BIOACTIVE CONSTITUENTS AND ANTIOXIDANT ACTIVITIES OF *ERYTHRINA STRICTA* ROXB. SEEDS

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Abstract. *Erythrina stricta* Roxb., an underutilized legume tree species, is traditionally employed in various medicinal applications. This study systematically examines the phytochemical analysis and antioxidant potential of *E. stricta* seeds. The water extract stands out with the highest phenolic and flavonoid content, measuring 91.99 mg gallic acid equivalent (GAE)/g extract and 50.64 mg quercetin equivalent/g extract, respectively. Additionally, the acetone extract exhibits the highest alkaloid content at 40.48 mg atropine equivalent/g extract. Consistent with the phytochemical findings, the water extract demonstrates superior antioxidant capacity, as determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, total antioxidant activity, and ferric reducing antioxidant power (FRAP) assay. In conclusion, this study underscores *E. stricta* seeds as a robust source of both nutrients and phytochemicals, warranting further exploration and consideration for potential applications.
Keywords: *antioxidant activity, Fabaceae, legumes, underutilized fruits, phytochemical analysis*

Introduction

Several plant species especially trees that are underutilized, and neglected are rich sources of nutrients and phytochemicals which are having tremendous scope to be utilized for therapeutic plants (Murthy et al., 2021; Knez et al., 2023). One such group of plants is *Erythrina* species. The genus *Erythrina*, which includes over 120 species, are perennial tree species found worldwide in tropical regions (de Luca et al., 2018). The majority of *Erythrina* species are neglected and underutilized species, they are rich in phytochemicals with pharmacological effects, including sedative, hypotensive, and central nervous system depressants (Faggion et al., 2011; Santos Rosa et al., 2012). Extracts of varied plant parts of *Erythrina* species are also traditionally used as antimicrobials, anti-inflammatory, and pain-relieving agents (Togola et al., 2008; Mukungu et al., 2016; Bodofsky et al., 2020). Varied phytochemicals such as phenolics, flavonoids, tannins, xanthones, terpenoids, and alkaloids have been reported from different species of *Erythrina* species (Rambo et al., 2019; Fahmy et al., 2020).

Erythrina stricta Roxb. is an underutilized and neglected species that is naturally distributed in Southeast Asia including India, Bangladesh, Cambodia, China, Laos, Myanmar, Nepal, Thailand, Tibet, and Vietnam (POWO, 2023). It is a medium-sized tree (*Fig. 1A*) with a bright red colored inflorescence and flowers (*Fig. B*) and it is popularly

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called an 'Indian coral tree'. Pods are elongated and (*Fig. 1C*) contain 3 to 5 kidney-shaped seeds that are yellowish to brown at maturity (*Fig. 1D*).

