Research Article

QUANTITATIVE PHYTOCHEMICAL ESTIMATION AND ANTIOXIDANT STUDIES ON AERIAL PARTS OF NARAVELIA ZEYLANICA DC

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ABSTRACT

Naravalia zeylanica DC (Ranunculaceae) is a climbing vine distributed in hilly areas used by the tribal to cure various ailments such as skin diseases rheumatoid arthritis, wounds and ulcer. The plants were identified and collected from kolli hills, Tamilnadu. The present work focused on evaluating phytochemical properties and Anti oxidant properties of the entire plant. In the quantitative estimation of phytoconstituents total phenols, alkaloids, tannin, saponin and flavonoids were estimated for crude powder. The anti oxidant properties of chloroform and ethanolic extract were evaluated by various method such as DPPH free radical scavenging activity, Nitric oxide scavenging activity, FRAP assay (Ferric reducing antioxidant power) activity, Ferric Thiocyanate (FTC) Method and Thiobarbituric Acid (TBA) Method. In the DPPH assay the Ic_{50} value of Chloroform & ethanolic extract 95.2µg/ml & 57.5µg/ml as opposed to that of ascorbic acid 52.3µg/ml. Both the extracts decreased the amount of nitrite generated from the decomposition of sodium nitroprusside. The nitric oxide scavenging assay showed the half maximum inhibitory concentration of ethanolic extract quite equivalent to standard. In ferric reducing antioxidant power assay a linear increase in reducing power was observed over the concentration range 20 – 100 µg/ml of extracts. In FTC and TBA method the results indicated that both extracts possessed anti-oxidant activity but ethanolic extract showed moderate activity. Phytochemical investigation indicated the presence of flavonoids, Phenols and high concentration of tannin in this plant that cause greater reducing power caused by the above constituents.

KEY WORDS Naravalia zeylanica, Quantitative phytochemical estimation, Anti-oxidant

INRODUCTION

Naravalia zeylanica DC (Ranunculaceae) is a woody climber with tuberous roots opposite, ovate, cordate leaflets, small flowers arranged in panicles and red coloured achenes along with long feathery styles, occurring in the hot to warm regions in India[1]. The whole plant is traditionally used in vitiated vata, pitta, inflammation, skin diseases, arthritis, headache, colic, wounds and ulcers[2]. Leaf paste is consumed to treat Chest pain. The vines when crushed give a pungent odour which is inhaled to cure cold, all type of headaches including migraine[3]. From the review the leaf of this plant reported for anti ulcer activity[4]. Based upon the ethnomedical importance, present work focused to reveal the quantitative phytochemical studies and anti-oxidant property of aerial parts of *Naravalia zeylanica.*

MATERIALS AND METHODS

COLLECTION OF PLANT

Naravalia zeylanica DC was collected from Kolli hills of Namakkal district, Tamilnadu, India. The plant was identified and authenticated by National Institute of Herbal Science, Chennai. The aerial part of the plants were collected in the month of August and shade dried.

EXTRACTION

Coarse powder of the plant material was extracted by cold maceratioin method using successive solvents such as petroleum ether, chloroform and ethanol in increasing polarity for 48 hours respectively. The extracts were concentrated and dried under reduced pressure.

QUATITATIVE ESTIMATION OF PHYTOCONSTITUENTS

After the confirmation of presence of phenols, alkaloids, flavonoids, saponins and tannins by preliminary phytochemical tests, the coarse powder of the plant material was taken for quantitative estimation.

Determination of total phenols by spectrophotometric method:

The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was taken into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The samples were made up to mark and left to react for 30 min for colour development[5-6]. This was measured at 505 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l

solutions of gallic acid in methanol: water (50:50, v/v).

Alkaloid determination using Harborne (1973) method:

5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed[7].

Flavonoid determination by the method of Bohm and Kocipai- Abyazan (1994):

10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight[8].

Saponin determination:

The method used was that of Obadoni and Ochuko (2001). 20 g of samples powder was put into a conical flask and 100 ml of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage[9].

Determination of tannins content

Dried plant material (0.5 g) was extracted with 300 ml of diethyl ether for 20 hours at room temperature. The residue was boiled for 2 h with 100 ml of distilled water, and then allowed to cool, and was filtered. The extract was adjusted to a volume of 100 ml in a volumetric flask. The content of tannins in the extract was determined colorimetrically using Folin–Denis reagent, and by measuring absorbance of the blue complex at 760 nm, using tannic acid solution as a standard solution[10].

