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Phytochemical screening and antioxidant studies of leaf and bark extracts of *Poeciloneuron indicum* Bedd. (Clusiaceae)

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Abstract
In the present study, the phytochemical constituents and antioxidant activity of various extracts from bark and leaves of *Poeciloneuron indicum* Bedd. were investigated by different *in-vitro* methods. Phytochemical studies have confirmed the presence of tannins, alkaloids, terpenoids and flavanoids in almost all the aqueous and ethanolic extracts of bark and leaves. The total estimate of phenolic compounds, flavonoids and free radical assays were performed. The phenolic compounds differed from 0.62±0.23 to 47.70±1.80 mg GAE/g in various solvent extracts tested. Ethanol bark extract of the plant species showed potent antioxidant activity with percent scavenging of 96.07%. The maximum amount of reducing ability using reducing power assay was observed in ethanol and aqueous bark extract (5.01±0.21 OD and 5.00±0.05 OD). Aqueous leaf extract (4.01±0.20 OD) also showed similar results in OD at 700nm for reducing power assay.

Keywords: *Poeciloneuron indicum*, antioxidants, phytochemical, phenolics, flavonoids, DPPH

1. Introduction
Medicinal plants constitute the major components of most indigenous medicines. A large number of medical preparations contain ingredients mostly of plant origin. Synthetic antioxidants have toxic effects to some extent. "Recently, there is an increased demand for the naturally occurring antioxidants, used in foods or medicinal preparations, to replace the synthetic antioxidants" (Ito *et al*., 1983) [10]. " In addition the natural antioxidants have capacity to improve quality of food and stability and they also act as nutraceuticals to cease the free radical chain reaction in biological systems, and thus providing additional health benefits to consumers" (Nahak and Sahu, 2010) [16]. So the uptake of natural antioxidants such as vitamins, carotenoids, polyphenols and flavonoids has been largely recommended. Green plants produce and preserve a variety of biochemical products, many of which are extractable and used as medicine or as, raw materials for cure of various diseases and scientific investigations. Many secondary metabolites of plants are commercially extracted for important clinical uses in a number of medicinal preparations. About 122 (47 tropical and 78 subtropical) major plant drugs have been identified for which no synthetic match is currently available. "The scientific study of traditional medicines and their derivation of drugs through bio prospection has given a systematic conservation of the concerned medicinal plants in the long run. They are thus of great importance" (Hebbar and Nalini, 2013) [8]. "Such plants represent a potential source of new compounds with antioxidant activity. Plants like *Polycias fruticosa* (L) Harms (Araliaceae), *Gymnema indorum* (Lour.) Decne. (Asclepiadaceae), *Mentha arvensis* L. (Lamiaceae), *Piper sarmentosum* Roxb. (Piperaceae) have shown potent antioxidant activity" (Chanwitheesuk *et al*., 2005) [3]. "The term 'antioxidants' refers to compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the activity or propagation of the oxidative chain reactions" (Moussa *et al*., 2011) [15].

1.1 Description of the plant
Poeciloeuron indicum Bedd. an endemic tree species distributed sparsely in the Western Ghats, is a plant belonging to the family Clusiaceae. It has been identified using flora of Karnataka (Saldanha C. J., 1984) [23]. It is a large evergreen tree with brown or brownish grey bark. Trees are buttressed, often with stilt roots, leaves simple, opposite decussate, inflorescence is a terminal panicle, fruit a capsule, beaked and one seeded. (Fig 1) Wood is quite strong and used for construction work such as beams, pillars, railway sleepers etc. In Kannada it is known as Balagi, and Kirballi
The present study intends to evaluate the effectiveness of plant extracts and the antioxidant properties of this endemic plant. Survey of literature has clearly revealed that this plant has not been evaluated so far for its bioactive potential although there is literature pertaining to the medicinal use of the plant. Root made into a paste in goat's milk and taken internally on the first and second day of menstruation acts as an oral contraceptive. It is also known to be used as an anti-cancerous agent

Fig 1: *Poeciloneuron indicum* Bedd.

2. Materials and Methods

2.1 Plant material

Poeciloneuron indicum Bedd. was collected from the natural forests of Western Ghats, Shimogga district, southern Karnataka. The plant parts such as bark and leaves were excised with sharp knife and placed in zip lock plastic pouches, labeled and brought to the laboratory for further analysis.

