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Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Indoneesiella echioides* (L.) Nees Leaves

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ABSTRACT

Indoneesiella echioides (or) *Andrographis echioides* (L) Nees is an important herb widely distributed in south India. This is commonly known as False Water willow. *Indoneesiella echioides* (L) Nees is a traditional Indian medicine; the whole plant is highly medicinal value such as the leaf juice of this plant is used to cure fever. The plant was extracted using n-hexane, chloroform, ethyl acetate, acetone, butanol, ethanol and methanol. The present study was to be determined the preliminary phytochemical screening, antioxidant and antimicrobial activities of *Indoneesiella echioides* (L) Nees leaves.

***Keywords*:** *Indoneesiella echioides,* Antioxidant activity, Antimicrobial activity, Phytochemical screenings

1. INTRODUCTION

Indoneesiella echioides (L) Nees (Acanthaceae), is also known as *Andrographis echioides* (L) Nees. This is commonly known as False Water Willow, is an abundantly growing in south India. *Indoneesiella echioides* (L) Nees is highly medicinal important.

The species of *Indoneesiella* is used in goitre, liver diseases [1], fertility problems, bacterial [2], malarial and fungal disorders. The leaf juice of this plant is used to treating fevers [3]. Several *Indoneesiella* species (about 40 species) has been used in treatment of influenza, malaria, dyspepsia and respiratory diseases. The *Indoneesiella* species also used to antidote for poisonous stings of some insects [4,5]. The leaf juice is mixed and boiled with coconut oils used to control falling and greying of hair [6]. Phytochemistry of *Indoneesiella echioides* has been investigated and reported to contain several flavonoids [7,8] and labdane diterpenoids [9-14].

In previous literatures are reported to only flavonoids as major components in *Indoneesiella echioides* (L) Nees extracts [15-18]. It has been reported that variety of phytoconstituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthene's [19]. Hence the present investigation was carried out to determine the preliminary phytochemical compounds, antioxidant, and antimicrobial activities (Photos 1 & 2).

Photo 1. *Indoneesiella echioides* (L).

Photo 2. *Indoneesiella echioides* (L) – flower.

2. MATERIALS AND METHODS

2. 1. Collection of plant materials

The leaves of *Indoneesiella echioides* (L) Nees was collected from Poondi village, Thanjavur District, Tamil Nadu. The botanical identity of the plant of was confirmed by Dr. S. John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli.

2. 2. Preparation of Extracts

The fine powder (5 Kg) was extracted with 95% ethanol at room temperature for ten days. The extract was filtered and concentrated under reduced pressure in a rotary evaporator and extracted for various solvents in increasing order of polarity from n-Hexane, Chloroform, Ethyl acetate, Acetone, Butanol, Ethanol and Methanol. The yields of fractions are dried and weighed. The dried fractions of ethanol, methanol and crude extracts were subjected to screening of phytochemical and antioxidant, antimicrobial activity.

2. 3. Phytochemical screening

The preliminary phytochemical analysis of *Indoneesiella echioides* (L) Nees was carried out as per standard methods (Table 1).

2. 4. *In - vitro* antioxidant activity (DPPH Method)

1,1-Diphenyl-2-Picryl-hydrazyl (DPPH) is free radical but stable [20,21]. DPPH is violet in colour which donates a hydrogen ion in to the solution [22]. The colour change is maintained by spectrophotometer and free radical scavenging activity is calculated. The method described by Shimada *et al*., 1992; [23] was used. Stock solution of 25 µg/mL of DPPH in methanol was made.

Different concentrations of *Indoneesiella echioides* leaf extract (20, 40, 60 and 80 µg/mL) were chosen for *in-vitro* antioxidant activity. L-Ascorbic acid was used as the standard. Briefly, a 2 mL aliquot of DPPH methanol solution (25 µg/mL) was added to 0.5 mL sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517 nm in a (UV 1800 – SHIMADZU) spectrophotometer.

Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity and % scavenging was calculated by the following equation.

$$\text{Radical scavenging activity (\%)} = 100 - \frac{A_C - A_S}{A_C} \cdot 100$$

where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

Statistical analysis

Tests were carried out in triplicate for 3-5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a non-linear regression algorithm.