Table 1: Quantitative Estimation of Phytoconstituents

CONSTITUENT	PERCENTAGE (%w/w)
Alkaloids	0.86±0.023
Total Phenols	0.72±0.012
Tannin	8.72±0.044
Flavonoids	0.56±0.037
Saponins	2.86±0.023

*Each value is presented as mean ± S.E. (n = 3).

ANTI OXIDANT ACTIVITY

After the completion preliminary phytochemical test, chloroform and ethanolic extracts were taken for the anti-oxidant studies.

1. DPPH(2,2-diphenyl-l-picryl hydrazyl) Radical Scavenging Assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging protocol. DPPH solution (0.004% w/v) was prepared in 95% ethanol. A stock solution of chloroform extract, ethanolic extract and standard ascorbic acid were prepared in the concentration of 10mg/100ml (100µg/ml). From stock solution 2ml, 4ml, 6ml, 8ml & 10ml of this solution were taken in five test tubes respectively. With same solvent made the final volume of each test tube up to 10 ml whose concentration was then 20µg/ml, 40µg/ml, 60µg/ml, 80µg/ml & 100µg/ml respectively. 2 ml of freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes. The reaction mixture was incubated in the dark for 15 min and thereafter the optical density was recorded at 523 nm against the blank. For the control, 2 ml of DPPH solution in ethanol was mixed with 10ml of ethanol and the optical density of the solution was recorded after 30 min. The assay was carried out in triplicate. The decrease in optical density of DPPH on addition of test samples in relation to the control was used to calculate the antioxidant activity, as percentage inhibition (%IP) of DPPH radical. The capability of scavenging DPPH radical was calculated using the following equation[11-13]

$$\text{DPPH Scavenged (\%)} = \frac{(\text{A control} - \text{A test})}{(\text{A control})} \times 100$$

Where "A control" is the absorbance of the control reaction and "A test" is the absorbance of the sample of the extracts. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

2. Nitric oxide scavenging activity assay

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously

generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 M sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of chloroform and ethanolic extracts at various concentrations and the mixture incubated at 25 o C for 2hrs. From the incubated mixture 0.5 ml was taken out and added into 1ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. finally, 1ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radicals scavenging activity was calculated using the same above equatuion[14-15].

3. Ferric reducing antioxidant power (FRAP Assay)

In ferric reducing antioxidant power assay, 1 ml of test sample of chloroform & ethanolic extract in different concentration were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes. The reaction mixtures were incubated in a temperature-controlled water bath at 50^0C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 µl of 0.1% FeCl3. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A_{700} after blank subtraction[16].

4. Ferric Thiocyanate (FTC) Method

The standard method as described by Kikuzaki and Nakatani was used[17]. A mixture containing 4 mg of the chloroform & ethanolic extract in 4 ml of 99.5% ethanol (final concentration 0.02%). 4.1ml of 2.52% linoleic acid in 99% ethanol, 8 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with screw cap and then placed in an incubator at 40^0 C in the dark separately. To 0.1 ml of this mixture 9.7 ml of 75% ethanol (v/v) and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes later the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to reaction mixture; (the absorbance of red color indicated the antioxidant activity) was measured at 500 nm for every 24 hours until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was used, and for the standard 4mg of the sample was replaced by 4 mg of Vitamin C.

5. Thiobarbituric Acid (TBA) Method

TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100^0C), melonaldehyde binds with TBA to form a red complex that can be measure at 532 nm. 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% TBA solutions were added to 2 ml of the mixtures containing the sample prepared in the FTC method. This mixture was kept in water bath (100^0C) for 10 minutes and after cooling to room temperature, was centrifuged at 3000 rpm for 20 minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay. The percentage of antioxidant activity was calculated by following formulae for both FTC and TBA[18].

$$\text{PERCENTAGE OF ACTIVITY} = \frac{\text{Absorbance of (Control – Test)}}{\text{Absorbance control}} \times 100$$

Table 2: DPPH Radical Scavenging Activity

Concentration (µg/ml)	Chloroform Extract		Ethanolic Extract		Standard (Ascorbic acid)	
	% Inhibition	IC_{50} µg/ml	% Inhibition	IC_{50} µg/ml	% Inhibition	IC_{50} µg/ml
20	15±0.33		21±0.88		24±0.33	
40	25±0.28		36±0.23		43±0.17	
60	33±0.13	95.2	56±0.18	57.5	58±0.23	52.3
80	42±0.23		69±0.42		71±0.35	
100	53±0.67		82±0.56		84±0.76	

*Each value is presented as mean ± S.E. (n = 3).