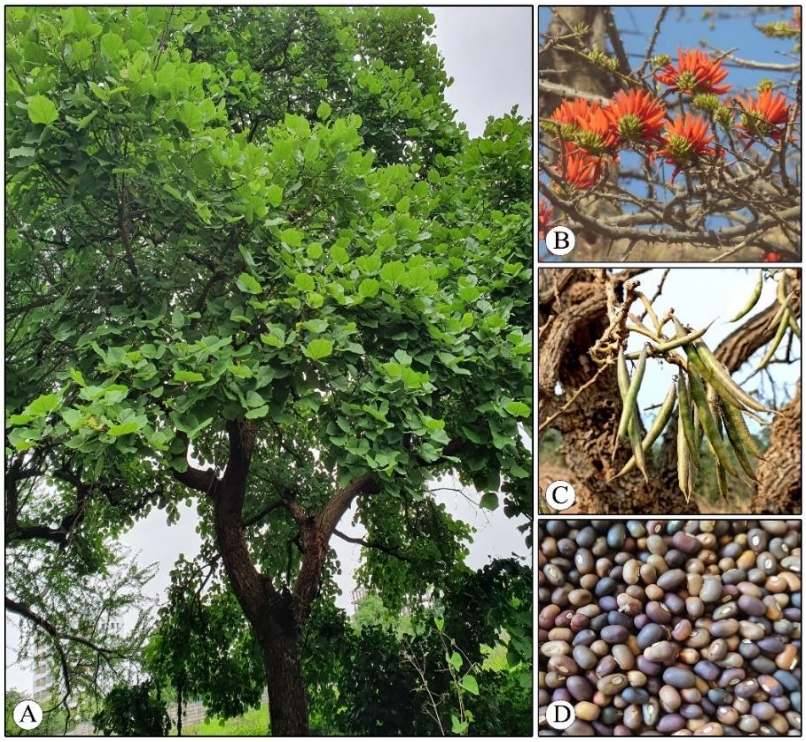

Figure 1. *Morphology of Erythrina stricta. A. Habit; B. Inflorescence and flowers, C. Mature pods; D. Seeds*

Erythrina stricta is cultivated as an avenue tree and to enrich soil fertility in open and wastelands. The varied parts of this tree are utilized for medicinal purposes, for example, leaves are used to alleviate joint pains, earache, toothache, and eye infections (Umamaheswari et al., 2009), and the bark is beneficial in the treatment of asthma, epilepsy, rheumatism, eczema, and dermatitis (Umamaheswari et al., 2009; Kichu et al., 2015; Akter et al., 2016). Plenty of seeds are produced by each tree and they go to waste in nature without utilization. A recent study on the nutritional composition of the seeds portrayed them as a good source of major nutrients and could be used as a seasonal food (Murthy et al., 2024). The seeds consist of 13.43% oil, 26.81% of protein, 18.71% of fiber, and 6.04% of ash with an energy value of 284.15 Kcal/100 g. It is also rich in calcium (5 mg/g dry weight [DW]), magnesium (6 mg/g DW), iron (787 µg/g DW), copper (32.70 µg/g DW), manganese (55.20 µg/g DW), boron (36.80 µg/g DW) and zinc (497 µg/g DW). The seed oil is rich in nutraceuticals, such as carotenoids and lignans, and consists of 70.9% unsaturated fatty acids (Murthy et al., 2024). However, the phytochemical constituents and biological activities have not been reported for this plant. Therefore, the major objective of the current study was to analyze the phytochemicals of matured seeds of *Erythrina strica*. In the present study, we carried out a qualitative analysis of different phytochemicals and a quantitative analysis of phenolics, flavonoids, and alkaloids in different solvent extracts. In addition, we analyzed the antioxidant activities of extracts using 2,2′-diphenyl-1-picrylhyrazyl radical (DPPH) assay,

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phosphomolybdenum method [total antioxidant activity (TAA]), and ferric reducing antioxidant power assay (FRAP assay).

Materials and Methods

Plant materials and chemicals

In the vicinity of Shiggavi, in the Haveri district of Karnataka, India, a single population of 25 plants was used to gather the pods of *Erythrina stricta* (15.010372N, 75.129678E). The pods were collected randomly from five trees and pooled together. The seeds (*Fig. 1D*) were separated from the pods and dried to make them moisture-free in an oven (OV4-S, Jiotech, Daejeon, Republic of Korea) at 40 ± 2 ℃. Dried seeds were powdered using a mechanical grinder and stored in air-tight polythene bags at room temperature until further use. Chemicals such as Folin-Ciocalteu reagent, $NaNO_3$, bromocresol green, gallic acid, quercetin, ascorbic acid, and atropine used in the present study were procured from Himedia laboratories, Mumbai, India, whereas $AlCl_3$ was purchased from Sigma-Aldrich, Bengaluru. All the other chemicals and solvents used were of analytical grade.

Phytochemical analysis

Extraction

The defatted seed cake was extracted successively with three solvents, viz. acetone, methanol, and water in increasing order of their polarity (acetone < methanol < water) using a Soxhlet apparatus for 8 h with each solvent. The residue obtained from one solvent extraction was used for the subsequent solvent. After the complete extraction, the solvents were evaporated using a rotary evaporator (Buchi, Rotavapor R-100, Flawil, Switzerland), the extracts were kept in an oven at 40 ± 2 ℃ to remove the traces of solvents and stored at 4 ℃ until they used.

