

# Comparative Evaluation of *in vitro* antioxidant activities of various extracts from *Chomelia asiatica* (Linn) and *Pavetta indica* (Linn)

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## Abstract:

The study was designed to evaluate and compare the antioxidant properties of six different plant's extracts of *Chomelia asiatica* and *Pavetta indica*. The antioxidant activity was evaluated by DPPH assay, superoxide anion scavenging activity, nitric oxide scavenging activity with reference standard rutin, Quercetin, ascorbate respectively and estimate the amount of total phenol. The methanolic extract of *Pavetta indica* showed strong antioxidant activity by inhibiting DPPH scavenging activity, nitric oxide radical scavenging activities when compared with standard rutin and ascorbate as well as Ethyl acetate extract of *Chomelia asiatica* exhibited higher ability in scavenging superoxide anion radical, when compared to the standard quercetin. In addition, Ethyl acetate extract of *Chomelia asiatica* and methanolic extract of *Pavetta indica* was found to contain noticeable amount of total phenols, which play a major role in controlling antioxidants. It is concluded that a aerial parts of Ethyl acetate extract of *Chomelia asiatica* and methanolic extract of *Pavetta indica*, which contains large amounts of phenolic compounds, exhibits high antioxidant and free radical scavenging activities.

**Key words:** *C. asiatica* *P. indica*, DPPH assay Nitric oxide scavenging, Superoxide anion, Total phenol.

## INTRODUCTION

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. Free radicals are highly reactive compounds, they are chemical species associated with an odd or unpaired electron and can be formed when oxygen interacts with certain molecules. They are neutral, short lived, unstable and highly reactive to pair with the odd electron and finally achieve stable configuration. Once formed these highly reactive radicals can start a chain reaction they are capable of attacking the healthy cells of the body, causing them to lose their structure and function. It is commonly recognized that antioxidants radicals can neutralize potentially harmful reactive free radicals in body cells before they cause lipid and protein oxidation and may reduce potential mutation and therefore, help prevent cancer or heart diseases[1]. Natural antioxidants have a wide range of biochemical activities, including inhibition of ROS (reactive oxygen species) generation, direct or indirect scavenging of free radicals, and alteration of intracellular redox potential[2]. Natural antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The use of natural antioxidants present in food and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value[3].

*Chomelia asiatica* (Linn) is a common species which occurs in india, srilanka and china. The leaves or powder extracts of *Chomelia asiatica* are used as an antimicrobial activities[4]. It had been reported analgesic and anti-inflammatory activities[5]. The parts of *Tarenna asiatica* (Rubiaceae) plants are traditionally used to promote suppuration[6], as anthelmintic[7] and antiulcer agent[8]. The phytochemical constituents of it are reported to be antiseptic[9]. *Pavetta indica* Linn. belongs to the family Rubiaceae. The entire plant used medicinally as a bitter tonic, diuretic, inflammation, rheumatism, jaundice and ulcer[10]. In the indigenous system of medicine, it is reported that the decoction of the leaves are used to relieve haemorrhoidal pain, as a lotion for nose, analgesic, antipyretic, appetizer and the ulceration of mouth[11,12]. In literature, it has been reported as an antibacterial, antiviral and antimalarial[13]. *P. indica* leaves are used in the treatment of liver disease, pain from piles, urinary diseases and fever[14]. Golwala *et al.* (2009) reported analgesic activity[15], antidiabetic activity[16], antimicrobial[17] activity

of leaf extract of *P. indica*. Its root extract also have diuretic and purgative activity[18] (Kumar, 2006). However, no data are available in the literature on the antioxidant activity of aerial parts of *Pavetta indica* (Linn). Therefore we undertook the present investigation to evaluate and compare the antioxidant properties of six different plant's extracts from *Chomelia asiatica* and *Pavetta indica* through various *in vitro* models.

