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COMPARATIVE ANTIOXIDANT ACTIVITY PROFILE OF *BUTEA MONOSPERMA (LAM)* AND *ERYTHRINA VARIEGATA (LAM)* LEAF EXTRACTS

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ABSTRACT

Medicinal plants are of great importance and becoming potential source for phyto-constituents with varied pharmacological activities. Identification of such plants of potential use in medicine is of significance in this era. *Butea monosperma (Lam)* (Palash) or *Erythrina monosperma* belonging to family *leguminosae-papilioneae* is a medium-sized deciduous tree commonly known as 'flame of the forest'. *Erythrina variegata (Lam) or Erythrina indica* species is grown for its variegated leaves and the name "coral tree" is used as a collective term for these plants. The qualitative preliminary phytochemical analysis of the hydroethanolic leaf extracts of *B.monosperma* and *E.variegate* showed the presence of glycoside, flavonoid, tannins, alkaloid, phenols, protein, amino acids and saponins in both plant samples. Quantitative studies revealed that the 50% hydroethanolic leaf extract of *B.monosperma* showed higher constituents of phytochemicals when compared with *E.varigatea* leaf extract. Both the plant extracts showed significant free radical scavenging activity against the tested free radicals such as hydrogen peroxide, DPPH and reducing power (FRAP) assay. The present study revealed that *B.monosperma* showed highest Antioxidant activity profile than *E.variegate*.

KEYWORDS

Medicinal plants, Flame of the forest, Coral tree, Phenols, Flavonoids and Free radical scavenging.

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INTRODUCTION

Butea monosperma (Lam) is extensibly used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. This plant is reported to possess antifertility, aphrodisiac and analgesic activities. Roots are useful in filariasis, night blindness, helminthiasis, piles, ulcer and tumors (Figure No.1a)[1,2]. Flowers are useful in diarrhoea, astringent, diuretic, depurative, tonic,

leprosy, skin diseases, gout, thirst, burning sensation (Figure No.1b). The stem bark is useful in indigenous medicine for the treatment of dyspepsia, diarrhoea, dysentery, ulcer, sore throat and snake bite[3,4].

The genus *Erythrina* comprises of about 110 species of trees and shrubs. "Coral tree" is used as a collective term for these plants. Coral tree is indigenous to the old World tropics, possibly originally from India to Malaysia[5]. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement tree for other tree crops such as coffee and cacao[6]. The *Erythrina variegata (Lam) or Erythrina indica* species is grown for its variegated leaves (Figure No.2a), as well as its seasonal showy red flowers (Figure No.2b)[7]. Studies on phytochemicals of *Erythrina variegata (Lam)* species have demonstrated alkaloids and flavonoids as major constituents[8,9]. Different parts of *E. Varigatea* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic[10]. It has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts and wounds[11,12].

MATERIAL AND METHODS

Collection of plant materials

The Plant *Butea monosperma (Lam)* was collected from Kollengode, Palakkad district of Kerala state, India in the month of April and *Erythrina variegata (Lam)* was also collected from the College campus and Authenticated by Botanical Survey of India at Tamil Nadu Agricultural University (TNAU), Coimbatore. The plant samples were thoroughly washed in the running tap water to remove the adhering dust particles and dried under shades for about two weeks. It was ground into fine particles and stored in airtight container and used for the further investigations.

Preparation of plant crude extracts

Extraction of dried powders of leaves of *Butea monosperma (Lam)* and *Erythrina variegata (Lam)* was carried out in succession with increasing polarity of solvents viz. petroleum ether, chloroform, ethanol, methanol, hydro ethanol and water. Then it was kept under room temperature for 48 hours. The supernatant was taken and filtered using Whatman No.1 filter paper.

Phytochemical screening of plant extracts

The preliminary phytochemical screening of the plant extracts were carried out by the method of Sadasivam and Manickam (1996)[13].

Estimation of Total Phenol Content (TPC)

Total phenol content was estimated by the method of Singleton and Rossi (1965)[14]. Weighed exactly 0.5g of sample and ground it with a motor and pestle in 5ml of ethanol. Centrifuged at 2000rpm for 10min and collected the supernatant in 50ml volumetric flask. Evaporated the solvent and the residue was dissolved in known volume of water and used for the assay.

Estimation of Total Flavonoid Content (TFC)

Total flavonoid content was estimated by the method of Ordon *et al*, (2006)[15]. 0.5g of the plant sample was taken and ground with a motor and pestle in 5ml of ethanol. Centrifuged at 2000rpm for 10min and the supernatant was collected in 50ml volumetric flask. The residue was collected after the evaporation of the solvent and dissolved in known volume of water and used for the assay.