2.2 Preparation of extracts for phytochemical screening

"The collected plant parts were dried under shade and also at 40˚C in a hot air oven, before blending, to remove the water content and then powdered. Approximately 500g of the powdered materials were placed in plastic zip lock covers for further use. 50g of shade dried bark and leaf powder was extracted with soxhlet apparatus in the order of polarity. (Polarity of the compounds-Chloroform>Ethanol>Water).

2.3 Phytochemical Screening

Preliminary phytochemical screening of the crude powder and different solvent extracts were determined using standard procedures" (Harborne, 1998) [7]. The extracts were evaluated qualitatively to see the presence of phytochemical compounds such as saponins, tannins, flavonoids, terpenoids, steroids, alkaloids, cardiac glycosides, phlobatannins, anthraquinones and reducing sugars.

2.4 Estimation of total phenolic content

The total phenolic compounds of plant extracts was estimated by Folin-Ciocalteau (FC) method as per the procedure of Volluri *et al*. (2011) [26] with some modifications. "Different concentrations of the plant extracts (50-250 µg/mL) and the standard gallic acid (5-25 µg/mL) were taken in test tubes and 1.0 mL of FC reagent was added, after 3-5 min 2.0 mL of sodium carbonate (20%; w/v) was added and the mixture was allowed to stand for 30-45 min under dark. After the prescribed period of incubation the absorbance was taken at 765 nm in a spectrophotometer (T-60 UV-visible spectrophotometer, TTL-Technologies.). The concentration of total phenolics was expressed in terms of mg GAE/g gallic acid equivalents."

2.5 Estimation of total flavonoid content

The total flavonoid content of plant extracts was estimated by standard procedures of Kim (Kim *et al*., 2003). with slight modifications. "Different concentrations of the plant extracts (50-250 µg/mL) were mixed with 4ml of distilled water, followed by addition of 0.3 ml of 5% $NaNO_2$ and 10% $AlCl_3$. The mixture was incubated for 5 min, at room temperature. After incubation, 2 ml of 1 mM of NaOH was added and the total volume was made up to 10 ml using distilled water. The absorbance was measured at 510 nm using UV-Vis spectrophotometer against reagent blank. Flavonoid content was expressed as the amount equivalent to quercetin."

2.6 Radical scavenging activity by DPPH assay

Radical scavenging activity by DPPH method was evaluated according to the methods given by Pannangpetch *et al*. (2007) [18]. "Aliquots of standard (5-25µg/mL) and plant extracts (20-100 µg/mL) were taken and the volume was made up to 250 µL using distilled water or methanol. To this one mL of DPPH was added and the tubes were kept under dark for 10 min. The incubated mixture was read at the absorbance of 517 nm using UV-Vis spectrophotometer. Percent radical scavenging was calculated based on the extent of reduction in the color.

Per cent radical scavenging activity = $A_c - A_s / A_c \times 100$

Where A_c = absorbance of the control; A_s= absorbance of the sample."

2.7 Determination of reducing power

The reducing power of the extract was evaluated by the established method described by

Oyaizu (Oyaizu, 1986) [17] with slight modification. "Plant extract (10 µl stock) was mixed with phosphate buffer and the volume was made upto 200 µl. Further, 200 µl of 1% potassium ferricyanide was added and incubated at 50ºC for 20 min. After incubation, 200 µl ml of 10% TCA was added to terminate the reaction and later 0.6 ml of distilled water was added. The solution was mixed with 94µl of 0.1% ferric chloride. The precipitate was removed by centrifugation at 8000 rpm for 10 min. The absorption of clear supernatant was measured at 650 nm against reagent blank. The reducing potential was calculated using the standard graph constructed using various concentration of Ascorbic acid (10-100 µg). Reducing potential is expressed as the amount equivalent to Ascorbic acid."