## MATERIALS AND METHODS

### Collection and Identification of Plant materials

The aerial parts of *Chomelia asiatica* (Linn), were collected from Senkottai, Tirunelveli District of Tamil Nadu, India and aerial parts of *Pavetta indica* (Linn), were collected from kalakkadu, Tirunelveli District, Tamil Nadu, India. Both the plants were subjected to taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. Both the plant materials were dried under shade, segregated, pulverized by a mechanical grinder separately and passed through a 40 mesh sieve.

### Preparation of Extracts

The above powdered materials were successively extracted with Petroleum ether (40-60°C) by hot continuous percolation method in Soxhlet apparatus[19] for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78°C) for 24 hrs and then marc was subjected to Methanol for 24 hrs. The extracts were concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

### Evaluation of antioxidant activity by *in vitro* techniques:

#### DPPH photometric assay[20]

The effect of extract on DPPH radical was assayed using the method of Mensor *et al* (2001). A methanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of the different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$\text{Scavenging activity(\%)} = \frac{A_{518} \text{ Control} - A_{518} \text{ Sample}}{A_{518} \text{ Control}} \times 100$$

Where  $A_{518}$  control is the absorbance of DPPH radical+ methanol;  $A_{518}$  sample is the absorbance of DPPH radical+ sample extract/ standard.

#### Superoxide anion scavenging activity[21]

Superoxide radical ( $O_2^-$ ) was generated from the photoreduction of riboflavin and was deducted by nitro blue tetrazolium dye (NBT) reduction method. Measurement of superoxide anion scavenging activity was performed based on the method described by Winterbourne et al (1975)<sup>19</sup>. The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5 mM NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer). The control tubes were also set up where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

#### Nitric oxide radical scavenging activity[22]

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions. The reaction mixture (3ml) containing 2 ml of sodium nitroprusside (10mM), 0.5 ml of phosphate buffer saline (1M) were incubated at 25°C for 150 mins. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 min for completing diazotization. Then 1 ml of naphthylethylene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions which can be estimated by the use of Griess Illosvery reaction at 540 nm.

#### Total phenol[23]

The measurement of total phenol is based on Mallick and Singh (1980)<sup>21</sup>. To 0.25g of sample, added 2.5 ml of ethanol and centrifuged at 2°C for 10 mins. The supernatant was preserved. Then, the sample was re-extracted with 2.5 ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then, added 3 ml of water to the dried supernatant. To which added 0.5 ml of Folin's phenol reagent and 2 ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1 min. the absorbance was measured at 650 nm in a spectrophotometer.

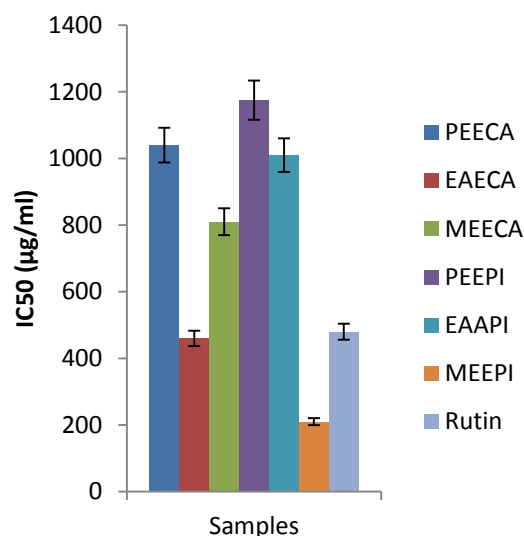
### RESULTS AND DISCUSSIONS

DPPH is a stable free radical at room temperature often used to evaluate the antioxidant activity of several natural compounds. The reduction capacity of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The  $IC_{50}$  value of various extract on DPPH radical scavenging activity of various extract were presented as shown in the Table 1. The  $IC_{50}$  of the petroleum ether extract of *C. asiatica*, ethyl acetate extract of *C. asiatica*, methanolic extract of *C. asiatica* were found to be 1040 $\mu$ g/ml, 460 $\mu$ g/ml, 810 $\mu$ g/ml and petroleum ether extract of *Pavetta indica*, ethyl acetate extract of *Pavetta indica*, methanolic extract of *Pavetta indica* and Rutin were found to be 1010 $\mu$ g/ml, 210 $\mu$ g/ml, 480 $\mu$ g/ml and 480 $\mu$ g/ml respectively

