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Anti-oxidant and Anti-glycation potential of extracts from leaves of *Chonemorpha fragrans* G. Don. and *Ellertonia rheedii* Wight

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Abstract

Because of wide variety of phytochemicals, *Chonemorpha fragrans* and *Ellertonia rheedii* members of the Apocynaceae family were frequently utilized in Indian traditional medicine. The present study aims at screening of extracts from leaves of *Chonemorpha fragrans* and *Ellertonia rheedii* for anti-oxidant and anti-glycation activities. The EtOH extract at 100 µg/ml showed maximum activity of 70.78% and 65.83% respectively for *E. rheedii* and *C. fragrans*. The extracts of both the plants exhibited excellent H_2O_2 scavenging, NO scavenging and Xanthine oxidase inhibition. Further, the extracts from both the selected plants also exhibited excellent anti-glycation activity with IC_{50} 82.34 and 87.66 µg/ml respectively. The results were outstanding, and both plants may be implicated in the treatment of carious diseases.

Key words : *Chonemorpha fragrans*, *Ellertonia rheedii*, anti-oxidant activity and anti-glycation activity.

Many metabolic illnesses have been treated with aromatic herbs, medicinal plants, and their byproducts since ancient times. Research on phytoconstituents from many plants has shown that they can regulate oxidative stress damage by scavenging free radicals and reactive oxygen species[13]. Higher consumption of foods strong in antioxidants reduces the chance of acquiring degenerative diseases, according to prior research showing a link between sickness incidence and nutrition[18,20]. For the purpose of future drug discovery and development, state-of-the-art research is therefore required to ascertain the phytochemical makeup and evaluate the therapeutic potential of bioactive chemicals derived from botanicals. Numerous herbal preparations are used as infusions as home remedies to prevent a range of ailments due to their exceptional anti-oxidative properties[2,7].

Chonemorpha fragrans, is one of the

plant species of the genus Chonemorpha, commonly known as the frangipani vine or climbing frangipani. It is a robust, usually evergreen, climbing shrub that can reach the summits of the tallest trees in Southeast Asian forests, with stems 30 meters (98 feet) or longer (National Biodiversity Centre). It has big glossy leaves and fragrant white flowers. China, India (including the Himalayas), Indonesia, Malaysia, Myanmar, Sri Lanka, and Thailand are its native lands. It is grown primarily in frost-free areas across the world and is widely utilized in Ayurveda, an Indian traditional medicine. Alkaloids such as funtumafrine, chonemorphine, and camptothecin (CPT) are found in the plant. A number of synthetic medications that are camptothecin's analogues are used in chemotherapy for different kinds of cancer. chloronemorphine aids in the treatment of intestinal infections and other ailments[3,8,14,17]. *Ellertonia rheedii* Wight was a native plant of the Apocynaceae family that grows up to 3000 feet in elevation in the Western Ghats, extending from South Karnataka to Travancore. The branches of this climbing shrub are reddish-white-lenticellate[14]. A woody climber used for vein disease, the branches are twining terate leaves up to 8 ×3 cm^2, and the name itself suggests the drug's long-term use and effectiveness. The plant is widely used in the treatment of varicose veins[1].

Therefore, we here in report the anti-oxidant and anti-glycation potential of *C. fragrans* and *E. rheedii* and report the results of our exploratory study.

Plant material :

The leaves of C. fragrans and E. rheedii were collected in Western Ghats near Sringeri, Karnataka (13° 25' 36.84" N 75° 15' 18.3456" E). A voucher specimen was deposited in AVK College Herbarium (AVK BOT 1023 & 1024).

Solvent extraction :

The leaves of *C. fragrans* and *E. rheedii* were separately subjected to extraction using ethanol as a solvent. The extracts were filtered and stored at 4°C under nitrogen atmosphere for further experimentation.

Free radical Scavenging Activity (FRSA) using DPPH *:*

According to published findings[10], the 1,1-diphenyl-2picrylhydrazyl (DPPH) radical scavenging method was used to assess the extracts' capacity to scavenge free radicals and performed in quituplicate. As a reference standard, ascorbic acid was employed. 2ml of a 1.0 mmol/L DPPH solution in methanol, 1ml of extracts (10-500 µg/ml), and a ascorbic acid were all present in the reaction mixture. For 20 minutes, the mixture was incubated at 37°C. The absorbance was recorded in comparison to a reagent blank at 517nm. Scavenging ability was expressed using the formula –