Table 1: Phytochemical constituents of *Poeciloneuron indicum* in different solvent extracts**.**

Solvents/ Tests	Chloroform		Ethanol		Aqueous	
	L	B	L	B	L	B
Saponins	- -		- -		- -	
Tannins	- -		+ +		+ +	
Flavonoids	- -		+ +		+ +	
Terpenoids	+ +		+ +		+ +	
Alkaloids	- +		+ +		+ +	
Steroids	+ +		+ +		- -	
Phlobatannins	- -		+ +		- -	
Cardiac Glycosides	+ +		- -		- -	
Carbohydrates	+ +		+ +		+ +	
Proteins	- -		- -		- -	

+ = present, - = absent, L = leaf, B = bark

Table 2: Total phenolic content and antioxidant assays of *Poeciloneuron indicum* in different solvents*

Tests/ Extracts	TPC (mg GAE/g extract)	TFC (mg Quercetin/g extract)	DPPH (% Scavenging)	Reducing Power Assay (OD at 700nm)
Leaf Extract				
Chloroform	2.51±0.09	47.37±1.15	34.38	0.05±0.05
Ethanol	26.19±0.66	113.16±1.11	70.10	0.95±0.16
Aqueous	36.37±0.32	142.11±1.08	80.93	4.01±0.20
Bark extracts				
Chloroform	0.62±0.23	121.05±0.00	27.83	0.05±0.05
Ethanol	47.70±1.08	457.89±0.10	96.07	5.01±0.21
Aqueous	25.42±1.33	252.63±1.15	82.74	5.00±0.05

*All values are expressed as mean ± standad error mean (SEM) (n=3)

3. Results and Discussions

3.1 Phytochemical screening

In the present investigation, the phytochemical constituents and antioxidant evaluation of *Poeciloneuron indicum* was carried out. Phytochemicals are the secondary metabolites of plants which have no direct role in the growth and development of a plant but serve as defense agents against any pathogen. The results provided in table 1, indicate that the plant species studied contain carbohydrates, tannins, flavonoids, alkaloids, cardiac glycosides, steroids and terpenoids and phlobatannins.

3.2 Total Phenolic content

"Phenolics are ubiquitous secondary metabolites in plants possessing a wide spectrum of biochemical activities such as antioxidant properties, anti-mutagenic and anti-carcinogenic activities. It is reported that the phenolics are responsible for the difference in the antioxidant activity of the plant" (Cai *et al*., 2004) [2]. "They exhibit antioxidant activity by inactivating lipid free radicals or preventing the decomposition of hyper-oxides into free radicals" (Pokorny, 2001; Pitchaon *et al*., 2007) [20, 19]. The total Phenolic components and antioxidant activities of *Poeciloneuron indicum* Bedd. in various solvent extracts are represented in table 2. The total Phenolic components varied significantly between the solvent extracts. The amount of total Phenolics was measured by the Folin-Ciocalteau method. High Phenolic content was seen in the ethanol bark extracts (47.70±1.08 mg GAE/g extract) and aqueous leaf extract (36.37±0.32 mg GAE/g extract), whereas low content was seen in chloroform bark extracts (0.62±0.23 mg GAE/g extract) and chloroform leaf extracts (2.51±0.09 mg GAE/g extract). Among the plant extracts, the ethanol and aqueous bark extracts showed high Phenolic content and antioxidant activity.

3.3 Total Flavonoid content

"Flavonoid constituents are considered to be the most vital antioxidant components of herbs and a significant correlation between concentration of plant flavonoids and the total antioxidant capacity has been reported". (Kiranmai et al., 2012; Torres et al., 2006). High flavonoid content was seen in ethanol bark extract (457.89±0.10) and aqueous bark extract (252.63±1.15) whereas low content was seen in chloroform bark (121.05±0.00) and chloroform leaf extracts(47.37±1.15).Among the plant extracts the ethanol and bark extracts showed high flavonoid content and the antioxidant activity.