**Table 1 The  $IC_{50}$  value of various extract on DPPH assay**

S.No	Sample	$IC_{50}$ ( $\mu$ g/ml) ( $\pm$ SEM)*
1	Petroleum ether extract of <i>Chomelia asiatica</i> (PEECA)	1040 $\pm$ 23.56
2	Ethyl acetate extract of <i>Chomelia asiatica</i> (EAECA)	460 $\pm$ 17.23
3	Methanolic extract of <i>Chomelia asiatica</i> (MEECA)	810 $\pm$ 14.37
4	Petroleum ether extract of <i>Pavetta indica</i> (PEEPI)	1175 $\pm$ 20.20
5	Ethyl acetate extract of <i>Pavetta indica</i> (EAEPI)	1010 $\pm$ 17.21
6	Methanolic extract of <i>Pavetta indica</i> (MEEPI)	210 $\pm$ 4.48
7	Standard ( <b>Rutin</b> )	480 $\pm$ 13.84

\*All values are expressed as mean  $\pm$  SEM for three determinations



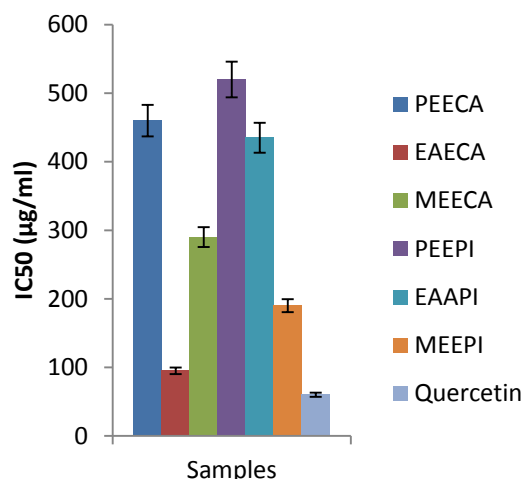
#### Superoxide anion scavenging activity

The  $IC_{50}$  value of superoxide anion radical scavenging activity of various extract were presented as shown in the Table 2. The  $IC_{50}$  of the petroleum ether extract of *C. asiatica*, ethyl acetate extract of *C. asiatica*, methanolic extract of *C. asiatica* were found to be 460 $\mu$ g/ml, 95 $\mu$ g/ml, 290 $\mu$ g/ml and petroleum ether extract of *Pavetta indica*, ethyl acetate extract of *Pavetta indica*, methanolic extract of *Pavetta indica* and Rutin were found to be 520 $\mu$ g/ml, 435 $\mu$ g/ml, 190 $\mu$ g/ml and 60 $\mu$ g/ml respectively

**Table: 2 The  $IC_{50}$  value of various extract on superoxide anion scavenging activity method**

S.No	Sample	$IC_{50}$ ( $\mu$ g/ml) ( $\pm$ SEM)*
1	Petroleum ether extract of <i>Chomelia asiatica</i> (PEECA)	460 $\pm$ 4.26
2	Ethyl acetate extract of <i>Chomelia asiatica</i> (EAECA)	95 $\pm$ 2.10
3	Methanolic extract of <i>Chomelia asiatica</i> (MEECA)	290 $\pm$ 7.37
4	Petroleum ether extract of <i>Pavetta indica</i> (PEEPI)	520 $\pm$ 10.12
5	Ethyl acetate extract of <i>Pavetta indica</i> (EAEPI)	435 $\pm$ 8.14
6	Methanolic extract of <i>Pavetta indica</i> (MEEPI)	190 $\pm$ 7.35
7	Standard ( <b>Quercetin</b> )	60 $\pm$ 4.42