Table 1. Preliminary phytochemical constituents of *Indoneesiella echioides* (L) Nees leaves.

S. N.	Phytochemicals	Hexane Extract	Chloroform Extract	Ethyl acetate Extract	Acetone Extract	Ethanol Extract	Butanol Extract	Methanol Extract
1.	Alkaloids	-	-	-	Present	Present	-	-
2.	Flavonoids	-	-	-	-	-	-	-
3.	Terpenes	Present	Present	-	-	-	-	-
4.	Triterpenoid saponins	-	Present	Present	Present	-	-	-
5.	Saponins	-	Present	Present	Present	Present	Present	Present
6.	Glycosides	-	-	-	-	-	-	-

7.	Steroids	Present	Present	Present	Present	-	-	-
8.	Carbohydrates	-	-	-	-	-	-	-
9.	Phenolic compounds	Present	Present	Present	Present	-	Present	Present
10.	Tannins	-	-	-	-	-	-	-
11.	Amino acids	-	-	Present	Present	Present	-	Present

Table 2. DPPH radical scavenging activity of ethanolic extract of *Indoneesiella echioides* (L) Nees leaves.

S. N.	Concentrations (µg / mL)	Ethanolic extract	Ascorbic acid (Standard)
1	20	14.09 ±0.98	25.6 ±2.04
2	40	31.81 ±2.22	61.26 ±4.90
3	60	61.81 ±4.32	88.98 ±7.11
4	80	72.77 ±5.09	99.34 ±7.94
	IC_{50}	54.74	35.03

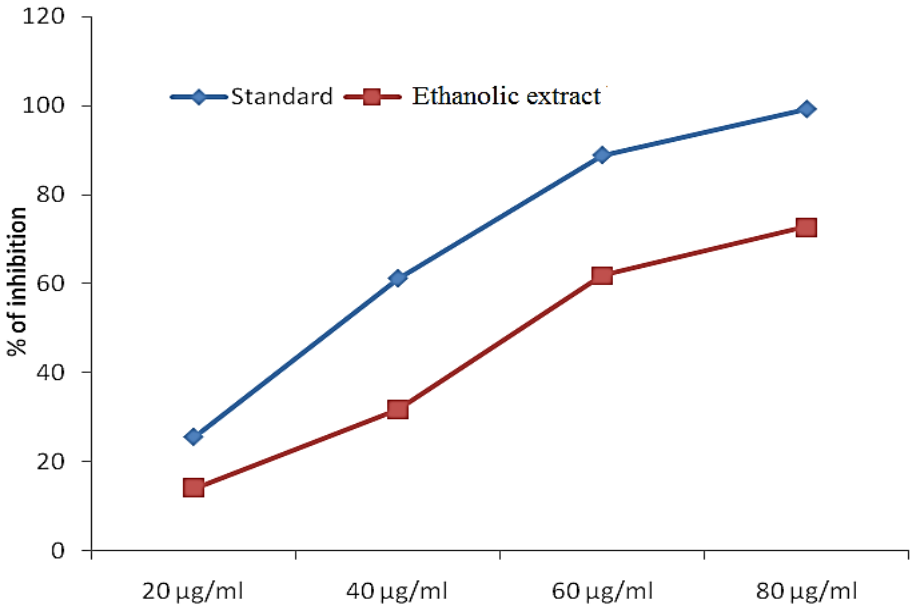

Fig. 1. Comparison of % scavenging of DPPH by ascorbic acid *vs* ethanolic extract at different concentrations.

Table 3. DPPH radical scavenging activity of methanolic extract of *Indoneesiella echioides* (L) Nees leaves.

S. N.	Concentrations (µg / mL)	Methanolic extract	Ascorbic acid (Standard)
1	20	9.95 ±0.69	25.6 ±2.04
2	40	27.81 ±1.94	61.26 ±4.90
3	60	45.4 ±3.17	88.98 ±7.11
4	80	64.5 ±4.51	99.34 ±7.94
	IC_{50}	64.45	35.03