Qualitative phytochemical analysis

The qualitative phytochemical analysis was carried out as mentioned by Harborne (1998). Fehling's test identified carbohydrates, while the presence of alkaloids was determined using Wagner's and Mayer's tests. Keller-Killani, gelatin, NaOH, foam, H_2SO_4, and Salkowski's tests were performed to confirm the existence of cardiac glycosides, phenolics, tannins, flavonoids, saponins, terpenoids, and phytosterols, respectively.

Quantitative phytochemical analysis

Total phenolics

A method described by Murthy et al. (2022) with minor modifications was followed to determine the total phenolics. To brief, 0.5 mL (0.33 mg/mL concentration) of the extract was diluted to 3 ml with distilled water and 0.1 ml of 2 N Folin-Ciocalteau reagent was added. After 6 min (minutes) of incubation, 0.5 ml of 20% sodium carbonate (Na_2CO_3) was added. Tubes were allowed to stand in a warm water bath for 30 min and the developed color absorbance was measured at 760 nm using a UV-Vis (ultraviolet-visible) spectrophotometer (Hitachi U-3310, Ibaraki, Japan). Gallic acid was used as standard.

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Flavonoids

The flavonoid content in the different extracts was estimated according to Pekal and Pyrzynkaet (2014) method. To brief, 0.5 mL (1 mg/mL concentration) of extract was diluted to 3 mL by using distilled water followed by the addition of 0.15 mL of $NaNO_3$ and subsequent 5 min incubation at room temperature. Then, 0.3 ml of $AlCl_3$ (10%) and 2 mL of NaOH (1 M) were added and the solutions were vortexed before measuring the absorbance at 510 nm. Quercetin was used as standard.

Alkaloids

The alkaloid content was quantitatively estimated by adopting the method of Shamsa et al. (2008). Bromocresol green solution was prepared by dissolving 6.98 mg of bromocresol green powder in 0.3 ml of NaOH and the final volume of the solution was adjusted to 100 mL with the distilled water. To 1.0 mL (5 mg/mL concentration) a known quantity of sample, 5 mL of above prepared bromocresol green solution was added and subsequently, 5 mL of phosphate buffer (2 M sodium phosphate and 0.2 M citric acid with pH adjusted to 4.7) was added. Then, 5 mL of chloroform was added and shaken vigorously; the chloroform layer was collected and the absorbance was detected at 470 nm. Atropine was used as the standard.

Antioxidant activities

DPPH radical scavenging activity

To the 0.1 ml of different concentrations of extract (3 mg/mL for acetone and methanol extracts and 0.5 mg/mL for water extract), 1.9 ml of 0.1 mM DPPH solution prepared in methanol was added. Then the tubes were shaken well and dark incubated for 15 min. The decrease in colour intensity of the DPPH solution was read at 517 nm. Gallic acid was used as standard, and activity was expressed as mg gallic acid equivalent (GAE)/g of extract (Yadav et al., 2022).

Total antioxidant activity (TAA)

The phosphomolebdenum method was followed to carry out the total antioxidant assay (Prieto et al., 1999). To the 0.15 ml of extracts of different concentrations (3 mg/mL for acetone and methanol extracts and 0.1 mg/mL for water extract), 1.5 ml of reagent solution (containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added. Tubes were incubated at 95 °C for 90 min and color developed was measured at 695 nm. Ascorbic acid was used as standard, and activity is expressed as mg ascorbic acid equivalent per gram (mg AAE/ g) extract.

FRAP activity

FRAP assay was performed by following the method described by Benzie and Strain (1999). To 0.1 mL of the extract (3 mg/mL for acetone and methanol extracts and 0.1 mg/mL for water extract), 3 ml of FRAP reagent containing 300 mM acetate buffer (pH 3.6), 10 mM of TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ (10:1:1 ratio). Tubes containing sample and FRAP reagent were vortexed and allowed to stand for 6 min at room temperature and absorbance was read at 593 nm against a blank solution. Ascorbic acid was used as a standard, and the activity of the extracts is expressed as mg ascorbic acid equivalent (AAE)/g extract.

