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Evaluation of *In Vitro* Antioxidant Activity of Various Extracts of Aerial Parts of *Chomelia asiatica* (Linn)

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ABSTRACT

The study was designed to examine the *in-vitro* antioxidant activities of various extracts of aerial parts of *Chomelia asiatica.* The antioxidant activity was evaluated by total antioxidant activity, FRAP with reference standard ascorbate respectively and estimate the amount of total flavonoids. The ethyl acetate extract of *Chomelia asiatica* was found to more effective in the total antioxidant activity. The IC_{50} of the ethyl acetate extract of *Chomelia asiatica* and ascorbate were found to be 260µg/ml and 480µg/ml respectively. An IC_{50} value was found that ethyl acetate extract of *Chomelia asiatica* is more effective in scavenging superoxide radical than that of methanol and petroleum ether extract. But when compare to the all the three extracts with ascorbate (standard), the ethyl acetate extract of the *Chomelia asiatica* showed the similar result. In addition, the ethyl acetate extract of *Chomelia asiatica* was found to contain a noticeable amount of total flavonoids, which play a major role in controlling antioxidants. It is concluded that a aerial parts of ethyl acetate extract of *Chomelia asiatica*, which contains large amounts of flavonoids compounds, exhibits high antioxidant and free radical scavenging activities. These *in vitro* assays indicate that this plant extracts is a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Aerial parts of *Chomelia asiatica*, total antioxidant, FRAP, total flavonoids.

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INTRODUCTION

Antioxidants are often used in oils and fatty foods to retard their autoxidation. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have restricted use in foods as they are suspected to be carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years[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]. Naturals antioxidants mainly come from plants in the form of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienbols) 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 belongs to the family Rubiaceae, commonly known as *Tharani in tami*: *Kuppipoovu, Tharana* in makayalam4. Leaves simple, opposite, decussate; stipules triangular with apiculate tip, interpetiolar, caducous and leaving scar; petiole 0.5-2 cm, canaliculate, glabrous; lamina 8-18 x 4-8 cm, elliptic to elliptic-ovate, apex shortly and abruptly acuminate with blunt tip, base attenuate, margin entire and revolute, glabrous beneath; midrib usually raised above and canaliculate when dry; secondary_nerves 9-12 pairs, hairy domatia present at axils of secondary_nerves; tertiary_nerves broadly reticulate. The flower Inflorescence terminal corymbose cymes; flowers cream to yellow, subsessile. The fruits are Berry, 2-celled; seeds many[5].

Chomelia Gaertn is a genus of about 370 species distributed in tropical and subtropical Africa, asia, Madagascar and pacific islands. *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[6](Jayasinghe et al., 2002). It had been reported analgesic and anti-inflammatory activities (Amutha et al., 2012) [7]. The parts of *Tarenna asiatica* (Rubiaceae) plants are traditionally used to promote suppuration (Anonymous, 1976)[8]as anthelmintic (Ramarao and Henry, 1996) [9] and antiulcer agent (Rao et al., 2006) [10]. The phytochemical constituents of it are reported to be antiseptic (Vinoth-kumar et al., 2011)[11]wound healing (Anjanadevi and Menaga, 2013) [12] and antioxidant (Ramabarathi et al., 2014) [13]. Besides, the extract of shoots, leaves and fruits are purportedly active against Mycobacter phlei (Rajakaruna et al., 2002).[14]

However, no data are available in the literature on the antioxidant activity of aerial parts of *Chomelia asiatica* (Linn). Therefore we undertook the present investigation to examine the antioxidant activities of various extract of aerial parts of *Chomelia asiatica* (Linn) through various *in vitro* models.

MATERIAL AND METHOD

Collection and identification of plant materials

The aerial parts of *Chomelia asiatica* (Linn), were collected form Senkottai, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The aerial parts of *Chomelia asiatica*(Linn), were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve.

Preparation of extracts

The above powered materials were successively extracted with Petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus for 24 hrs. Then the marc was subjected to Ethyl acetate (76-78^{0}C) for 24 hrs and then mark 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[15].