Free radical scavenging activity (%) = $(A_s - A_t) / A_s \times 100$

Where A_s= Absorbance of standard compound and A_t = Absorbance of sample

Free radical Scavenging Activity (FRSA) using Hydrogen Peroxide (H_2O_2) *:*

With minor adjustments and in quintuplicate, the extracts' capacity to scavenge

hydrogen peroxide was tested in accordance with published reports[16]. For the investigation, a 43 mM hydrogen peroxide concentration in 1M phosphate buffer (pH 7.4) was made. After adding extracts at varying concentrations (10–500 µg/ml) to a hydrogen peroxide solution (0.6 ml, 43 mM), the absorbance at 230 nm was measured 10 minutes later against a blank made with phosphate buffer devoid of hydrogen peroxide. As a reference, ascorbic acid was used. The free radical scavenging activity was determined by assessing the % inhibition using the following formula –

% inhibition = (Control- Test) /control ×100

β -carotene bleaching assay :

The β-carotene bleaching experiment was carried out in quintuplicate and in accordance with the established procedure [19]. 20 µl of linoleic acid and 200 µl of Tween 20 were combined with 2 ml of β-carotene (200 µg/ml in $CHCl_3$) in a round-bottom flask, thoroughly mixed, and evaporated for 10 minutes at 40°C. Next, 100 ml of HPLC-grade distilled water was added. An aliquot of 5 ml of the resultant emulsion was put into test tubes with different extract concentrations (10-500µg/ml) after the liquid had been vortexed. A UV–VIS spectrophotometer (Shimadzu UV1900) was used to measure the absorbance at 470 nm every 15 minutes while the combination was submerged in a water bath at 50°C for two hours. All experiments were performed in quintuplicate. The total antioxidant activity was calculated based on the following formula –

Anti-oxidant activity %=1– $(A_0–A_t)/(A_0^0– A_0^t)$

Where, A_0 = Absorbance of control; A_t = Absorbance of sample

Nitric oxide scavenging activity :

The Griess-Illosvory reaction (Garrat, 1964) was used to determine the extracts' NO scavenging capacity. After thoroughly mixing different quantities of extracts (1-100 µg/ml) with sodium nitroprusside (in 0.9% PBS), the mixture was incubated for two hours at 30 ^oC. Gallic acid was used as the positive control, and the mixture devoid of the extracts was used as the negative control. Griess reagent (0.5 ml; 1% sulfanilamide, 2% H_3PO_4, and 0.1%N-(1-naphthyl) ethylenediamine dihydrochloride) was added following incubation, and the absorbance (OD) values were found at 550 nm. Subsequently, IC_{50} values (the inhibitory concentration required to reduce 50%of the nitric oxide formation) was measured for all the extracts. All experiments were performed in quintuplicate.

Xanthine oxidase inhibition Assay :

The extracts' capacity to inhibit xanthine oxidase was tested in quintuplicate using the previously described methodology[6]. To reach a final concentration of 200 µg/ml, all extracts were dissolved in buffer before being employed for the inhibitory activity. Xanthine (0.120 mM), sodium pyrophosphate buffer (80 mM, pH 8.5), and the enzyme xanthine oxidase (0.1 unit) made up the reaction mixture. At 295 nm, the uric acid that was produced was measured. As a negative control, a blank with zero enzyme activity was made; it contained the test mixture but no plant extracts. Allopurinol served as the reference substance (positive control). Subsequently, IC_{50} values for all the compounds and extracts were determined. The xanthine oxidase inhibition

potential was expressed as the inhibition percentages and can be given as

$$\%\text{Inhibition} = \left(1 - \frac{Test\ Inclination}{Blank\ Inclination}\right) \times 100$$

Where,
Test inclination = Absorbance change per min of test compounds (linear change)
Blank inclination = Absorbance change per minute of the blank (linear change)

Anti-glycation activity :

The Anti-glycation assay was carried out as per the method in the literature with slight modifications[5] and in quintuplicate. Briefly, BSA (1 mg/mL) was incubated with 0.25M fructose and 0.25M glucose in 0.1M phosphate-buffered saline (PBS), pH 7.4, in darkness at 50°C for 4 days. Before incubation, the solutions of extracts and reference standard (Aminoguanidine) were dissolved in 50% DMSO was added to the mixture. Glycation of BSA takes place and the formed glycated protein was evaluated at two different wavelengths like 335 nm (Fluorescence, excitation wavelength) and 385 nm (emission wavelength). Various concentrations of extracts ranging from 1- 100 µg/ml providing 50% AGE inhibitions (IC_{50}) were calculated from the calibration curve of inhibition percentage against the extract concentration.

The inhibition percentage of AGEs was determined using the following formula

$$AGE\ \% = (F_{control} - F_{control\ blank}) \times 100 / (F_{extract} - F_{extract\ blank})$$

Where,
($F_{control} - F_{control\ blank}$) is the difference between the fluorescent intensity of BSA incubated with or without glucose and fructose ($F_{extract} - F_{extract\ blank}$) is the difference between the fluorescent intensity of BSA and sugars incubated with or without plant extracts.

