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Quantitization of antioxidant potency in various plant parts of *Embelia tsjeriam-cottam*, an important medicinal plant

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Abstract
The entire world is in search of natural compounds having medicinal potency due to lesser side effect issues. To this effort *E. tsjeriam-cottam* is an emerging shrub having high medicinal potency. In this study antioxidant potency of different parts of *E. tsjeriam-cottam* was quantified through measuring the non enzymatic antioxidant parameters and the antioxidant activity of crude extracts were compared with the purified embelin isolates. For validation process, two types of extraction procedures viz. Soxhlet and water bath method were used and parameters like Phenol, Flavonoid, DPPH, FRAP, Reducing power were quantified. From the results, crude extracts along with isolated compounds had shown better antioxidant potency particularly in fruit followed by root, stem bark and leaf. Crude extracts were found to be superior to purified isolates in terms of antioxidant potency. As both crude extracts and purified isolates showed remarkable antioxidant potency hence can be used in medicinal industries.

Keywords: *E. tsjeriam-cottam,* Antioxidant, DPPH, FRAP, Reducing Power

1. Introduction
Natural bio-resources from plants play a vital role in the nurturance of mankind especially by mitigating the increasing demand of herbal therapeutics and hence increasing an inclination of global trend for medicinal formulations from synthetic to natural resources. Reactive oxygen species induce oxidative damage to bio molecules causing several diseases like atherosclerosis, cancer, DNA and protein damage, lipid peroxidation, ageing, inflammatory activities etc. Antioxidants are biomolecular compounds, particularly the secondary metabolites acting as electron donor, singlet oxygen quencher, enzyme inhibitor, or metal chelating agent. These have the ability to countervail the pernicious effect of the reactive oxygen species by neutralizing free radical intermediates and inhibit other oxidation reactions [1]. Plants are endowed with several natural antioxidant molecules viz. Phenolic acids, Flavonoids, Coumarins, alkaloids, amines, carotenoids etc [2], which can terminate or retard the chain reactions of oxidation processes by scavenging free radicals [3]. In current scenario of Morden and synthetic medicinal era, use of some synthetic antioxidants has become curtailed due to their certain noxious effects [4-6] along with the increasing upsurge of interest in the use of therapeutic potential medicinal plants as antioxidants [7] has enlightened the path towards exploring new natural source for antioxidant compounds of plant origin diligently.

mbelin is a naturally occurring alkyl substituted hydroxyl-benzoquinone found as the active principal element in the plants of Myrsinaceae family. This bioactive compound is unique and vital due to having several ethno medicinal, Phytochemical and pharmacological activities. The versatility of embelin could be evidenced from the fact that it is having antihelminthic, contraceptive activities [8], anti-inflammatory [9-12] and anticancer potency [13-15] as well as photosensitizing property [16]. It has further been shown to protect cells against UVB-induced oxidative damage [17]. With regard to antioxidant activity in *Embelia tsjeriam-cottam,* some reports are available [18-21]. The present piece of work is taken forward to establish the comparative illustration of antioxidant potency of the crude extracts of *E. tsjeriam-cottam* along with the purified, isolated embelin compound to validate their effectiveness as a vital role in natural antioxidant molecules category.

2. Materials & Methods

2.1. Materials

Embelia tsjeriam-cottam plants and its various plant parts (Seeds, Leaves, Stem bark & Roots) were collected from Ghana Reserve Forest of Kalahandi district, Odisha (19°52′15"N and 83°53'30"E). The studied plant species was compared with herbarium specimens present in the institutional herbarium (bearing voucher specimen no 4897) and also verified through the reference book "The Flora of Odisha". The plants were procured in RPRC nursery for further mass multiplication and conservation purpose [22].

2.2. Methods

2.2.1. Pre-treatment of Samples

All the plant parts of both the selected medicinal plants, (Leaf, stem bark, fruit and root samples) were collected, cleaned in running tap water. All the samples were in hot air oven (Wiswd Instruments) at 50°C for 12 hrs [23] and pulverized to fine granules by use of mechanical grinding. The dried pulverized samples were then stored in freezer (Voltas deep freezer, Model No-405L CF) in airtight containers for further extraction.

2.2.2. Sample Extraction

2.2.2.1. Extraction-1

Powdered samples (leaf, stem bark, fruit and root) were extracted using Soxhlet apparatus (JSGW, Model No-13948) for 16-18 hrs using Methanol and Chloroform as solvent systems (24-25). Filtrates were collected and condensed to 4-7 ml using desiccator (TARSON, Model No-Rocker 410) and then condensed to semi solid form by the help of dry bath (Bangalore Genei, Model No-SLN-DB 120) and kept as stock solution [26].

2.2.2.2. Extraction-2

The powdered samples (leaf, stem bark, fruit and root) were extracted at 60º C for a period of 12-14 hrs with Methanol and Chloroform as solvent systems separately through water bath (Rivotek, Model No-211074). Filtrates were collected and condensed in the same way and kept as stock solution [27-28].

