IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 7 Ser. 11 (July. 2024), PP 04-09
www.iosrjournals.org

Physicochemical, Preliminary Phytochemical and Evaluation of In-Vitro Free Radical Scavenging Potential of Whole Plant of Waltheria indica (Linn).

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Abstract
Objective: *The objective of the present study was to evaluate the physicochemical, phytochemical constitution and antioxidant activity of ethanolic extract of whole plant of Waltheria indica(Linn.)*
Materials and Methods: *Various physicochemical parameters such Total ash value, Water soluble ash value, Acid insoluble ash value. Qualitative analysis of Phytochemical constituents' viz. tannins, saponins, flavonoids, steroids, alkaloids, quinones, terpenoids and cardiac glycosides. The antioxidant activity of whole plant of Waltheria indica (Linn) was investigated in various in-vitro methods. The antioxidant activity was evaluated by total antioxidant activity (Phosphomolybdic acid method), FRAP assay with reference standard ascorbate and total flavonoids content respectively.*
Results: *In physicochemical study, the parameters such as total ash, acid insoluble ash, water-soluble ash were carried out. The total ash was found 11.47±0.72% and acid insoluble ash was found 4.92 ±0.14% and water-soluble was found 8.35±0.69%. The results of phytochemical analysis suggest that presence of tannins, saponins, flavonoids, steroids, alkaloids, terpenoids and cardiac glycosides. The ethanolic extract of Waltheria indica (Linn) was found to moderate effect in the total antioxidant activity. The IC50 values of the ethanolic extract of Waltheria indica (Linn) and ascorbate were found to be 460µg/ml and 420µg/ml respectively. The ethanolic extract of Waltheria indica was found moderate effective in FRAP assay. But when compare to the ethanolic extract with ascorbate (standard), the ethanolic extract of the Waltheria indica (Linn) showed the better result. The ethanolic extract of Waltheria indica (Linn), contains high amount of flavonoids. These in-vitro assays indicate that this plant extract is a better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.*
Discussion: *Thus the ethanolic extract of whole plant of Waltheria indica(Linn.) shows good physicochemical, phytochemical constitution and antioxidant activity.*
Keywords: *Waltheria indica (Linn), In-vitro antioxidant, Total antioxidant activity, FRAP assay, Total flavonoids.*

Date of Submission: 18-07-2024 Date of Acceptance: 28-07-2024

I. Introduction

The medicinal value of the plant is due to the presence of various bioactive chemical constituents such as alkaloids, tannins, flavanoids and phenolic compounds (1). Therefore, the plants with the medicinal values have to be investigated to understand their safety and efficacy (2). Medicinal plants are the primary source of medicine for the treatment of human diseases in many rural areas of the developing countries (3). About 80% of the world population relies on the traditional medicine for their primaryhealth care (4).

The physico-chemical parameters such as moisture content, pH (1% aqueous), total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive and preliminary phytochemical screening for the

presence of alkaloid, flavonoid, glycoside, phenol, saponin, resin, steroid, tannin were carried out by following the standard procedures. (5-8)

Antioxidant plays an essential role in the prevention of human diseases. Naturally occurring antioxidants in leafy vegetables and seeds, like ascorbic acid, vitamin E and phenolic compounds, possess the ability to diminish the oxidative damage associated with numerous diseases, including cancer, cardiovascular disease, cataracts, atherosclerosis, diabetes, arthritis, immune deficiency diseases and ageing (9). Current reports indicated that there is an inverse relationship between the dietary intake of antioxidant rich foods and the incidence of human diseases (10).

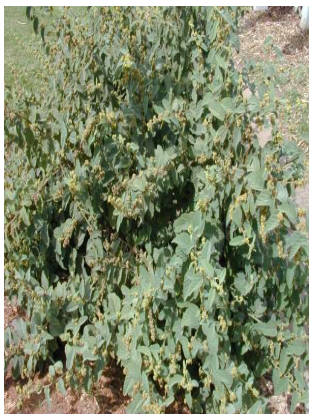
Collection and Identification of Plant materials

Waltheria indica(Linn.) belongs to the family *Sterculiacae.* It is valuable plant in traditional systems of medicine. The whole plant 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 (11-12). Waltheria indica contains different chemical groups including alkaloids, flavonoids, sterols, terpenes, cardiao 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 (13). *Waltheria indica(Linn.)* is commonly known as Shengalipoondu (Tamil), *Waltheria americana* (Synonym: English). *Waltheria indica L*. or sleepy morning, also known as velvet leaf, marsh-mallow, monkey bush, boater bush, leather coat. (14).

II. Material And Methods

Plant Profiles: The Whole plant of *waltheria indica* were collected from 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.

Waltheria Indica(Linn.)



Preparation of Extracts

The powdered materials were successively extracted with ethanol by hot continuous percolation method in Soxhlet apparatus 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. The extracts were suspended in 2% tween 80. (15)

Physicochemical Analysis: The following physicochemical parameters were carried out (WHO, 2002); The Ayurvedic Pharmacopoeia of India 2008 in dried powder and Whole Plant of *Waltheria Indica (Linn)*.