The antioxidant efficacy of extracts was evaluated by standard methods. Among the extracts, EtOH extract of *E. rheedii* at 100 µg/ml showed maximum activity of 70.78% followed by the EtOH extract of *C. fragrans* (65.83%) (Table-1). While, ascorbic acid, the standard compound showed 100% activity at 80 µg/ml. Further the extracts showed maximum H_2O_2 scavenging activity of 86-90% at 100 µg/ml, while, the reference standard ascorbic acid exhibited 100% activity at 80µg/ml for the assay (Table-2). These results indicate that extracts of *C. fragrans* and *E. rheedii* have antioxidant potential comparable with that of the ascorbic acid. Remarkably, the β-carotene bleaching assay results demonstrated a higher antioxidant activity of with IC_{50} of IC_{50} of 67.73 µg/ml 76.73 µg/ml respectively for *E. rheedii* and *C. fragrans* extracts (Table-3).

NO *scavenging assay :*

Nitric Oxide (NO) scavenging assay based on the scavenging ability of the extracts was evaluated in a dose dependent manner using Gallic acid as a positive control sample. As evident from the table-4, the extracts also showed excellent NO scavenging activity with IC_{50} of 70.12 and 75.91 µg/ml. respectively for *C. fragrans* and *E. rheedii* extracts. While Gallic acid showed IC_{50} of 26.61 µg/ml. Further,

Table-1. DPPH scavenging activity of extracts of *C. fragrans and E. rheedii*

Concentration(µg/ml)	*C. fragrans* extract	*E. rheedii* extract	Ascorbic acid
10	8.11 ± 0.61*	10.22 ± 0.68*	24.13±0.77*
20	15.23 ± 0.92**	22.51 ± 0.87*	36.53 ± 0.98**
40	23.08 ± 1.12*	34.08 ± 1.05**	5167 ±1.21**
60	40.64 ± 1.16**	44.85 ± 1.22**	80.72 ± 1.37*
80	44.16 ± 1.68*	56.73 ± 2.06*	100
100	65.83 ± 1.97*	70.78 ± 2.18**	100

Data expressed as Mean ± S.E.;

*Test samples were significant ($P < 0.05$); **Test samples were significant ($P < 0.01$);

Table-2. H_2O_2 scavenging activity of extracts of *C. fragrans and E. rheedii*

Concentration(µg/ml)	*C. fragrans* extract	*E. rheedii* extract	Ascorbic acid
10	12.34 ± 0.69*	14.42 ± 0.76*	24.13±0.77
20	24.65 ± 0.93**	28.36± 0.91*	36.53 ± 0.98
40	32.43 ± 1.22*	42.68 ± 1.24**	5167 ±1.21
60	51.09 ± 1.79*	63.83 ± 1.29*	80.72 ± 1.37
80	65.26 ± 2.37*	77.17± 1.33*	100
100	86.03 ± 2.41*	90.01± 2.38*	100

Data expressed as Mean ± S.E.;

*Test samples were significant ($P < 0.05$); **Test samples were significant ($P < 0.01$);

Table-3. β-carotene bleaching assay of extracts

S. No.	Sample/Extract	IC_{50} (µg/ml)
1.	*C. fragrans* extract	76.72 ± 1.56* (63.189 – 89.344)
2.	*E. rheedii* extract	67.73 ± 1.29** (57.118 – 77.271)

*Test samples were significant ($P < 0.05$); **Test samples were significant ($P < 0.01$)

Data expressed as Mean ± S.E.;

The numbers in parenthesis represents 95% confidence limits

Table-4. NO scavenging activity of essential oil and extracts from *E. monogynum*

S. No.	Sample/Extract	IC_{50} (µg/ml)	Relative Potency
1.	*C. fragrans* extract	75.91 ± 1.51** (61.198 – 89.232)	0.334
2.	*E. rheedii* extract	70.12 ± 1.47* (59.183 – 81.412)	0.362
7.	Gallic acid	25.39 ± 1.05** (18.172 – 32.244)	1

*Test samples were significant ($P < 0.05$); *Test samples were significant ($P < 0.01$)
Data presented as Mean ± S.E.; Relative potency = IC_{50} standard/IC_{50} sample;
The numbers in parenthesis represents 95% confidence limits

Xanthine oxidase inhibition :

The extracts were evaluated as potential XO inhibitors and compared favourably with the positive control sample, Allopurinol and the results were summarized in Table-5. The extracts from both the selected plants also exhibited excellent anti-glycation activity with IC_{50} 82.34 and 87.66 µg/ml respectively.