2.2.3. Estimation of Total Phenol Content (TPC)

Phenol content was estimated following the method of [29], modified by [30]. Absorbance of the final solution mixture of extracted and purified samples was measured at 515 nm wavelength and the values were expressed as mg GAE/gm dry wt. Gallic acid (1mg/ml) was used as standard.

2.2.4. Estimation of Total Flavonoid Content (TFC)

Total Flavonoid content (TFC) was measured by the Aluminium Chloride method [31]. Absorbance of the extracted and purified samples was measured at 510 nm wavelength and results were expressed as mg QE/gm dry wt. Quercetin (1mg/ml) was used as standard.

2.2.5. Estimation of DPPH Free Radical scavenging activity

DPPH free radical scavenging activity of the extracted and purified samples was measured by following method of [32]. The absorbance of the mixture sample was measured at 517 nm wavelength and the results were expressed as % radical scavenging activity and were calculated through the following equation [20].

% scavenging activity = [(Absorbance $_{Blank}$ – Absorbance $_{Sample}$)/Absorbance $_{Blank}$] ×100

2.2.5.1. EC50 value in DPPH assay

The EC50 values of each extracted and purified samples were determined graphically. The EC50 was defined as the concentration in µg of dry sample/ml that inhibits the formation of DPPH radicals by 50% [33].

2.2.6. Estimation of Phosphomolybdenum Reduction Assay

The antioxidant content of the extracted and purified samples was evaluated by the phosphomolybdenum method [34]. The absorbance of the solution was measured at 695 nm wavelength. The antioxidant content was measured from standard curve of ascorbic acid and the values were expressed as mg AAE/gm dry wt.

2.2.7. Estimation of Ferric reducing antioxidant power (FRAP) assay

FRAP assay, for measuring the total antioxidant capacity, was determined by method of [35]. The absorbance of reaction mixture was measured at 593 nm wavelength and the FRAP values were expressed in mM ascorbic acid equivalent (AAE)/gm dry wt. derived from standard curve.

2.2.8. Estimation of reducing power activity

The reducing power of the sample was determined using method of potassium ferricyanide and ferric chloride [36] with little modifications [37]. The absorbance was measured at 700 nm wavelength. Ascorbic acid was used as standard. The extract concentration providing 0.5 of absorbance was calculated from the graph of absorbance at 700 nm against extract concentration and expressed as EC50 [33].

2.3. Statistical Approach

In present study, the results of the non enzymatic parameters of antioxidant activity of both the crude plant extracts and the purified embelin samples were analyzed through Two Way ANOVA (Repetitive Measures) using GRAPHPAD PRISM software version 6.0. All the data are expressed as Mean ± SD. All the percentile values were converted into angular transformation for statistical analysis. The variations in results were analyzed at 99.9% significant level.

3. Results

3.1. Total Phenol Content (TPC) in crude extracts & purified Isolates of different parts of *Embelia tsjeriam-cottam*

The total phenol content was assessed from different plant parts of both the crude extracts and purified isolates were evaluated and was expressed in terms of mg GAE/gm dry wt. /ml. In case of the crude extracts of *E. tsjeriam-cottam,* TPC content was found to be in a range of 10.32-29.6 mg GAE/gm dry wt. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), TPC content was found to be highest in Fruit parts (29.6 mg GAE/gm dry wt.), when extracted with chloroform. While in case of leaves the least TPC content was found in methanolic extracts (10.32 mg GAE/gm dry wt.). When the purified isolates of these crude extracts were evaluated for TPC content, it was found to be in a range of 1.12-17.71 mg GAE/gm dry wt. Highest TPC content was found in Fruit parts (17.71 mg GAE/gm dry wt.), when extracted with chloroform. While in case of leaves the least TPC content was found in methanolic extracts (1.12 mg GAE/gm dry wt.). The fruit part showed highest total phenol content followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along

with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of total phenol content. When both the crude extracts and purified isolates were compared for TPC content, the crude extracts was found to be superior to the purified isolates (Table-1, Figure-1). All data were analyzed statistically at 99% interval level through two ways RM ANOVA along with Sidak's multiple comparisons test. In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with P value < 0.0001, where as the column factor i.e. the crude and purified isolates were found to be significant at P value 0.0002.