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Statistical analysis

Each experiment was repeated three times and results are expressed as mean values with standard error. Descriptive statistics, viz., mean and standard error were calculated using Microsoft Excel 2019 and Duncan's multiple range test was carried out by using IBM SPSS software version 20.

Results and Discussion

Phytochemical composition

Erythrina species are well-known for their rich bioactive compounds, especially the erythrinan alkaloids. They made the species recognized as potent medicinal plants worldwide and included in the traditional medicine system (Umamaheswari et al., 2009; Kichu et al., 2015; Akter et al., 2016). Like its allied species, *E. stricta* is also rich in various phytochemicals and has also been reflected in its antioxidant activities. A comprehensive extraction of beneficial phytocompounds may be challenging when using a solitary solvent. Consequently, to facilitate the extraction of both polar and non-polar compounds, we conducted the seed extraction using three solvents—acetone, methanol, and water—each possessing a different polarity. Notably, each solvent yielded a substantial amount of extract. Extraction with methanol and water yielded 12.57 and 12.81% of the extract, respectively, whereas acetone yielded 0.73%. The extraction with water is more suitable for the seeds of *E. stricta* as it gives the highest yield. Also, the water extract is rich in phytochemicals and possesses superior biological activities, compared to the extracts of methanol and acetone. A similar attempt of extracting a plant material with many solvents having a range of polarity was made by several investigators and found to be useful (Molole et al., 2022; Murthy et al., 2023). Further, the qualitative analysis of acetone, methanol, and water extracts showed the presence of a range of phytochemical groups, such as carbohydrates, phenolics, tannins, flavonoids, and alkaloids (*Table 1*). However, some groups, viz., glycosides, saponins, terpenoids, and phytosterols, do not show their presence in any of the extracts.

Table 1. *Qualitative phytochemical analysis of Erythrina stricta seeds*

Metabolite	Test/s	Acetone extract	Methanol extract	Water extract
Carbohydrate	Fehling's test	+	+	+
	Benedict's test	+	+	+
Glycosides	Cardiac glycosides	-	-	-
Phenolics and tannins	Gelatine test	+	+	+
Flavonoids	NaOH test	+	+	+
Saponin	Froth test	-	-	-
Terpenoids	Terpenoids	-	-	-
Phytosterol	Salkowski's test	-	-	-
Alkaloids	Mayer's test	-	-	-
	Wagner's test	+	+	+

The seeds accommodate an acceptable quantity of three phytochemical groups: phenolics, flavonoids, and alkaloids. The water extract exhibited the highest concentration of phenolics and flavonoids compared to the methanol and acetone extracts. Nevertheless, the acetone extract contained excessive alkaloids (*Table 2*). The phenolic

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content of the water extract was 91.99 mg GAE/g extract, followed by 60.52 and 51.92 mg GAE/g extract in acetone and methanol extracts, respectively. 50.64, 45.29, and 25.10 mg QE/g extracts of flavonoids were found in water, methanol, and acetone extracts, respectively. Alkaloid content was highest, 40.48 mg atropine equivalent (AE)/g extract in acetone extract, and minimum was found in water extract, 2.42 mg AE/g extract. Considering these values in relation to the dry weight of the seed, phenolic compounds constitute 22.30 mg GAE/g DW, whereas flavonoids and alkaloids constitute 14.31 mg QE and 4.03 mg AE/g DW, respectively.

Table 2. *Phytochemical composition of Erythrina stricta seed extracts*

	Extract yield (g/100 g DW)	**Total phenolics (mg GAE/g extract)**	**Flavonoids (mg QE/g extract)**	**Alkaloids (mg AE/g extract)**
Acetone extract	0.73	60.52 ± 3.01^{b}	25.10 ± 1.04^{b}	40.48 ± 1.34^{a}
Methanol extract	12.57	51.92 ± 1.36^{c}	45.29 ± 0.75^{a}	22.45 ± 0.45^{b}
Water extract	12.81	91.99 ± 2.25^{a}	50.64 ± 3.14^{a}	2.42 ± 0.14^{c}