\*All values are expressed as mean  $\pm$  SEM for three determinations



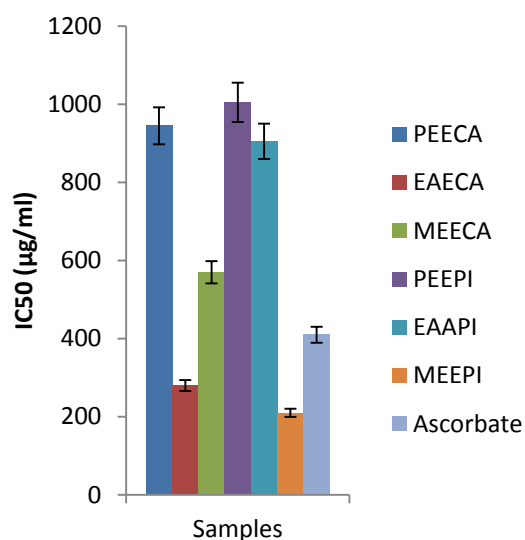
**Nitric oxide scavenging activity**

The reduction of nitric oxide radical by the various extract and ascorbate IC<sub>50</sub> values were illustrated in Table 3. The IC<sub>50</sub> of the petroleum ether extract of *C. asiatica*, ethyl acetate extract of *C. asiatica*, methanolic extract of *C. asiatica* were found to be 945µg/ml, 280µg/ml, 570µg/ml and petroleum ether extract of *Pavetta indica*, ethyl acetate extract of *Pavetta indica*, methanolic extract of *Pavetta indica* and Rutin were found to be 1005µg/ml, 905µg/ml, 210µg/ml and 410µg/ml respectively

**Table: 3 The IC<sub>50</sub> value of various extract on Nitric oxide scavenging activity method**

S.No	Sample	IC <sub>50</sub> (µg/ml) (±SEM)*
1	Petroleum ether extract of <i>Chomelia asiatica</i> (PEECA)	945± 8.90
2	Ethyl acetate extract of <i>Chomelia asiatica</i> (EAECA)	280± 7.25
3	Methanolic extract of <i>Chomelia asiatica</i> (MEECA)	570± 10.50
4	Petroleum ether extract of <i>Pavetta indica</i> (PEEPI)	1005±15.12
5	Ethyl acetate extract of <i>Pavetta indica</i> (EAAP)	905±13.86
6	Methanolic extract of <i>Pavetta indica</i> (MEEPI)	210±8.35
7	Standard (Ascorbate)	410 ±9.42

\*All values are expressed as mean ± SEM for three determinations



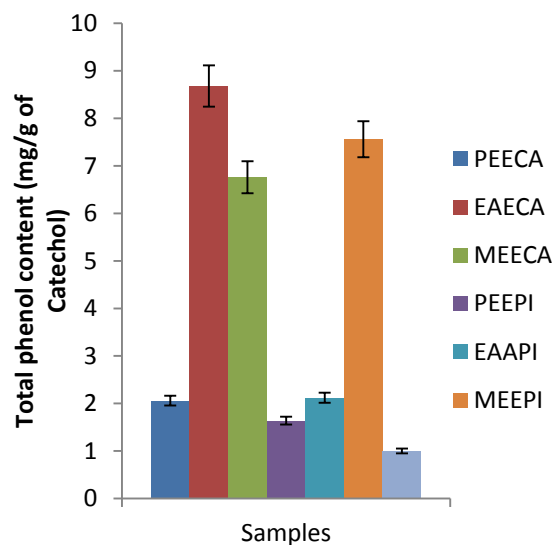
**Total phenol**

The total amount of phenolic content of various extract were presented in Table 4. The total phenolic content of the petroleum ether extract of *C. asiatica*, ethyl acetate extract of *C. asiatica*, methanolic extract of *C. asiatica* were found to be 2.06 ± 0.032, 8.68 ± 0.046, 6.76 ± 0.054µg/ml and petroleum ether extract of *Pavetta indica*, ethyl acetate extract of *Pavetta indica*, methanolic extract of *Pavetta indica* were found to be 1.64 ± 0.010, 2.12 ± 0.021 and 7.56 ± 0.026 respectively