3.2. Total Flavonoid Content (TFC) in crude extracts & purified samples of *Embelia tsjeriam-cottam*

Total Flavonoid content was measured in crude extracts as well as the purified isolates, derived from various plant parts (Fruit, Leaf, Stem bark and Root) of *E. tsjeriam-cottam* through Aluminium chloride colorimetric assay. TFC content was found to be in a range of 12.59 -57.39 mg QE/gm dry wt. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), TFC content was found to be highest in Fruit parts (57.39 mg QE/gm dry wt.), when extracted with chloroform. While in case of leaves the least TFC content was found in methanolic extracts (12.59 mg QE/gm dry wt.). When the purified isolates of these crude extracts were evaluated for TFC content, it was found to be in a range of 2.51-14.13 mg QE/gm dry wt. Highest TFC content was found in Fruit parts (14.13 mg QE/gm dry wt.), when extracted with chloroform. While in case of leaves the least TFC content was found in methanolic extracts (2.51 mg QE/gm dry wt.). The fruit part showed highest total Flavonoid content followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of total Flavonoid content. When both the crude extracts and purified isolates were compared for TFC content, the crude extracts were found to be superior to the purified isolates, which indicated its antioxidant potency clearly (Table-1, Figure-2). All data were analyzed statistically at 99% interval level through two ways RM ANOVA along with Sidak's multiple comparisons test. In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with P value < 0.0001, where as the column factor i.e. the crude and purified isolates were found to be significant at P value 0.0007.

Table 1: Total Phenol Content (mg GAE/g dry wt.) and Total Flavonoid Content (mg QE/g dry wt.) of Crude extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

	Extraction Process	Solvents Used	TPC Content (mg GAE/gm dry wt.) in *Embelia tsjeriam-cottam*		TFC Content (mg QE/gm dry wt.) in *Embelia tsjeriam-cottam*	
			Crude	Pure	Crude	Pure
FRUIT	Soxhlet	Methanol	27.79±2.13	15.79±0.49	52.05±2.97	13.17±0.40
		Chloroform	29.6±7.62	17.71±0.80	57.39±1.93	14.13±0.40
	Water bath	Methanol	25.44±3.06	4.64±0.16	41.6±4.23	12.32±0.42
		Chloroform	25.6±6.71	9.01±2.91	49.6±4.81	12.53±0.42
LEAF	Soxhlet	Methanol	13.95±1.32	1.44±0.16	19.31±0.97	4.43±0.46
		Chloroform	15.52±0.16	1.65±0.09	20.8±0.32	4.85±0.33
	Water bath	Methanol	10.32±0.44	1.12±0.16	12.59±0.48	2.51±0.24
		Chloroform	12.05±1.87	1.23±0.09	18.56±1.66	3.63±0.18
STEM BARK	Soxhlet	Methanol	17.17±3.11	1.71±0.09	23.89±0.36	8.8±0.16
		Chloroform	17.5±1.05	1.92±0.16	25.71±0.80	9.12±0.16
	Water bath	Methanol	15.7±4.80	1.55±0.09	21.65±0.18	7.15±1.12
		Chloroform	16.64±5.08	1.7±0.18	22.4±0.32	8.32±0.97
ROOT	Soxhlet	Methanol	22.83±1.54	2.61±0.24	38.9±5.14	10.61±0.48
		Chloroform	23.59±2.31	3.093±0.49	39.47±4.03	11.52±0.32
	Water bath	Methanol	18.7±3.45	2.24±0.27	26.77±0.48	9.92±0.64
		Chloroform	21.23±0.80	2.9±0.46	27.63±0.48	10.13±0.56

NB- Data are expressed as Mean ± SD, (where n=3)

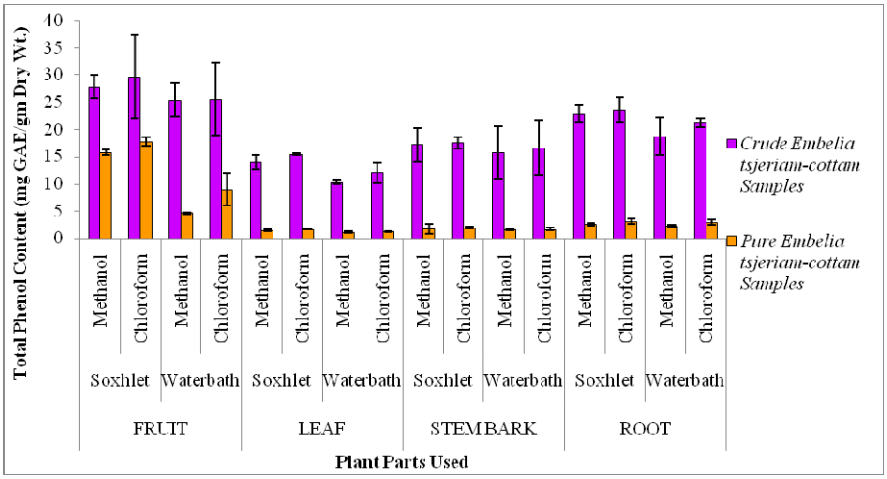

Fig 1: Total Phenol Content (mg GAE/gm Dry Wt.) of Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

