0.007	47			
FAleaf	Between Groups	0.144	1	0.144	74.206	0.000
	Within Groups	0.089	46	0.002		
	Total	0.233	47			
FAroot	Between Groups	0.179	1	0.179	76.105	0.000
	Within Groups	0.108	46	0.002		
	Total	0.287	47			
FAbark	Between Groups	5.300	1	5.300	123.162	0.000
	Within groups	1.980	46	0.043		
	Total	7.280	47			

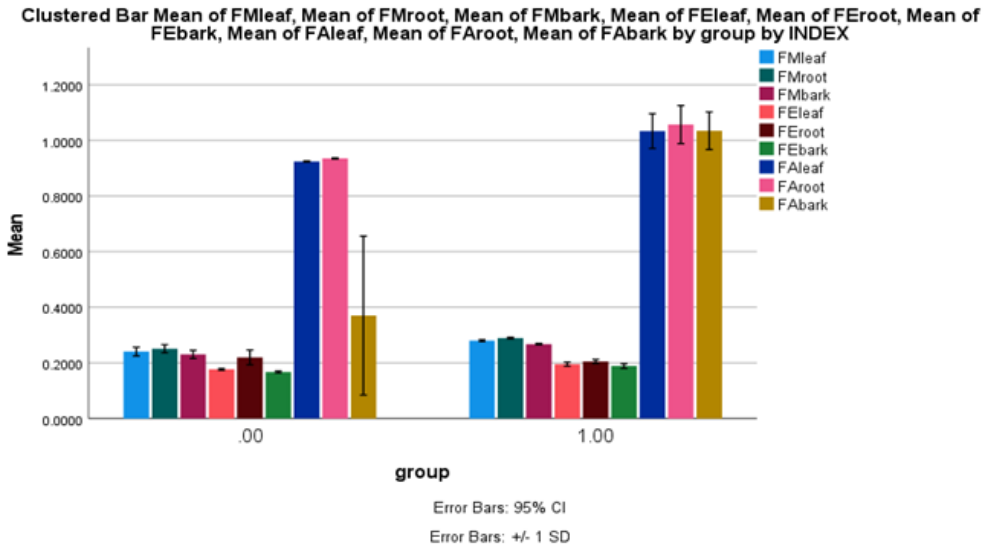

Fig. 3. Comparison of flavonoids in root and bark with leaf of *Cardiospermum halicacabum* in terms of mean. The mean of flavonoid content in root using acetone concentration appears to be better than in leaf and bark and standard deviation of flavonoid content in root is slightly better than the flavonoid content in leaf and bark. X Axis: flavonoid content in leaf Vs tannin content in root and bark. Y Axis: Mean with SD of±1. (FM-Flavonoids in Methanol, FE-Flavonoids in ethanol, FA-Flavonoids in acetone)

Discussion

There appears to be a statistically significant difference in the mean of phenols, tannins and flavonoid content of leaf, root and bark (p<0.01, Independent samples) depicts in Table 2,4,6. The results showed higher extraction of phytochemical compounds due the novel solvent extraction method. Methanol extract of leaf appeared to have higher phenol (0.32mg/ml) content compared to ethanol extract of leaf (G et al. 2018). Ethanol extract of leaf appeared to have higher tannin content (0.59mg/ml) (Jeyadevi et al. 2013). Aqueous extract of C. halicacabum and B.monosperma leaves appeared to show the presence of most phytoconstituents compared to ethanol extract and methanol extract. The aqueous extract is one of the best extracts followed by ethanol extract (Rameshwari et al. 2020). Phytochemical screening reveals that the

C.halicacabum extract contains glycosides, carbohydrates, flavonoids, phytosterols, phenolic compounds and saponin (Zalke et al. 2013).

Our institution is passionate about high quality evidence based research and has excelled in various fields ((Vijayashree Priyadharsini 2019; Ezhilarasan, Apoorva, and Ashok Vardhan 2019; Ramesh et al. 2018; Mathew et al. 2020; Sridharan et al. 2019; Pc, Marimuthu, and Devadoss 2018; Ramadurai et al. 2019). We hope this study adds to this rich legacy.

The various factors affecting this study may be due to the concentration of the solvents which reduces the extraction of bioactive compounds(Heinrich et al. 2017). The physical parameters like moisture content and solubility of the powdered extract can also influence the separation of components (Ajaya Kumar et al. 2004). Secondary metabolites which are present in the extract cause reduction in the phytochemical contents.

Conclusion

The Present examination shows the capacity of different extracts of *Cardiospermum halicacabum* in screening of phytochemicals and bioactive compounds. Among all the extracts, root extract appears to be highest phytochemical and phenolic content when compared with leaf and bark extract.

Declarations

Conflict of Interests

No conflict of interest in this manuscript.

Authors Contributions

Author PVP was involved in data collection, data analysis, manuscript writing. Author JRD was involved in conceptualization, data validation and critical review of manuscript.

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