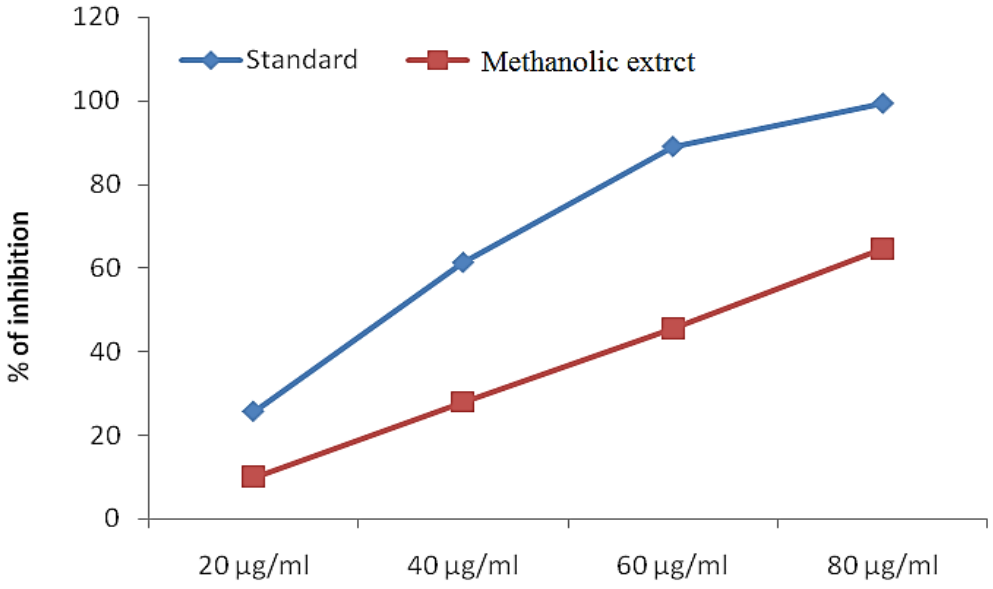

Fig. 2. Comparison of % scavenging of DPPH by ascorbic acid *vs* methanolic extract at different concentrations.

Table 4. DPPH radical scavenging activity of crude extract of *Indoneesiella echioides* (L) Nees leaves.

S. N.	Concentrations (µg / mL)	Crude extract	Ascorbic acid (Standard)
1	20	22.72 ±1.46	25.6 ±2.04
2	40	40.20 ±2.86	61.26 ±4.90

3	60	63.62 ±4.13	88.98 ±7.11
4	80	86.36 ±5.45	99.34 ±7.94
	IC_{50}	47.06	35.03

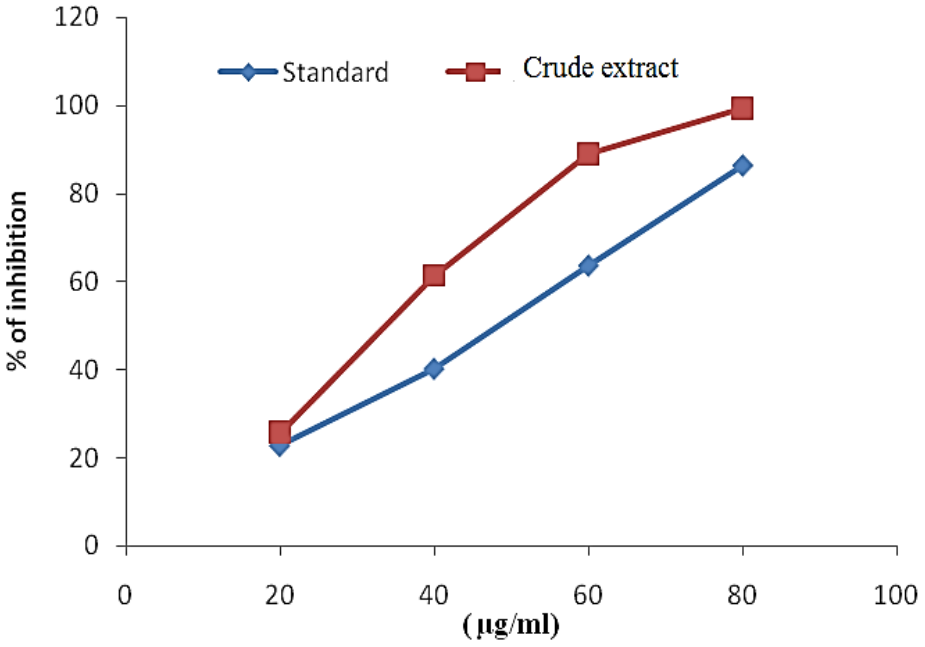

Fig. 3. Comparison of % scavenging of DPPH by ascorbic acid *vs* crude extract at different concentrations.