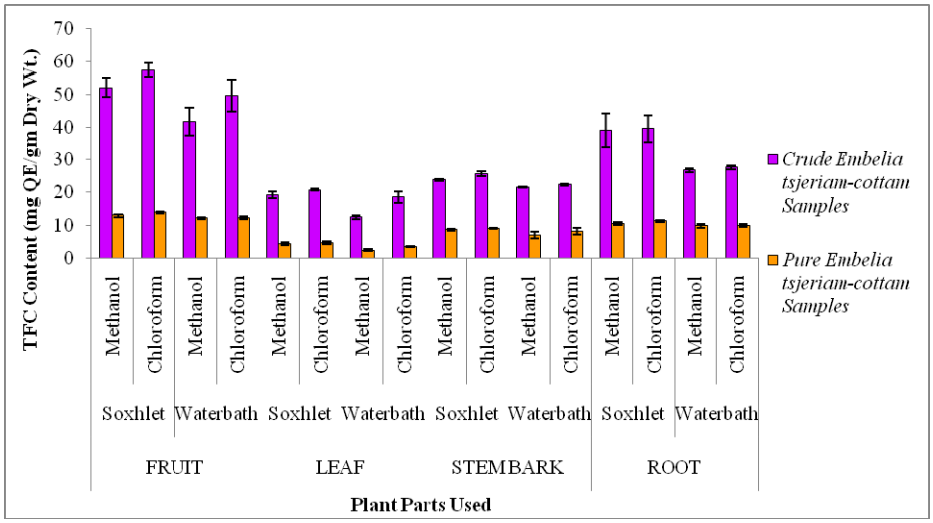

Fig 2: Total Flavonoid Content (mg QE/gm Dry Wt.) of Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

3.3. Total Antioxidant Content (TAC) in crude extracts & purified samples of *Embelia tsjeriam-cottam*

The total antioxidant content was assessed from different plant parts of both crude and purified isolates of *E. tsjeriam-cottam* and was expressed in terms of mg AAE/gm dry wt., after calculating from standard curve of Ascorbic Acid. In case of the crude extracts of *E. tsjeriam-cottam,* TAC content was found to be in a range of 0.59-3.09 mg AAE/gm dry wt. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), TAC content was found to be highest in Fruit parts (3.09 mg AAE/gm dry wt.), when extracted with chloroform. While in case of leaves the least TAC content was found in methanolic extracts (0.59 mg AAE/gm dry wt.). When the purified isolates of these crude extracts were evaluated for TAC content, it was found to be in a range of 0.17-0.46 mg AAE/gm dry wt. Highest TAC content was found in Fruit parts (0.46 mg AAE/gm dry wt.), when extracted with chloroform. While in case of leaves the least TAC content was found in methanolic extracts (0.17 mg AAE/gm dry wt.). The fruit part showed highest total antioxidant content in terms of non enzymatic evaluation followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of total antioxidant content. When both the crude extracts and purified isolates were compared for TAC content, the crude extracts was found to be superior to the purified isolates (Table-2, Figure-3). All data were analyzed statistically at 99% interval level through two ways RM ANOVA along with Sidak's multiple comparisons test. In the multiple comparison analysis, the row factor i.e. the plant parts extracted through different processes with various solvent systems was found to be highly significant with P value < 0.0001, where as the column factor i.e. the crude and purified isolates were found to be significant at P value 0.0017.

3.4. Reducing Power Activity in crude extracts & purified samples of *Embelia tsjeriam-cottam*

The reducing power activity of the crude extracts and the purified compounds were expressed in terms of EC50 in µg dry wt. /ml, which means effective concentration at which the extract concentration provides 0.5 of absorbance (EC50) and was calculated from the graph. In case of the crude extracts of *E. tsjeriam-cottam,* reducing power activity was found to be in a range of 12-36 µg dry wt. /ml. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), reducing power activity was found to be best in Fruit parts (12 µg dry wt. /ml), showing lower effective coefficient, when extracted with chloroform, while leaves yielded the least reducing power activity in methanolic extracts (36 µg dry wt. /ml), showing higher effective coefficient. When the purified isolates of these crude extracts were evaluated for reducing power activity, it was found to be in a range of 19-53 µg dry wt. /ml. Best reducing power activity was found in Fruit parts (19 µg dry wt.), showing lower effective coefficient, when extracted with chloroform and in case of leaves the least reducing power activity was found in methanolic extracts (53 µg dry wt. /ml), showing higher effective coefficient. The fruit part showed highest reducing power activity followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of reducing power activity. When both the crude extracts and purified isolates were compared for reducing power activity, the crude extracts were found to be superior to the purified isolates (Table-2, Figure-4).