In vitro Radical scavenging assay

DPPH Radical scavenging assay

DPPH Radical scavenging assay was done by the method of Blois, (1958)[16]. Various concentrations of plant samples and Butylated Hydroxyl Toluene (BHT- reference standard) were taken and the volume was adjusted to 1ml by adding methanol. 5ml of 0.1mM methanol solution of DPPH was added and vortexed. Then, allowed to stand at room temperature for 20mins. The control was prepared in the same way without any extract and methanol was used for the baseline correction. Changes in the absorbance of the samples were measured at 517nm. The inhibition percentage was calculated by the formula:

Percentage radical scavenging activity = [(control OD – sample OD) / control OD] × 100

The concentration required for the 50% inhibition of radicals was expressed as IC_{50} value.

Reducing Power Capacity

The reducing capacity was estimated by the method of Decker and Welch (1990)[17]. The different concentrations of plant extracts and Ascorbic acid standard solution were prepared. From that 1ml of sample was taken and added phosphate buffer and 1% potassium ferricyanide. The contents were incubated at 50°C for 20mins. After incubation, added 10% TCA and centrifuged the tubes at3000rpm for 10mins. The supernatant was collected, added 0.1% $FeCl_3$ and distilled water. The intensity of the red color formation was read at 700nm in a UV/ Visible spectrophotometer.

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay was estimated by the method of Ruch *et al*, (1989)[18]. Different concentrations of plant extract samples (2-10mg/ml) were added to hydrogen peroxide solution. Absorbance of hydrogen peroxide at 230nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. The free radical scavenging activity was determined by evaluating % inhibition.

Percentage radical scavenging activity = [(control OD - sample OD) / control OD] × 100

RESULTS AND DISCUSSION

Phytochemical screening of plant extracts

The preliminary phytochemical screening of *Butea monosperma (Lam)* and *Erythrina variegata (Lam)* was carried out in succession with increasing polarity of solvents viz. petroleum ether, chloroform, ethanol, methanol, hydroethanol and water. On the basis of therapeutic potential of secondary metabolites, the phytochemical characters of the *B.monosperma* and *E.variegata* have been investigated and represented in the Table No.1.

Secondary metabolites are reported to have many biological and therapeutic properties. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity[19]. The qualitative phytochemical analysis of plant crude extracts revealed that the *Butea monosperma* leaf extract showed the high content of phytochemicals than the leaf extract of *Erythrina variegata*. The present results have revealed that hydroethanolic and petroleum ether extracts possessed maximum alkaloid and flavonoid contents.

Total Phenol and Flavonoid Content

The quantitative analysis of hydroethanolic leaf extracts of *Butea monosperma* and *Erythrina variegata* has been presented in the Table No.2. Significant amount of Total Phenol (22mg and 20mg respectively) and Flavonoid (0.34mg and 0.24mg respectively) content were present in the plant crude extracts. Among the various phytoconstitutents, phenol content was found to be present in highest concentration in both plant crude extracts. Among the two plant extracts, *Butea monosperma* leaf extract showed significant amount of phytoconstituents than *Erythrina variegate.*

DPPH radical scavenging activity

DPPH radical scavenging activity of *E.variegata, B.monosperma* leaf extracts and ascorbic acid standard are given in the Figure No.3. The potential of L-Ascorbic acid to scavenge DPPH radical is directly proportional to its concentration gradient. Hydroethanolic leaf extracts of both the plants have shown increasing order of radical scavenging activity as compared with the standard. The radical scavenging activity of *B.monosperma* leaf extract is slightly higher than that of the ascorbic acid. At the same time, *E.variegata* extract produced lower scavenging activity than *B.monosperma* and the standard. The present results are in accordance with the works done by Lavhale *et al*, (2007)[20] and Kanakasabapathy Devaki, (2016)[21].

Determination of reducing power

Reducing power assay of *E.variegata, B.monosperma* leaf extracts and the standard are shown in the Figure No.4. The reducing power potential of *B.monosperma* and *E.variegata* leaf extracts was directly proportional to its concentration gradient as compared with ascorbic acid. The reducing power of *B.monosperma* was slightly higher than that of the standard. On the other hand, the reducing activity of *E.variegata* was clearly lower than that of the *B.monosperma* and L-Ascorbic acid. Similar results were observed in the

studies done by Raj Kumar and Leena (2012)[22] and Hemmalakshmi *et al*, (2016)[23].