Table-5. Xanthine oxidase inhibitory potential of extracts of *C. fragrans* and *E. rheedii*

Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)	Relative Potency
C. fragrans extract	87.66±1.31* (75.133 – 99.281)	150.279±1.62* (138.276 – 162.345)	0.208
E. rheedii extract	82.34±1.22* (71.244 – 83.282)	144.118±1.41* (130.524 – 158.397)	0.222
Allopurinol	18.28±0.53** (13.621 – 23.893)	31.44±0.59** (23.245 – 40.557)	1

*Test samples were significant ($P < 0.05$); **Test samples were significant ($P < 0.01$)
Data expressed as Mean ± S.E.
Relative potency = IC_{50} standard/IC_{50} sample;
The numbers in parenthesis represents 95% confidence limits

Anti-glycation activity :

The ability of *C. fragrans* and *E. rheedii* extracts to inhibit advanced glycation end products (AGE) formation was evaluated using the anti-glycation assay. In this study, bovine serum albumin (BSA), glucose and fructose were served as the model protein and glycating agents respectively. The formation of AGEs was evaluated by monitoring the production of fluorescent products formed at 335 nm and 385 nm, respectively and the results were depicted in table-6. It was evident from the data that all the tested extracts presented higher inhibitory effect of more than 90% inhibition of AGE formation at a concentration

Table-6. Anti-glycation activity of extracts from leaves of *C. fragrans* and *E. rheedii*

Compound	IC_{50} (µg/ml)	IC_{90} (µg/ml)	Relative Potency
C. fragrans extract	68.29±1.14* (58.991 – 78.407)	127.36±1.42* (115.826 – 139.389)	0.326
E. rheedii extract	60.72±1.22** (50.812 – 70.245)	114.26±1.36* (104.171 – 124.197)	0.366
Aminoguanidine	22.23±0.62** (17.231 – 27.334)	40.36±1.02* (30.514 – 40.712)	1

*Test samples were significant ($P < 0.05$); **Test samples were significant ($P < 0.01$)
Data expressed as Mean ± S.E.
Relative potency = IC_{50} standard/IC_{50} sample;
The numbers in parenthesis represents 95% confidence limits

of 100 µg/ml and with IC_{50} values of 68.28 and 60.72 µg/ml respectively. While, Aminoguanidine the positive control sample showed IC_{50} of 22.23 µg/ml.

Phenols and terpenes are well-known, significant chemicals found in plants that contribute to the antioxidant capacity of various botanicals[4]. Because they can react with active oxygen radicals like hydroxyl radicals, superoxide anion radicals, and lipid peroxy radicals and prevent lipid oxidation early on, phytochemicals—specifically, plant essential oils—are known to possess primary antioxidant action. According to reports, the rise in phenolic content can be utilized as a biomarker for air pollution screening[11]. Numerous studies have demonstrated that flavonoids are antioxidants and are valued nutraceuticals that can counteract the effects of free radical stress[4]. More phenols and flavonoids were found to be associated with the antioxidant activity capacity of the *C. fragrans* and *E. rheedii* plants in this investigation. It has been established that medicinal plants' flavonoids, phenols, and essential oils have the ability to inhibit xanthine oxidase[11]. The highest concentration of phenols can be found in medicinal and aromatic plants, which have also been shown to have xanthine oxidase inhibitory action. Furthermore, our findings concur with those of other research studies that have already been published. The extracts under investigation have shown remarkable and noticeable inhibition comparable to allopurinol suggesting these plants can be further investigated for the development of herbal medicinal formulation for gout disease. The prevention of fructosamine production was used to assess the potential of extracted sterols, essential oils, and extracts on BSA glycation. Additionally, elevated oxidative damage near glycated histone residues have been reported[11]. With >90% inhibition of AGEs, the extracts used this investigation demonstrated outstanding ant-glycation action. Consequently, both *C. fragrans* and *E. rheedii* plants may be investigated as a natural, bio-friendly sources of phytoconstituents that might be used as an alternative to traditional manufactured substances.

The present study involves screening of extracts from leaves of *C. fragrans* and *E. rheedii* for antioxidant potential, anti-glycation activity and xanthine oxidase inhibition potential. The extracts of the both the selected plants exhibited excellent anti-oxidant activity and this can be attributed to the presence of substantial amounts of phenols and flavonoids. Nitric Oxide (NO) scavenging assay based on the scavenging ability of the extracts, where both the extracts showed potent NO scavenging activity with IC_{50} values comparable to positive control sample gallic acid. The extracts further displayed excellent ant-glycation activity with >90% inhibition of AGEs. Therefore, *C. fragrans* and *E. rheedii* plants could be explored as bio-friendly natural source of phytoconstituents, which serves as a substitute (alternative) to the conventional synthetic compounds that might be implicated in the treatment of various diseases.

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