3.5. DPPH Radical Scavenging Activity (EC 50) in crude extracts & purified samples of *Embelia tsjeriam-cottam*

The DPPH radical scavenging activity of the crude extracts and the purified compounds were expressed in terms of the amount antioxidant required for decrease the initial absorbance of DPPH by 50 % and the values were expressed in terms of EC50 in µg of dry wt. /ml, which means effective concentration at which 50% of DPPH radicals are scavenged. In case of the crude extracts of *E. tsjeriam-cottam,* radical-scavenging activity in terms of EC 50 value was found to be in a range of 11-40 µg dry wt./ml. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), DPPH radical-scavenging activity was found to be best in Fruit parts (11 µg dry wt./ml), showing lower effective coefficient, when extracted with chloroform, while leaves yielded the least DPPH radical-scavenging activity in methanolic extracts (40 µg dry wt./ml), showing higher effective coefficient. When the purified isolates of these crude extracts were evaluated for DPPH radical-scavenging activity, it was found to be in a range of 27-90 µg dry wt. /ml. Best DPPH radical-scavenging activity was found in Fruit parts (27 µg dry wt. /ml), showing lower effective coefficient, when extracted with chloroform.

While in case of leaves the least DPPH radical-scavenging activity was found in methanolic extracts (90 µg dry wt. /ml). The fruit part showed highest DPPH radical-scavenging activity followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of DPPH radical-scavenging activity. When both the crude extracts and purified isolates were compared for DPPH radical-scavenging activity, the crude extracts were found to be superior to the purified isolates (Table-2, Figure-5).

Table 2: TAC Content (mg AAE/gm dry wt. /ml), Reducing Power and DPPH content (EC50 in µg of dry wt. /ml) in Crude extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

Plant Parts	Extraction Process	Solvents Used	TAC Content (mg AAE/gm dry wt./ml)		DPPH Content (EC 50 in µg of dry wt./ml)		Reducing Power (EC 50 in µg of dry wt./ml)	
			Crude	Pure	Crude	Pure	Crude	Pure
FRUIT	Soxhlet	Methanol	2.34±0.182	0.43±0.031	12	30	14	23
		Chloroform	3.09±0.065	0.46±0.021	11	27	12	19
	Water bath	Methanol	1.8±0.031	0.39±0.031	15	40	16	29
		Chloroform	1.9±0.351	0.41±0.083	13	37	15	24
LEAF	Soxhlet	Methanol	0.65±0.175	0.21±0.032	30	83	34	44
		Chloroform	0.67±0.0115	0.25±0.046	27	72	27	40
	Water bath	Methanol	0.59±0.112	0.17±0.042	40	90	36	53
		Chloroform	0.67±0.0115	0.19±0.042	30	80	29	52
STEM BARK	Soxhlet	Methanol	0.93±0.063	0.29±0.011	21	57	26	33
		Chloroform	0.95±0.054	0.31±0.011	20	55	24	29
	Water bath	Methanol	0.89±0.0115	0.27±0.046	26	66	23	38
		Chloroform	0.91±0.186	0.29±0.051	22	60	17	31
ROOT	Soxhlet	Methanol	1.29±0.122	0.35±0.011	15	45	16	29
		Chloroform	1.31±0.208	0.36±0.035	14	42	12	26
	Water bath	Methanol	1.07±0.0115	0.34±0.042	20	48	27	29
		Chloroform	1.09±0.023	0.35±0.006	18	47	23	27

NB-Samples are diluted 5 times before use. Data are expressed as Mean ± SD, (where n=3)

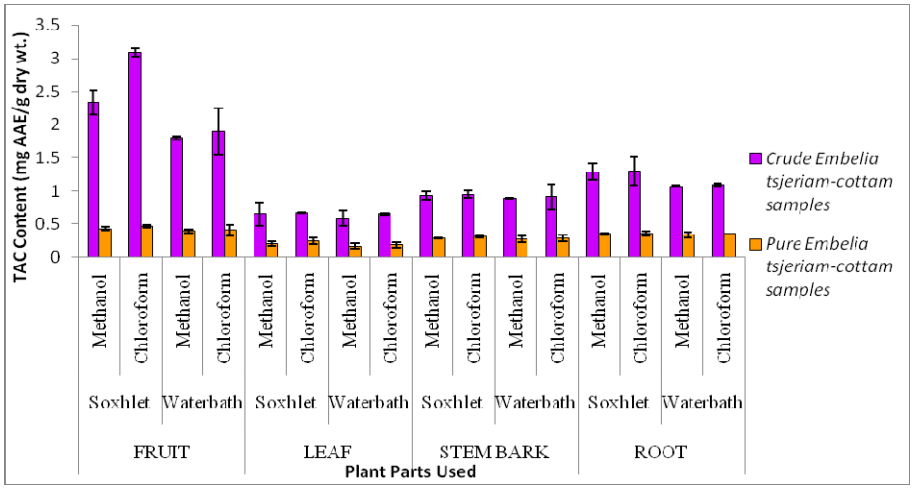

Fig 3: TAC Content (mg AAE/gm Dry Wt.) In Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