Hydrogen peroxide scavenging assay

Hydrogen peroxide scavenging assay of standard Ascorbic acid, *E.variegata* and *B.monosperma* leaf extracts are depicted in the Figure No.5. The potential of both the plant leaf extracts towards hydrogen peroxide scavenge activity increases with its increasing concentration when compared with standard ascorbic acid. Moreover, the H_2O_2 scavenging activity of *E.variegata* leaf extract was far lower than that of *B.monosperma* and standard ascorbic acid. Devansh *et al*, (2012)[24] and Hemmalakshmi *et al*, (2016)[25] have reported similar type of results on H_2O_2 scavenging activity.

Table No.1: Premilinary phytochemical screening of *B.monosperma* (Lam) and *E.variegata* (Lam)

S.No	Secondary Metabolites	Petroleum Ether		Chloroform		Ethanol		Methanol		Hydro Ethanol		Water	
		B	E	B	E	B	E	B	E	B	E	B	E
1	Alkaloids	++	++	++	++	-	-	++	-	++	-	++	+
2	Flavonoids	+	+	--	-	++	+	-	-	++	-	--	-
3	Proteins	++	+	+	+	-	+	+	-	-	+	++	-
4	Amino acids	++	+	+	-	-	-	+	+	-	+	++	-
5	Carbohydrate	-	+	+	-	+	+	+	+	++	-	+	++
6	Phenols	-	-	-	++	-	+	-	-	++	+	-	++
7	Tannins	-	-	-	++	-	-	-	+	+	-	-	+
8	Saponins	+	+	-	-	++	+	-	-	-	-	-	++
9	Glycosides	--	-	-	+	-	++	-	-	-	+	-	+

"B"- indicates *Butea monosperma* and "E" - indicates *Erythrina varigtae*

"+" - indicates presence of compounds; "-" - indicates absence of compounds and

"++" - indicates high concentration.

Table No.2: Quantitative analysis of hydroethanolic leaf extracts of *B.monosperma* and *E.variegate*

S.No	Secondary Metabolites	Result (mg)	
		B. monosperma	***E. variegata***
1	Phenols	22.0	20.0
2	Flavonoids	0.34	0.24

Figure No.1a: *B.monosperma* whole Tree

Figure No.1b: *B.monosperma* flower (Flame of the Forest)

Figure No.2a: *E.variegata* whole Tree

Figure No.2b: *E.variegata* flower

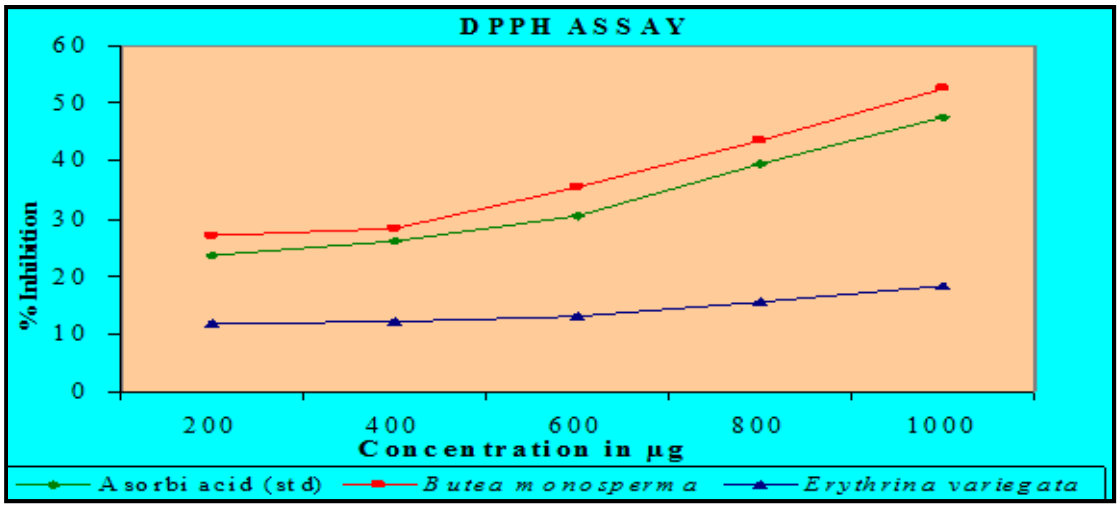

Figure No.3: DPPH radical scavenging of *E.variegata*, *B.monosperma* leaf extracts and Ascorbic acid