Each value represents the mean ± standard error of three replicates. Mean values followed by different letters in their superscript are significantly different from each other (p = 0.05) in the respective column according to Duncan's multiple range test. GAE – Gallic acid equivalent; QE – Quercetin equivalent; AE – Atropine equivalent

Phenolics are a prominent group of phytochemicals that exhibit various biological activities. The legume seeds are an important source of dietary polyphenols that contribute directly to antioxidant activities (Singh et al., 2017). The total phenolic content of *E. stricta* is substantially high when compared to that of previous reports on some well-known legumes, such as 1.98 to 4.83 mg/g DW in *Phaseolus vulgaris* (Luthria and Pastor-Corrales, 2006), 0.86 to 1.14 mg GAE/g in *Pisum sativum*, 4.86 to 9.60 mg GAE/g in *Lens culinaris*, 0.57 to 6.99 mg GAE/g in *Phaseolus vulgaris* and 1.57 to 5.57 mg GAE/g in *Glycine max* (Xu et al., 2007). However, the faba bean (*Vicia faba*) holds comparatively more total phenolic quantity in its seeds, i.e., 16.98 to 67.47 mg GAE/g extract (Chaieb et al., 2011). The seed coat is a reservoir of phenolic compounds in the legume seeds which contributes up to 10% to the total seed weight and its colour intensity is directly related to the phenolic quantity (Singh et al., 2017). Thus, a high phenolic content of the *E. stricta* seeds could be correlated to its dark seed coats (*Fig 1D*). Flavonoids are a group of polyphenolic compounds present in legume seeds that are reported to possess antioxidant, antihyperglycemic, and antidiabetic properties Murthy et al. (2021). Interestingly, total flavonoid content of *E. stricta* seeds is substantially higher than that of some well-known legumes, such as *Vicia faba* which ranged from 5.19 to 9.30 mg rutin equivalent/g DW (Chaieb et al., 2011), *Pisum sativum* (0.12 to 0.17 catechin equivalent [CE]/g DW), *Lens culinaris* (3.10-4.20 mg CE/g DW), *Phaseolus vulgaris* (0.92-4.24 CE/g DW) and *Glycine max* (1.13 to 4.04 CE/g DW) (Xu et al., 2007). The genus *Erythrina* is well-known for its alkaloids, erithrinan type, and a total of 143 alkaloids are reported, primarily in their seeds and also in stems, roots, leaves, and flowers, demonstrating cytotoxic, anti-inflammatory, insecticidal, antiviral, and nervous system-related activities (Rambo et al., 2019; Fahmy et al., 2020). The alkaloid quantity in *E. stricta* seeds is lower than that in its allied species, such as 5.3 mg/g DW in *E. americana* and 7.7 mg/g DW in *E. breviflora* (Sotelo et al., 1993), as well as an important food crop *Lupinus angustifolius*, which exhibited up to 18.3 mg/g DW in seeds

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(Sotelo et al., 1993). The alkaloids are one of the major antinutrients in the legume seeds, along with lectins, phytate, oxalate, cyanogenic glycosides, and others (Samtiya et al., 2020). The suggested maximum alkaloid content for safe feeding purposes is 0.2 mg/g DW (Kamel et al., 2016). To achieve this, simple processing techniques such as heating, soaking, washing, grinding, and fermentation have proved very effective (Casado et al., 2023). For instance, Carvajal-Larenas et al. (2014) reduced 94.9% of alkaloids in *Lupinus mutabilis* seeds through a series of processes, such as soaking, cooking, washing, and grading, completed in 5.7 days. Similarly, the European Union recommends processing methods such as washing, soaking, boiling, heating, grinding, and other methods to reduce the alkaloid content of *Papaver somniferum* seeds by up to 100% European Union (2014). Considering the seed oil, protein, minerals, and phytochemicals, *E. stricta* provides excellent opportunities to explore as a new nutritive source in rural areas.

