



In-vitro Antioxidant potential of whole plant of ethanolic extract of *Waltheria indica*(Linn)

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Abstract : In-vitro antioxidant activities of ethanolic extract of whole plant of *Waltheria indica* (Linn) was investigated by various methods. The antioxidant activity was evaluated by Nitric oxide radical scavenging activity, Hydroxyl radical scavenging activity and Estimation of total phenolic content. The ethanolic extract of *Waltheria indica* (Linn) and reference standard ascorbate IC₅₀ values was found to be 850 µg/ml and 420 µg/ml in nitric oxide radical scavenging activity. Hydroxyl radical scavenging activity of ethanolic extract and reference standard ascorbate IC₅₀ values was found to be 210 µg/ml and 420 µg/ml. The total phenolic content of ethanolic extract was found to be 7.82 mg/g respectively. The above result possess significant antioxidant activity when compare to the above all standard.

Keywords : Antioxidant, Hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity, Estimation of total phenol.

Introduction

Oxidant stress is a result of imbalance between the antioxidant defence system and the formaldehyde of reactive oxygen species, may damage life important membrane lipids, protein, DNA and carbohydrates [1]. Oxidative stress can result either from an increase production of reaction [2]. Plant products whether volatile or non – volatile are valuable sources of novel bio active compounds useful in combating various sources such as cancer, cell damage, inflammation, viral infection, allergic response as well as in the provision of primary health care in most developing country [3].

Waltheria indica is commonly known as astamilname(Shengalipoondu), *Waltheria americana* (Synonym: English). *Waltheria indica* L. or sleepy morning, also known as velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat, buff coat, and many other names [4].

Waltheria indica belongs to the family *Sterculiaceae*. It is valuable plant in traditional systems of medicine. The whole plant is used to treat disease such as, anti-inflammatory, analgesic antibacterial, antifungal, rheumatism, antidiarrheal, antimalarial, antiviral, anticonvulsant, anti-anemia, used in asthma and teeth infection and Sedative activities [5-6].

Waltheria indica contains different chemical groups including alkaloids, flavonoids, sterols, terpenes, cardiac glycosides, saponins, anthraquinones and carbohydrates. *Waltheria indica* contains in the leaves and roots. Saponins, alkaloids, anthraquinones, flavonoids, tannins, phenols and cardiac glycosides at varied degrees [7].

Therefore we the present investigation to examine the antioxidant activities of ethanolic extract of whole plant of *Waltheria indica* (Linn).through various *in-vitro* models.

Experimental

Material and Methods

Chemicals

The extract used in this study are the whole plant of *Waltheria indica*(Linn), were collected form Kilikulam, Tirunelveli District of Tamil Nadu. Soxhlet apparatus,Centrifuge, Spectrophotometer Ethanol, sodium nitroprusside, sulphanilic acid, DMSO, Folins phenol.

Collection and Identification of Plant materials

The whole plant of *Waltheria indica*(Linn), were collected form Kilikulam, Tirunelveli District of Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The whole plant of *Waltheria indica*(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 ethanol by hot continuous percolation method in Soxhlet apparatus [8]for 24 hrs. The extract was 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:

1) Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitride ions, which were measured by the method of Garret (1964). The reaction mixture (3ml) containing 2ml of sodium nitroprusside (10mM), 0.5ml of phosphate buffer saline (1M) were incubated at 25°C for 150 mins. After incubation, 0.5ml of the reaction mixture containing nitride was pipetted and mixed with 1ml of sulphanilic acid reagent (0.33%) and allowed to stand for 5 mins for completing diazotization. Then 1 ml of naphthylethylenediaminedihydrochloride (1%NEDA) was added, mixed and allowed to stand for 30 mins. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions which can be estimated by the use of Griessillosvery reaction at 540nm.

2) Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This methods involves in-vitro generation of Hydroxyl radical using Fe³⁺ /ascorbate/EDTA/H₂O₂ system using fenton reaction. Scavenging of this Hydroxyl radical is presence of antioxidant is measured. In one of the methods the Hydroxyl radical formed by the oxidation is made to react with DMSO (dimethyl sulphaoxide) to yield formaldehyde. Formaldehyde formed produces intense yellow colour with nash reagent (2M ammonium acetate in distilled water). The intensity of yellow colour formed is measured at 41nm.

3) Estimation of total phenol

The measurement of total phenol is based on malick and singh (1980). To 0.25g of sample, added 2.5ml of ethanol and centrifuged at 2°C for 10 mins. The supernatant was preserved. Then the sample was re-extracted with 2.5ml of 80% ethanol and centrifuged. The pooled supernatant was evaporated to dryness. Then added 3ml of water to be dried supernatant. To which added 0.5ml of folins phenol reagent and 2ml of sodium carbonate (20%). The reaction mixture was kept in boiling water bath for 1mins. The absorbance was measured at 650m in a spectrophotometer.

Results and Discussion

1) Nitric oxide scavenging activity

The reduction of nitric oxide reduces by ethanolic extract of plant of *Waltheria indica*(Linn), and ascorbate were illustrated Table I. The IC₅₀ values of the extract of *Waltheria indica*, are ascorbate were found to be 84µg/ml are 420µg/ml respectively.

Table - I

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Ethanolic extract)	Standard (Rutin)
1	125	38.60±0.015	28.86 ± 0.07
2	250	41.25± 0.16	31.20 ± 0.04
3	500	45.16±0.02	62.64 ± 0.02
4	1000	56.62±0.03	76.24 ± 0.25
		IC₅₀ = 840µg/ml	IC₅₀ = 420µg/ml

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

2) Hydroxyl radical scavenging activity

The percentage of hydroxyl scavenging activity of ethanolic extract of *waltheria indica* presented the IC₅₀ values of extract of *waltheria indica* and ascorbate were formed to be 210µg/ml and 420 µg/ml respectively.

Table – II

S.No	Concentration (µg/ml)	% of activity (±SEM*)	
		Sample (Ethanolic extract)	Standard (Rutin)
1	125	40.00±0.02	28.86 ± 0.07
2	250	51.30± 0.6	31.20 ± 0.04
3	500	59.52±0.02	62.64 ± 0.02
4	1000	65.75±0.04	76.24 ± 0.25
		IC₅₀ = 210µg/ml	IC₅₀ = 420µg/ml

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

3) Total Phenol

The total phenol content of ethanolic extract of *waltheria indica* was presents table – III. Based on the report of ethanolic extract of *Waltheria indica* was formed 7.82µg/ml of phenolic content.

Table – III :The total phenolic content of ethanolic extract of whole plant of waltheria indica(Linn)

S.No	Extract	Total phenolic content (mg/ml)	Total phenolic content ± SEM
1	Ethanolic extract	7.83	7.82mg/ml
		7.94	
		7.68	

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

Conclusion

The result of above investigation indicated that the ethanolic extract of whole plant of *waltheriaindica*(Linn), showed strong antioxidant activity, however, phytochemical screening of ethanolic

extract shower of triterpenoids, flavonoids and phenolic compounds. So, it can be concluded that these components might be involves in the antioxidant activity of *waltheria indica* (Linn).

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