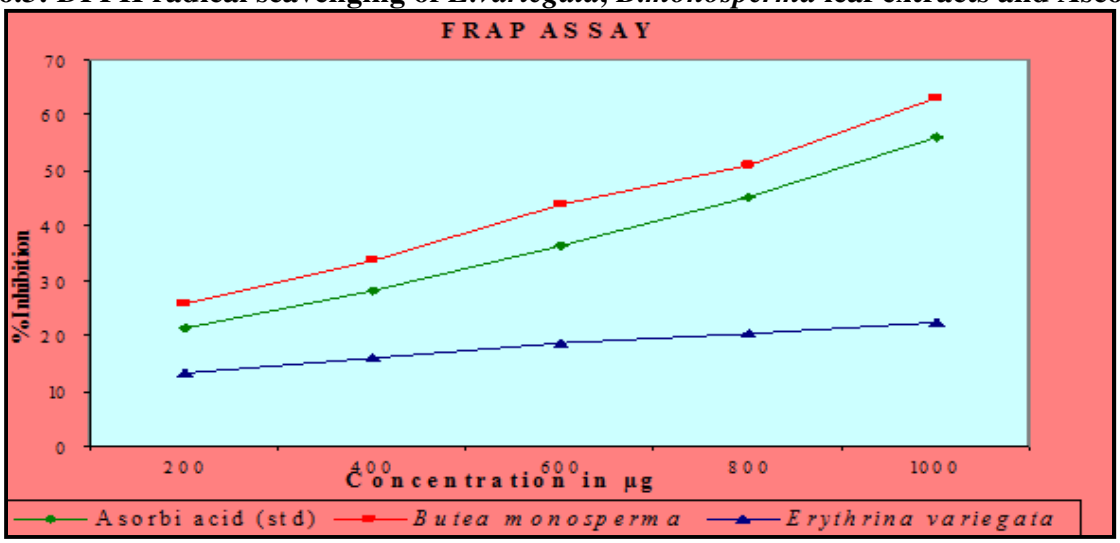

Figure No.4: Reducing power assay of *E.variegata*, *B.monosperma* leaf extracts and Ascorbic acid

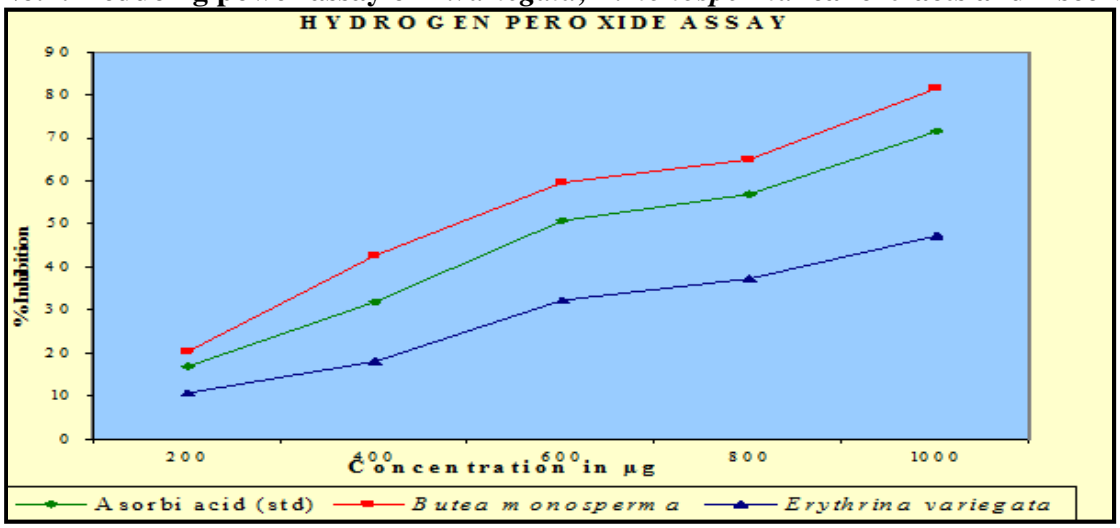

Figure No.5: H_2O_2 scavenging assay of *E.variegata, B. monosperma* leaf extracts and Ascorbic acid

CONCLUSION

The comparative study done on hydroethanolic leaf extracts of *Butea Monosperma* (Lam) and *Erythrina variegate* (Lam) has reported very relevant results on the preliminary phytochemical analysis and antioxidant property. The leaf extract of *Butea Monosperma* (Lam) has reported higher antioxidant activity than *Erythrina variegate* (Lam). This might be due to the presence of higher concentrations of alkaloids in the hydroethanolic leaf extract of *B.monosperma.* This comparative investigation clearly indicates that the active principles isolated from these two plants might be used as novel treatment for oxidative stress related diseases in future.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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