Determination of Total ash

Two grams of crude powder of *Waltheria Indica (Linn.)*, Whole Plants was taken in an evaporating dish and then dried in an oven at 105 ºC till constant weight was obtained. The weight after drying was noted and loss on drying was calculated. The percentage was calculated on the basis of sample taken initially.

Determination of water soluble ash

To the crucible containing the total ash, 25 ml of water was added and boiled for 5 min. The insoluble matter was collected on an ashless filter paper. It was washed with hot water and heated in a crucible for 15 min. Weight of insoluble matter was subtracted from the weight of total ash. The content of water soluble ash was calculated as mg/g of air dried material.

Determination of acid insoluble ash

The 25 ml of hydrochloric acid (70 g/L) was added to the crucible containing total ash. It was covered with a watch-glass and heated gently for 5 min to boil. The watch-glass was rinsed with 5 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ashless filter paper and it was washed with hot water until the filter was neutral. The filter paper containing the insoluble matter was transferred to the original crucible; it was dried on a hot plate and heated till constant weight was obtained. The residue was allowed to cool in a desiccator for 30 min and then weighed without delay. Acid insoluble ash was calculated as mg/g of air dried material.

Qualitative Phytochemical analysis

Qualitative chemical tests were carried out for crude powder and ME, to identify different phyto-constituents (16).

Tests for Alkaloids

1. Dragondroff's Test:

To 1ml of the extract, and 1ml of the Dragondroff's reagent was added, formation of orange red precipitate indicated the presence of alkaloids.

2. Wagner's Test:

To 1ml of the extract, and 1ml of the Wagner's reagent was added, formation of reddish brown precipitate indicated the presence of alkaloids.

3. Mayer's Test:

To 1ml of the extract, and 1ml of the Mayer's reagent was added, formation of full white precipitate confirmed the presence of alkaloids.

Tests for Carbohydrates

1. **Molish Test:**

To 2ml of the extract, and 1ml of the α-napthol solution was added, and concentrated sulphuric acid through the sides of test tupe. formation of Purple or reddish violet colour at the junction of two liquids revealed the presence of carbohydrates.

2. Fehling's Test:

To 1ml of the extract, and equal quantitities of fehling's solution A and B was added, upon heating formation of brick red precipitate indicated the presence of carbohydrates.

3. Benedict's Test:

To 5ml of the Benedict's reagent, and 1ml of the extract solution was added and Boiled for 2 minutes and cooled. formation of red precipitate showed the presence of carbohydrates.

Tests for Proteins and Amino acids

1. Biuret Test:

To 1ml of the extract, and add 1ml of the 40% sodium hydroxide solution was added followed by 2 drops of 1% copper sulphate solution. Formation of a violet colour showed the presence of Protiens.

2. Xanthoprotiens Test:

To 1ml of the extract, and 1ml of the concentrated nitric acid was added, A white precipitate is formed, it is boiled and cooled. 20% of sodium hydroxide or ammonia is subsquently added, Formation of orange colour indicated the presence of aroatic amino acids.

3. Lead Acetate:

To the extract, and 1ml of the lead acetate solution was added, Formation of white precipitate confirmed the presence of proteins.

Tests for Steroids

1. Salkowski Test:

Dissolve the extract in chloroform and equal volue of concentrated sulphuric acid was added. Formation of bluish red to cherry red colour in chloroform layer andd green fluorescence in the acid layer represented the steroid components in the tested extract.

Tests for Glycosides

1. Legal Test:

The extract test was dissolved in pyridine and sodiu nitroprusside solution was added to ake it alkaline. The formation of pink red to red colour showed the presence glycosides.

2. Borntrager's Test:

A few ml of dil HCL was added to 1ml of the extract solution. It was then boiled, filtered and filtrate was extracted with chloform. The chloroform layer was then treated with 1ml of ammonia. The formation of red colour showed the presence of anthraquinone glycosides.

3. Keller Killiani Test:

The extract was dissloved in acetic acid containig traces of ferric chloride and it was then transferred to a test tupe containing sulphuric acid. At the junction, formation of a reddish brown colour, White gradually becoe blue, Confirmed the presence of glycosides.

Tests for Saponins

About 1 ml of ethanolic extract was diluted separately with distilled water to 20 ml, and shaken in a graduated cylinder for 15 minutes. A 1 cm layer of foam indicated the presence of saponins.

Test for Flavonoids

Shinoda Test: To 1 ml of the extract, magnesium turning were added followed by 1-2 drops of concentrated hydrochloric acid. Formation of re colour showed the presence of flavonoids.

Tests for Tannins and Phenolic compounds

1. To 1ml of the extract, and ferric chloride was added, formation of dark blue or greenish black colour product showed the presence of tannins.
2. To 1ml of the extract, and pottasium dichromate solution was added, formation of a precipitate showed the presence of phenolic compounds.