Evaluation of antioxidant activity by *in vitro* techniques:

Total antioxidant activity (Phosphomolybdic acid method) [16]

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto et al., 1999)[16]. An aliquot of 0.4 ml of sample solution was combined in a vial with 4 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated in a water bath at 95^{0}C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The antioxidant activity was expresses relative to that of ascorbic acid.

FRAP assay[17]

A modified method of Benzie and Strain (1996)[17] was adopted for the FRAP assay. The stock solutions included 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution in 40 mM HCl and 20 mM $Fecl_{3.}$ $6H_2O$. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $Fecl_3$ $.6H_2O$. The temperature of the solution was raised to 37^0 C before using. Plant extracts (0.15 ml) were allowed to react with 2.85

ml of FRAP solution for 30 min in the dark condition. Readings of the colored product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 µM $Feso_4$. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Total flavonoids[18]

0.2g of the plant material was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively. The homogenate was filtered and these 2 ratios were combined. This was evaporated to dryness until most of the ethanol has removed. The resultant aqueous extract was extracted in a separating funnel with hexane or chloroform. The solvent extracted aqueous layer was concentrated 0.5 ml of aliquot of extract was pipette-out in a test tube. 4 ml of the vanillin reagent (1% vanillin in 70% conc. H_2SO_4) was added and kept in a boiling water bath for 15 mins. The absorbance was read at 360 nm. A standard was run by using catechol (110 µg/ml).

RESULTS AND DISCUSSION

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of petroleum ether extract of *Chomelia asiatica* presented in Table 1. The petroleum ether extract of *Chomelia asiatica* exhibited a maximum total antioxidant activity of 61.46 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the petroleum ether extract of *Chomelia asiatica* and ascorbate were found to be 755µg/ml and 410µg/ml respectively.

Table 1: Total antioxidant activity of Petroleum ether extract of *Chomelia asiatica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	Standard (Ascorbate)
1	125	21.34 ± 0.026	26.87 ± 0.076
2	250	29.16 ± 0.016	30.30 ± 0.054
3	500	39.54 ± 0.038	60.64 ± 0.022
4	1000	61.46 ± 0.042	65.23 ± 0.014
		IC_{50} = 755 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of ethyl acetate extract of *Chomelia asiatica* presented in Table 2. The ethyl acetate extract of *Chomelia asiatica* exhibited a maximum total antioxidant activity of 76.86 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} values of the ethyl acetate extract of *Chomelia asiatica* and ascorbate were found to be 260µg/ml and 410µg/ml respectively.

Table 2: Total antioxidant activity of Ethyl acetate extract of *Chomelia asiatica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	**Standard (Ascorbate)**
1	125	35.45 ± 0.023	26.87 ± 0.076
2	250	49.28 ± 0.040	30.30 ± 0.054
3	500	65.67 ± 0.052	60.64 ± 0.022
4	1000	76.86 ± 0.042	65.23 ± 0.014
		IC_{50} = 260 µg/ml	**IC_{50} = 410 µg/ml**

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

The percentage of total antioxidant activity of Ethyl acetate extract of *Chomelia asiatica* presented in Table 3. The methanolic extract of *Chomelia asiatica exhibited* a maximum total antioxidant activity of 87.12 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 55.23 % at 1000 µg/ml. The IC_{50} of the methanolic extract of *Chomelia asiatica and* ascorbate were found to be 485µg/ml and 410µg/ml respectively.

Table 3: Total antioxidant activity of Methanolic extract of *Chomelia asiatica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	**Standard (Ascorbate)**
1	125	24.34 ± 0.023	26.87 ± 0.076
2	250	32.42 ± 0.022	30.30 ± 0.054
3	500	53.24 ± 0.010	60.64 ± 0.022
4	1000	65.23 ± 0.034	65.23 ± 0.014
		IC_{50} = 485 µg/ml	**IC_{50} = 410 µg/ml**

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

Based on the result clearly indicated the ethyl acetate extract of *Chomelia asiatica was* found to more effective than petroleum ether and methanolic extract. But when compare all the extracts with standard the methanolic extract of *Chomelia asiatica was* found strong antioxidant activity. The IC_{50} of the methanolic extract of *Chomelia asiatica and* Ascorbate were found to be 100µg/ml and 410µg/ml respectively.