**The IC50 was obtained by linear regression equations.

Table 3: Nitric Oxide Scavenging Activity Assay

Concentration (µg/ml)	Chloroform Extract		Ethanolic Extract		Standard (Ascorbic acid)	
	% Inhibition	IC_{50} µg/ml	% Inhibition	IC_{50} µg/ml	% Inhibition	IC_{50} µg/ml
20	16±0.23		22±0.43		24±0.36	
40	26±0.40		34±0.33		40±0.26	
60	33±0.20	92.1	56±0.32	51.6	57±0.36	54.2
80	44±0.23		67±0.36		70±0.46	
100	53±0.43		82±0.53		84±0.47	

*Each value is presented as mean ± S.E. (n = 3).

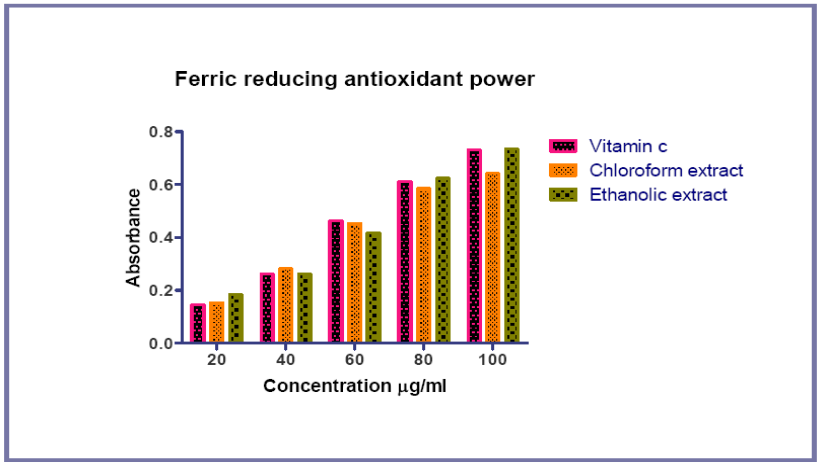
**The IC50 was obtained by linear regression equations

Table 4: Anti-oxidant Activity at the Final Day for FTC and TBA Method

EXTRACT	PERCENTAGE OF ACTIVITY	
	FTC	TBA
VITAMIN C	82.10	84.65
CHLOROFORM EXTRACT	75.68	72.36
ETHANOLIC EXTRACT	80.21	85.15

*Each value is presented as mean ± S.E. (n = 3).

Figure 1: FRAP Assay



RESULTS AND DISCUSSION

From the earlier report knew that *Naravalia zeylanica* DC was traditionally used for many diseases particularly the aerial parts of the plants were utilized in various skin diseases. The plant was identified and authenticated botanically. The aerial parts were shade dried and the coarse powder was extracted by cold maceration method using petroleum ether, chloroform and ethanol successively. All the extracts were concentrated under reduced pressure and the extractive values were also calculated. (Petroleum ether extract 7.52w/w %, Chloroform extract 9.16w/w% and Ethanol extract 16.12w/w %). Based upon the preliminary phytochemical test Quantitative determination phytoconstituents were carried out for the powdered plant material by various standard methods and found that alkaloid 0.86%w/w, total phenol 0.72%w/w, tannin 8.72%w/w, flavonoids 0.56%w/w and saponin 2.86%w/w were present in the aerial parts this plant(Table no 1).

On the basis phytochemical investigation chloroform and ethanolic extract were chosen for the anti-oxidant studies.

DPPH radical scavenging activity of chloroform and ethanolic extracts of aerial parts of *Naravalia zeylanica* and ascorbic acid are presented in table no2. The half maximal inhibitory concentration (IC_{50}) of chloroform extract, ethanolic extract and ascorbic acid were found to be 95.2µg/ml, 57.5µg/ml and 52.3µg/ml respectively. The ethanolic extract has profound reducing activity against stable free radicals.

The nitric oxide scavenging assay showed the half maximum inhibitory concentration of ethanolic extract quite equivalent to standard. The result indicated that the extracts might contain compounds able to inhibit nitric oxide and offers scientific evidence for the use of the plant in inflammatory condition (Table no 3).

In the FRAP assay a linear increase in reducing power was observed over the concentration range 20 – 100 µg/ml sample and ascorbic acid (Figure no 1). The ethanolic extract has potent reducing power and equivalent to ascorbic acid.

In FTC and TBA method the total anti-oxidant activity exhibited by the extract and standard were shown in table no 4. The results indicated that both extracts possessed anti-oxidant activity but ethanolic extract showed moderate as standard (Table no 4). The antioxidant activity has been attributed to various mechanisms such as prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging and the reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[19-20]

Phytochemical investigation indicates the presence of flavonoids, Phenols and high concentration of tannin in this plant. So the greater reducing power caused by the presence of flavonoids, hydrophilic polphenols and tannins.

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