Antioxidant activities

Antioxidants are crucial for maintaining human health as they defend against oxidative stress, averting various diseases like cancer, autoimmune disorders, Alzheimer's, and Parkinson's. Plant-derived bioactive compounds shield cells from oxidative harm by inhibiting or interacting with free radicals and reactive oxygen species (Murthy et al., 2023). The impressive presence of phytochemicals in the seed extracts of *E. stricta* was also reflected in their antioxidant activities assessed through three standard *in vitro* methods - DPPH radical scavenging activity, total antioxidant activity (TAA), and FRAP assay – and results are presented in *Figure 2*.

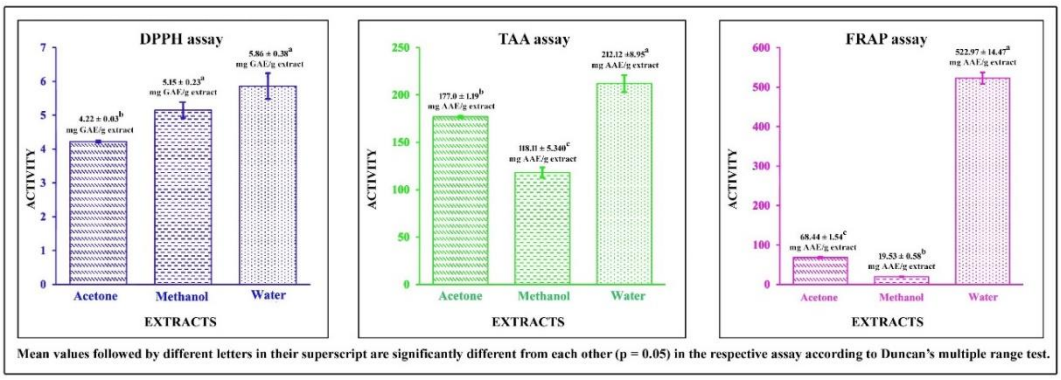

Figure 2. *Antioxidant activities of Erythrina stricta seed extracts*

Among all the extracts, water extract exhibited substantial activity in all the methods studied, followed by acetone and methanol extracts. Water extract had 5.86 mg GAE/g extract of DPPH radicle scavenging activity, followed by 5.15 and 4.22 mg GAE/g extract in methanol and water extracts, respectively. TAA was 212.12 mg AAE (ascorbic acid equivalent)/g extract in water extract and 177.0 and 118.11 mg AAE/g extract in acetone and methanol extracts, respectively. The FRAP activity of water extract was 522.97 mg AAE/g extract, followed by 68.44 and 19.53 mg AAE/g extract in acetone and methanol extract, respectively. The antioxidant properties of phenolics and flavonoids are widely recognized (Singh et al., 2017; Bodofsky et al., 2020). Similarly, the significant antioxidant activity observed in *E. stricta* seeds is likely attributable to their high phenolic and flavonoid content as the antioxidant properties of the extracts correlate with their

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phytochemical composition. Berger et al. (2007) reported 0.7 and 1.4 mg AAE/g DW of antioxidant capacity for peas and green beans, respectively, as determined by the FRAP assay. Likewise, Yadav et al. (2022) reported the antioxidant activity of *Balanites roxburghii* seeds, an underutilized plant, as 0.65 mg GAE/g DW in the DPPH assay, 20.27 mg AAE/g DW in TAA, and 117.9 mM Trolox equivalent in the FRAP assay. Thus, parallel to the phytochemical composition, the seeds exhibited impressive antioxidant activities. This paves the way to explore *E. stricta* as a new nutritive source and also as a functional food.

Conclusions

The investigation into *Erythrina strica*, an underutilized legume species prevalent in the Indian sub-continent, focuses on unraveling its phytochemical profile. Notably, *E. stricta* seeds exhibit a robust presence of bioactive phenolics and flavonoids, contributing significantly to their antioxidant activity. Despite the identification of antinutrient compounds, such as alkaloids, their mitigation through simple food processing methods has been demonstrated as effective. This study, thus, endeavors to elucidate the phytochemical composition of *E. stricta* seeds, positioning them as a promising candidate for a reservoir of bioactive compounds for potential therapeutic applications.

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