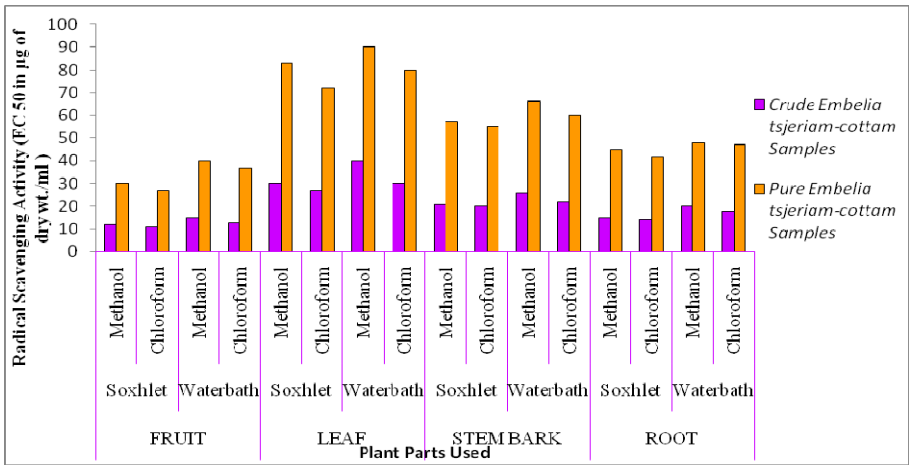

Fig 4: DPPH Radical Scavenging Activity (EC 50 Value) of Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

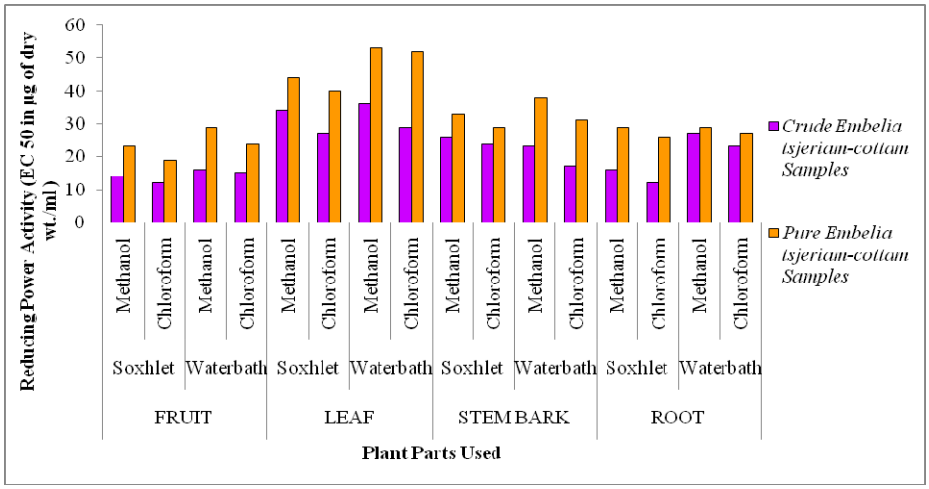

Fig 5: Reducing Power Activity (EC 50 In mg of Dry Wt. /ml) Of Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

3.6. Percentage of Radical Scavenging Activity (RSA) in crude extracts & purified samples of *Embelia tsjeriam-cottam*

The radical-scavenging activity (RSA) was assessed from different plant parts of both crude and purified isolates of *E. tsjeriam-cottam* and was calculated from formula. In case of the crude extracts of *E. tsjeriam-cottam,* radical-scavenging activity was found to be in a range of 40.75-56.2% dry wt. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), radical-scavenging activity was found to be highest in Fruit parts (56.2% dry wt.), when extracted with chloroform, while leaves yielded the least radical-scavenging activity in methanolic extracts (40.75% dry wt.). When the purified isolates of these crude extracts were evaluated for radical-scavenging activity, it was found to be in a range of 51.75-68.06% dry wt. Highest radical-scavenging activity was found in Fruit parts (68.06% dry wt.), when extracted with chloroform. While in case of leaves the least radical-scavenging activity was found in methanolic extracts (51.75% dry wt.). The fruit part showed highest radical-scavenging activity followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of radical-scavenging activity. When both the crude extracts and purified isolates were compared for radical-scavenging activity, the crude extracts were found to be superior to the purified isolates (Table-3, Figure-6).