Evaluation of Antioxidant activity by in-vitro Techniques:

Total antioxidant activity (Phosphomolybdic acid method)

The antioxidant activity of the sample was evaluated by the transformation of Mo (VI) to Mo(V) to form phosphomolybdenum complex (17). 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, 28mM sodium phosphate and 4 mM ammonium molybdate). This vials were capped and incubated in a water bath at 95ºc 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 [Ferric ion reducing antioxidant potential

A modified method of 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 $FeCl_3$. 6 H_2o (18). The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml $FeCl_3$ 2.6H2O. The temperature of the solution was raised to 370^{0}C before using. Ethanolic extract(0.15 ml) was 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 measured at 593 nm. The standard curve was linear between 200 and 1000 µM FeSo4. Results were expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Total Flavonoids

0.2g of the ethanolic extract was ground with ethanol-water in 2 different ratios namely 9:1 and 1:1 respectively (19). 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 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 mints. The absorbance was measured at 360 nm. A standard was run by using catechol (110 µg/ml).

III. Results And Discussion

Physicochemical Analysis

Total ash value - 11.47 ± 0.72 %
Water Soluble ash - 4.92 ± 0.14 %
Acid Insoluble ash - 8.35 ± 0.69 %

Phytochemical Analysis

Phytochemical Tests	**Results**
Tests for Alkaloids	
1) Dragondroff's Test:	+ve
2) Wagner's Test	+ve
3) Mayer's Test	+ve
Tests for Carbohydrates	
1) Molish Test	+ve
2) Fehling's Test	+ve
3) Benedict's Test	+ve
Tests for Proteins and Amino acids	
1) Biuret Test	+ve
2) Xanthoprotiens Test	+ve
3) Lead Acetate	+ve
Tests for Steroids	
Salkowski Test	+ve
Tests for Glycosides	+ve
1) Legal Test	+ve
2) Borntrager's Test	+ve
3) Keller Killiani Test	+ve
Tests for Saponins	+ve
Test for Flavonoids	+ve
Tests for Tannins and Phenolic compounds	+ve

Total antioxidant activity (Phosphomolybdic acid method)

The percentage of total antioxidant activity of ethanolic extract of waltheria indica presented in Table 1. The ethanolic extract of waltheria indica exhibited a maximum total antioxidant activity of 67.66 % at 1000 µg/ml whereas for ascorbate (standard) was found to be 54.22 % at 1000 µg/ml. The IC_{50} of the ethanolic extract of *Waltheria indica* and ascorbate were found to be 460µg/ml and 420µg/ml respectively.

Table - 1

S.No	**Concentration (µg/ml)**	**% of activity (±SEM*)**	
		Sample (Ethanolic extract)	**Standard (Ascorbate)**
1	125	21.48 ± 0.041	27.63 0.076
2	250	33.50± 0.062	33.53 0.054
3	500	48.18 ± 0.035	59.12 0.022
4	1000	67.66 ± 0.025	54.00 0.014
		IC_{50} = 460 g/ml	**IC_{50} = 420 g/ml**

FRAP Method

Free radical scavenging activity of the ethanolic extract of waltheria indica (Linn.) was determined by FRAP method. Table - 2, The antioxidant of scavenging activity of free radical potential shown maximum activity is 82.62 at 1000µg/ml for as Standard (Ascorbate) was found to be 98.07 at 1000 µg/ml. The IC_{50} of the ethanol extract of waltheria indica (Linn.) and standard(Ascorbate) was found to be 192µg/ml and 50µg/ml in better antioxidant is respectively.

Table - II

S.No	**Concentration (µg/ml)**	**% of activity (±SEM*)**	
		Sample (Ethanolic extract)	**Standard (Ascorbate)**
1	125	30.47 0.04	72.04 0.014
2	250	43.11 0.02	82.05 0.034
3	500	61.84 0.06	86.04 0.026
4	1000	82.62 0.02	98.07 0.041
		IC_{50} = 192 g/ml	**IC_{50} = 50 g/ml**

Total Flavonoids

Flavonoids present in food of plant origin are also potential antioxidants Most beneficial effects of flavonoids are attributed to their antioxidant and chelating abilities. The flavonoid content of Ethanol extract of whole plant of waltheria indica (Linn.) was presented table 3. Based on the report of Ethanolic extract of whole plant of waltheria indica (Linn.) was found 3.367mg/g of flavonoid compound.

Table - III

S.No	Extract	Total flavonoids content (mg/g) (± SEM)*
1	Ethanolic extract of Waltharia Indica	3.367 ± 0.036

IV. Conclusion

The present study was clearly indicated the ethanolic extract of whole plant of Waltheria indica(Linn.) showed moderate antioxidant activity by total antioxidant activity and FRAP assay when compared with standard ascorbate. In addition, the ethanolic extract of Waltheria indica(Linn.) was found to contain a noticeable amount of flavonoids, which play a major role in controlling antioxidants. In physicochemical study, the parameters such as total ash, acid insoluble ash, water-soluble were carried out. The results of phytochemical analysis suggest that presence of tannins, saponins, flavonoids, steroids, alkaloids, quinones, terpenoids and cardiac glycosides.

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