**Table 4: The estimation of total phenolic content**

S. No	Sample	Total phenol content (mg/g of Catechol) (±SEM)*
1	Petroleum ether extract of <i>Chomelia asiatica</i> (PEECA)	2.06 ± 0.032
2	Ethyl acetate extract of <i>Chomelia asiatica</i> (EAECA)	8.68 ± 0.046
3	Methanolic extract of <i>Chomelia asiatica</i> (MEECA)	6.76 ± 0.054
4	Petroleum ether extract of <i>Pavetta indica</i> (PEEPI)	1.64 ± 0.010
5	Ethyl acetate extract of <i>Pavetta indica</i> (EAAP)	2.12 ± 0.021
6	Methanolic extract of <i>Pavetta indica</i> (MEEPI)	7.56 ± 0.026

\*All values are expressed as mean ± SEM for three determinations



**DISCUSSION**

Antioxidant compounds may function as free radical scavengers, initiator of the complexes of pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation. The *in-vitro* antioxidant potential of various extracts were evaluated by DPPH free radical scavenging activity, superoxide anion radical scavenging activity, Nitric oxide scavenging activity method and estimation of total phenolic content. The studies were carried out taking rutin, quercetin and ascorbic acid as the standard antioxidant.

DPPH radical is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical scavenging ability of the various samples[24]. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H by the reaction[25]. DPPH radical scavenging activity were examined various extracts and

found IC<sub>50</sub> value reflects higher scavenging ability. Among the six different plant extracts tested, interestingly, in the DPPH radical scavenging activity of the Methanolic extract of *Pavetta indica* exhibited DPPH radical scavenging potential comparable with that of standard rutin. Superoxides could be produced in large amounts by various biological processes. It is known to be very harmful to cellular components as a precursor of the most reactive oxygen species (ROS), contributing to tissue damage and various diseases[26]. The Ethyl acetate extract of *Chomelia asiatica* exhibited higher ability in scavenging superoxide anion radical, when compared to the standard quercetin.

Nitric oxide is a diffusible free radical which is an important effector molecule in diverse biological systems[27]. So it is worthful to investigate the NO scavenging potential of the plant extract. The Methanolic extract of *Pavetta indica* exhibited higher ability in scavenging superoxide anion radical, when compared to the standard quercetin. Phenolic compounds are known as powerful chain breaking antioxidants[28]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[29]. The phenolic compounds may contribute directly to antioxidative action[30]. Based on the result the Ethyl acetate extract of *Chomelia asiatica*(Linn), methanolic extract of *Chomelia asiatica*(Linn) and methanolic extract of *Pavetta indica* was found higher content of phenolic components than that of other extracts of both the plants. It is well known that flavonoids and polyphenols are natural antioxidants but have also been reported to significantly increase SOD, glutathione and catalase activities. Furthermore it was shown that these compounds act as promoters for SOD, catalase and glutathione and cause the expression of SOD, glutathione and catalase[31].

#### CONCLUSION

The present study was clearly indicated the methanolic extract of *Pavetta indica* showed strong antioxidant activity by inhibiting DPPH scavenging activity, nitric oxide radical scavenging activities when compared with standard rutin and ascorbate as well as Ethyl acetate extract of *Chomelia asiatica* exhibited higher ability in scavenging superoxide anion radical, when compared to the standard quercetin. In addition, Ethyl acetate extract of *Chomelia asiatica* and methanolic extract of *Pavetta indica* was found to contain noticeable amount of total phenols, which play a major role in promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention of free radical-mediated diseases. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the plant extract.

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