3.7. Ferric Reducing Antioxidant Power (FRAP) in crude extracts & purified samples of *Embelia tsjeriam-cottam*

The FRAP activity was assessed from different plant parts of both crude and purified isolates of *E. tsjeriam-cottam* and *A. corniculatum* and was expressed in terms of mg AAE/gm dry wt., after calculating from standard curve of Ascorbic Acid. In case of the crude extracts of *E. tsjeriam-cottam,* FRAP activity was found to be in a range of 1.5-8.3 mg AAE/gm dry wt. Amongst all the plant parts selected (Fruit, Leaf, Stem bark and Root), FRAP value was found to be highest in Fruit parts (8.3 mg AAE/gm dry wt.), when extracted with chloroform, while leaves yielded the least FRAP value in methanolic extracts (1.5 mg AAE/gm dry wt.). When the purified isolates of these crude extracts were evaluated for FRAP activity, it was found to be in a range of 0.37-1.05 mg AAE/gm dry wt. Highest FRAP value was found in Fruit parts (1.05 mg AAE/gm dry wt.), when extracted with chloroform. While in case of leaves the least FRAP value was found in methanolic extracts (0.7 mg AAE/gm dry wt.). The fruit part showed highest FRAP activity followed by the root parts followed by stem bark and then finally by the leaf parts. The solvent systems used along with the methods involved for extraction of the crude content had also played a crucial role in variations in the presence of FRAP activity. When both the crude extracts and purified isolates were compared for FRAP activity, the crude extracts were found to be superior to the purified isolates (Table-3, Figure-7).

All data were analyzed statistically at 99.9% interval level through two ways RM ANOVA along with Sidak's multiple comparisons test. In the multiple comparison analysis, both the row factors and column factors i.e. the plant parts extracted through different processes with various solvent systems and the crude and purified isolates were found to be highly significant with P value < 0.0001.

Table 3: Percentage of Radical Scavenging Activity and Ferric Reducing Antioxidant Power (mM AAE/g dry wt.) of Crude extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

Plant Parts	Extraction Process	Solvents Used	% of Scavenging Activity in *Embelia tsjeriam-cottam*		Ferric Reducing Antioxidant Power (mM AAE/g dry wt.)	
			Crude	Pure	Crude	Pure
FRUIT	Soxhlet	Methanol	54.96	67.92	7.5 ± 0.4	0.99 ± 0.03
		Chloroform	56.2	68.06	8.3 ± 0.2	1.05 ± 0.02
	Water bath	Methanol	51.8	62.96	6.7 ± 0.2	0.76 ± 0.03
		Chloroform	51.72	65.97	7.1 ± 0.173	0.86 ± 0.026
LEAF	Soxhlet	Methanol	45.78	55.93	2.3 ± 0.2	0.43 ± 0.0173
		Chloroform	47.74	55.95	3.1 ± 0.264	0.47 ± 0.0264
	Water bath	Methanol	40.75	51.75	1.5 ± 0.2	0.37 ± 0.02
		Chloroform	45.12	52.95	1.8 ± 0.1	0.4 ± 0.056
STEM BARK	Soxhlet	Methanol	47.78	59.5	4.2 ± 0.36	0.55 ± 0.02

		Chloroform	49.1	60.3	4.6 ± 0.3	0.59 ± 0.03
	Water bath	Methanol	47.44	58.25	3.4 ± 0.2	0.49 ± 0.02
		Chloroform	47.6	59.2	3.7 ± 0.264	0.52 ± 0.0173
ROOT	Soxhlet	Methanol	51.24	62.39	5.6 ± 0.436	0.69 ± 0.03
		Chloroform	55.22	62.75	6.1 ± 0.2	0.72 ± 0.0173
	Water bath	Methanol	50.75	60.78	5 ± 0.173	0.62 ± 0.01
		Chloroform	51.34	61.56	5.3 ± 0.2	0.66 ± 0.0264

NB-Samples are diluted 5 times before use; Data are expressed as Mean ± SD, (where n=3)

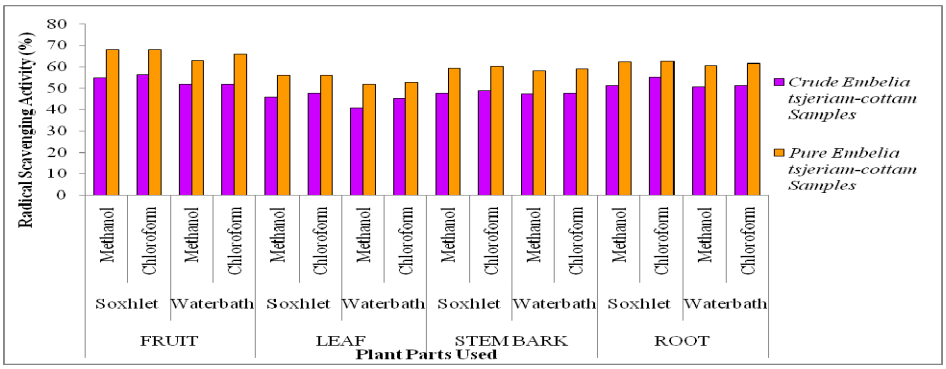

Fig 6: Percentage of Radical Scavenging Activity of Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