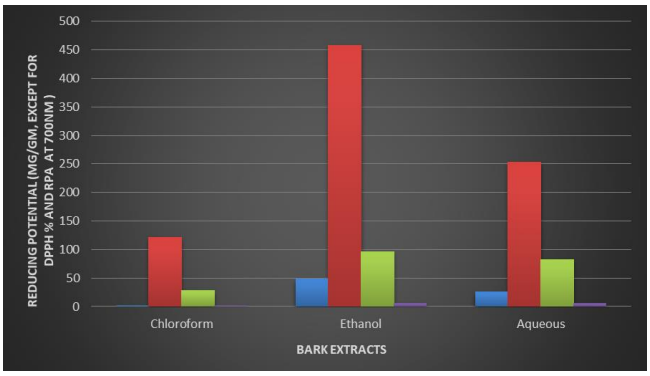
3.4 Antioxidant assays

"DPPH scavenging assay is one of the most preferred antioxidant method for determining the radical scavenging activity of plant material. It is based on reduction of the violet DPPH radical by the antioxidant via a hydrogen atom transfer mechanism to cause a change in the color to stable pale-yellow DPPH molecules. The remaining violet DPPH radical is measured by a UV-Vis spectrophotometer at approximately 515 –520 nm to determine the antioxidant activity. Free radical scavenging of Phenolic compounds is an important property underlying their various biological and pharmacological activities:" (Mayakrishnan *et al*., 2012) [13]. Ethanol and aqueous extracts of both the plant species showed free radical scavenging activity (Table 2), Percent scavenging values of ethanol (96.07%) and aqueous bark extract (82.74%) of *P. indicum* indicated high antioxidant activity. The aqueous leaf extract of *P. indicum* (80.93%) ml showed potent DPPH radical scavenging activity, in comparison with the standard. Chloroform extracts with weak total Phenolic content, exhibited weak radical scavenging activity.

"The reducing power of a compound is related to its electron transfer ability and may therefore, serve as a significant indicator of its antioxidant activity" (Ajila *et al*., 2007) [1]. In the reducing power assay, the Ethanol and aqueous bark extracts of *P. indicum* showed a concentration dependent antioxidant potential. In this biological assay, the presence of antioxidants in the extracts causes the reduction of the ferric cyanide complex in the ferrous form, leading to a color change in the test solution from yellow to different shades of green and blue, depending on the reducing power capability of each tested extract. Therefore, Fe^{2+} concentration can be monitored by measuring the formation of Pearl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates an increase in reducing power. In the present study, it depicts that the reductive effect of *P. indicum* increased with concentration of extracts. High absorbance at 700 nm indicates high reducing power. The highest reducing power activity was seen in the ethanol and aqueous bark extracts of *P.indicum* (5.01±0.21 and 5.00±0.05 mg BHT/g of extract respectively) and aqueous leaf extract (4.01±0.20 mg BHT/g of extract), whereas low reducing power activity was seen in the chloroform leaf and bark extract (0.05±0.05 and 0.05±0.05 mg BHT/g of extract respectively).

"Antioxidants are compounds which interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors" (Gulcin *et al*., 2005) [5]. "Reducing the capacity of a compound may serve as an indicator of its potential antioxidant capacity" (Meir *et al*., 1995) [14]. In the present study, the ethanol and aqueous extracts of *P. indicum* exhibited antioxidant activity, which has potential

application to reduce oxidative stress with consequent health benefits. “Phenolic compounds act as free radical acceptors and chain breakers. They interfere with the oxidation of lipids and other molecules by rapid donation of the hydrogen atom to radicals” (Dai and Mumper, 2010) [4]. Ethanol bark and aqueous bark extracts of *P. indicum* showed higher amounts of Phenolic compounds indicating strong antioxidant activity.



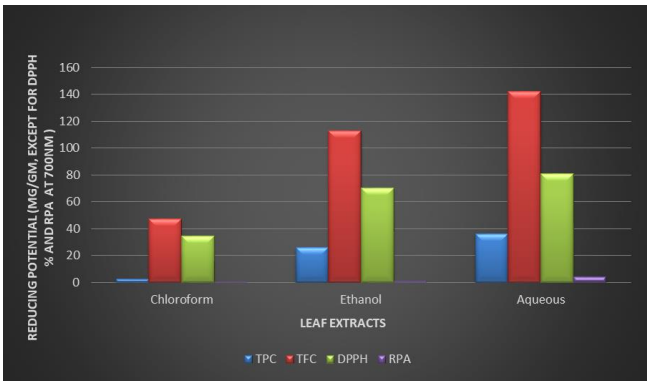


Fig 3 & 4: Graph depicting the total phenolic content and anti-oxidant assays of *P. indicum* bark and leaf extracts respectively in different solvent systems

Conclusion

In the present work, high antioxidant activity was observed in ethanol and aqueous extracts of bark and aqueous leaf extracts of *P. indicum* in comparison with other solvents. This may be attributed to the presence of hydrophilic antioxidants and the compounds present play a role in preventing human diseases in which free radicals are involved, such as cancer, ageing and cardiovascular diseases. Further studies on the fractionization of solvent extracts and characterization by spectroscopy techniques may reveal the compounds responsible for the antioxidant potentials.

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