FRAP assay

The antioxidant potential of *Chomelia asiatica* was ascertained from FRAP assay based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing ability of the petroleum ether extract of *Chomelia asiatica and* ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 4. The maximum reducing ability at 1000µg/ml for petroleum ether extract and ascorbate were found to be 58.56% and 98.07% respectively. The IC_{50} values of petroleum ether extract and ascorbate were recorded as 790µg/ml and 50µg/ml respectively.

Table 4: FRAP assay of Pet. ether extract of *Chomelia asiatica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Petroleum ether extract)	**Standard (Ascorbate)**
1	125	19.34 ± 0.024	72.04 ± 0.014
2	250	31.81 ± 0.038	82.05 ± 0.034
3	500	41.23 ± 0.025	86.04 ± 0.026
4	1000	58.56 ± 0.022	98.07 ± 0.041
		IC_{50} = 790 µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the ethyl acetate extract of *Chomelia asiatica and* ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 5. The maximum reducing ability at 1000µg/ml for ethyl acetate extract and ascorbate were found to be 65.18% and 98.07% respectively. The IC_{50} values of ethyl acetate extract and ascorbate were recorded as 290µg/ml and 50µg/ml respectively.

Table 5: FRAP assay of Ethyl acetate extract of *Chomelia asiatica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Ethyl acetate extract)	**Standard (Ascorbate)**
1	125	37.34 ± 0.016	72.04 ± 0.014
2	250	49.42 ± 0.015	82.05 ± 0.034
3	500	57.16 ± 0.030	86.04 ± 0.026
4	1000	65.18 ± 0.024	98.07 ± 0.041
		IC_{50} = 290 µg/ml	**IC_{50} = 50 µg/ml**

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

The reducing ability of the methanolic extract of *Chomelia asiatica and* ascorbate at various concentrations (125, 250, 500, 1000 µg/ml) were examined and the values were presented in Table 6. The maximum reducing ability at 1000µg/ml for methanolic extract and ascorbate were found to be 65.55% and 98.07% respectively. The IC_{50} values of methanolic extract and ascorbate were recorded as 385µg/ml and 50µg/ml respectively.

Table 6: FRAP assay of Methanolic extract of *Chomelia asiatica*

S.No	Concentration (µg/ml)	% of activity(±SEM)*	
		Sample (Methanolic extract)	**Standard (Ascorbate)**
1	125	35.34 ± 0.044	72.04 ± 0.014
2	250	46.48 ± 0.030	82.05 ± 0.034
3	500	53.99 ± 0.026	86.04 ± 0.026
4	1000	65.55 ± 0.016	98.07 ± 0.041
		IC_{50} = 385 µg/ml	**IC_{50} = 50 µg/ml**

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

Based on the above results indicated, the ethyl acetate extract of *Chomelia asiatica was* found to most effective than that of petroleum ether & methanolic extract. . But when compare to the all the three extracts with ascorbate (standard), the methanolic extract of the *Chomelia asiatica showed* the moderate result.

Total flavonoids

The total amount of flavonoids content of various extract of whole plant of *Chomelia asiatica was* presented in Table 7.

Table 7: The total flavonoids content of various extracts of whole plant of *Chomelia asiatica*

S.No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Petroleum ether extract of *Chomelia asiatica*	0.014 ± 0.012
2	Ethyl acetate extract of *Chomelia asiatica*	3.425 ± 0.008
3	Methanolic extract of *Chomelia asiatica*	1.241 ± 0.022

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

Based on the result the ethyl acetate extract of *Chomelia asiatica was* found higher content of flavonoids than that of petroleum ether and methanolic extract of *Chomelia asiatica* .

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