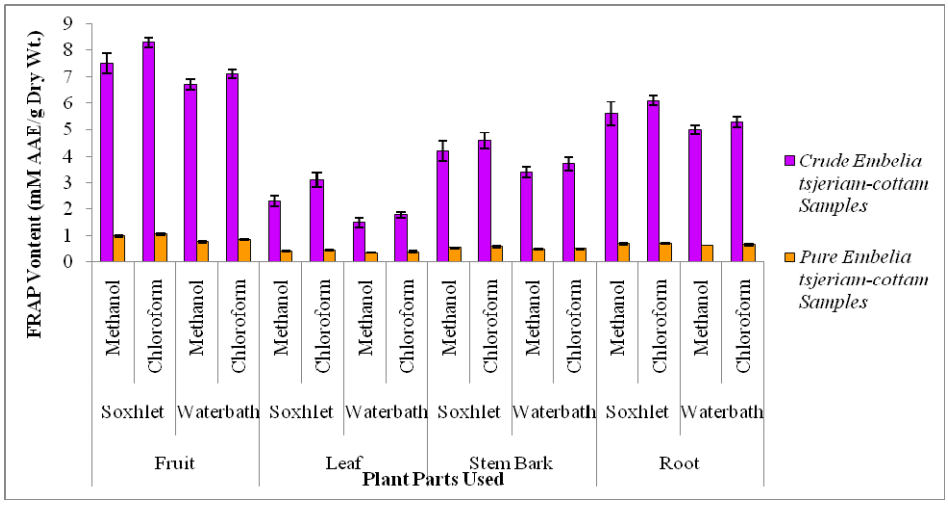

Fig 7: Ferric Reducing Antioxidant Power (Mm AAE/gm Dry Wt.) Activity of Crude Extracts & Purified Embelin Compounds of *Embelia tsjeriam-cottam*

4. Discussion

Antioxidant may have great relevance in the prevention and therapeutics of diseases. Due to over increasing toxicity of synthetic antioxidant compounds, interest in natural antioxidants, of plant origin, has greatly increased in recent year. Natural antioxidants are essentially the plant's secondary metabolites, capable of slowing or inhibiting the harmful effects of free radicals and high levels of oxygen produced during photosynthesis [38]. These natural secondary metabolites are being implemented in both plant self defence mechanism and healing several diseases for betterment of human health care system. Various Phytochemical and pharmacological studies strongly supported the fact that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. For this reason a growing interest toward natural antioxidants of herbal resources is being developed gradually [39]. *E. tsjeriam-cottam* is a well known and vulnerable medicinal plant having various medicinal attributes. The active principle compound embelin, isolated from the various plant parts of *E. tsjeriam-cottam*, is being widely used for treatment of several alignments. In this present study the antioxidant potency of the crude extracts of *E. tsjeriam-cottam* along with the purified embelin elutes from each extract were quantified through evaluation of several non enzymatic parameters like DPPH, FRAP, Reducing power etc. Utilization of crude plant extracts as antioxidants instead of using the eluted pure compounds is a favourable alternative from an economic and time saving point of view. In some cases these crude extracts have also been proved to be superior to that of the synthetic compounds [40] as the other bio constituents present in the crude extracts may act synergistically to produce higher antioxidant potency. Separation of desired bio active compounds present in the crude extract may also lead towards elimination eventual desirable and/or undesirable compounds [41]. In this case various plant parts viz. Fruit, leaf, stem bark and the root parts of the *E. tsjeriam-cottam* plants have been evaluated for its antioxidant potency along with the comparative account with the purified embelin elutes from the respective plant parts. From the experiments, in all the cases, the fruit part showed highest antioxidant activity followed by the root parts followed by stem bark and then finally by the leaf parts. The antioxidant activity of *E. tsjeriam-cottam* and its closely related species *E. ribes* has been quantified by

several other authors [18, 21, 42-45], but in most cases they have used only the fruits as target area to evaluate the antioxidant activity. However some scattered evidences regarding the antioxidant study in other plant parts besides the fruits [20, 46-47] are also present. More over the comparative analysis of the crude extracts with the purified embelin compounds for antioxidant activity was an interesting focus area to find out the more potent source as antioxidant compound. When both the crude extracts and purified isolates were compared, the crude extracts were found to be superior to the purified isolates. This fact was being supported by several other research findings [21, 48-50]. In all these experiments, the crude extracts showed best antioxidant activity. The fact was supported by the fact that other bio constituents present in the crude extracts may act synergistically to produce higher antioxidant potency [41].

5. Conclusion

The present piece of work gives a detailed view on the antioxidant activity of all the plant parts of *E. tsjeriam-cottam* keeping in view on its non enzymatic antioxidant activity. The crude extracts along with the isolated pure embelin compounds had shown better antioxidant potency particularly in the fruit parts followed by the root parts followed by stem bark and then finally by the leaf parts. When both the crude extracts and purified isolates were compared for antioxidant potency, the crude extracts were found to be superior to the purified isolates. Hence both the crude formulation and the purified compound can be used in drug formulation industries after further elaborate studies.

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7. References

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