2. 5. *In-vitro* antimicrobial activity (Disc diffusion method)

The ethanol, methanol and crude extracts were prepared in various concentrations such as 50, 100, 150 µg/mL respectively and used for antimicrobial activity.

2. 5. 1. Test microorganisms

Pure cultures of *Escherichia coli*, *Staphylococcus aureus* (Gram positive bacteria), *Pseudomonas aeruginosa*, *Proteus vulgaris* (Gram negative bacteria) specie of bacteria's and *Candida albicans*, *Aspergillus flavus* specie, of fungi's were procured from *Rontgen Laboratory*, Thanjavur. These microorganisms were identified and confirmed by Microbiologists, Department of Microbiology, Thanjavur Medical College, Thanjavur.

2. 5. 2. Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10 mL of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37 °C for 24 hours except for fungal which was incubated at 25

°C for 24 - 48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8 °C until use.

2. 5. 3. Preparation of plant extracts solutions for the experiment

The dried *Indoneesiella echioides* (L) Nees plant extract was weighed (10 mg/mL) and dissolved in sterile distilled to prepare appropriate dilution to get required concentrations of about 50 µL (50 µg), 100 µL (100 µg) and 150 µL (150 µg). Control used as respective solvent (Aqueous). They were kept under refrigerated condition unless they were used for the experiment. Standard solution as Chloramphenicol for bacteria and fluconazole (25 mg/mL distilled water - 30 µL) for fungi used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

2. 5. 4. Preparation of dried filter paper discs

Whattman filter paper (No: 1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, the discs were loaded with different concentrations of prepared plant extract solutions and again kept under refrigeration for 24 hours.

2. 5. 5. Application of discs to inoculated agar plates

Previously prepared paper discs were dispensed onto the surface of the inoculated agar plate. Each disc was pressed down firmly to ensure complete contact with the agar surface. The discs were placed on the medium suitably apart and the plates were incubated at 5 °C for 1 hour to permit good diffusion and then transferred to incubator at 37 °C for 24 hours. After completion of 24 hours, the plates were inverted and placed in an incubator set to respective temperature for 24 hours.

2. 6. Antimicrobial assay

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka et al., 2007) [24] using plant extracts. Petri plates were prepared by pouring 30 mL of NA/PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 minutes.

The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing *Escherichia coli*, *Staphylococcus aureus* (Gram positive bacteria), *Pseudomonas aeruginosa*, *Proteus vulgaris*(Gram negative bacteria) were spread on Nutrient agar plates for bacteria and *Candida albicans*, *Aspergillus flavus* were spread on potato dextrose agar for fungus strains.

Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50 µL, 100 µL and 150 µL) were laid down on the surface of inoculated agar plate.

The plates were incubated at 37 °C for 24 hours for the bacteria and 48 hours for fungus at room temperature (30 ±1) for 24-48 hour for yeasts strains. Each sample was tested in triplicate.

Table 5. Antibacterial activity of *Indoneesiella echioides* (L) Nees plant leaves against *Escherichia coli* bacteria at different concentrations.

Samples	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Chloramphenicol for bacteria) (mm)	Control (solvent)
Crude extract	7 ±0.49	8 ±0.56	10 ±0.70	12 ±0.84	0
Ethanolic extract	7 ±0.49	6 ±0.42	9 ±0.63	14 ±0.98	0
Methanolic extract	5 ±0.35	7 ±0.49	10 ±0.70	15 ±1.05	0

Values are expressed as Mean ± SD for triplicate.

Table 6. Antibacterial activity of *Indoneesiella echioides* (L) Nees plant leaves against *Staphylococcus aureus* bacteria at different concentrations.

Samples	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Chloramphenicol for bacteria) (mm)	Control (solvent)
Crude extract	8 ±0.56	9 ±0.63	10 ±0.70	15 ±1.05	0
Ethanolic extract	5 ±0.35	11 ±0.77	12 ±0.84	11 ±0.77	0
Methanolic extract	6 ±0.42	10 ±0.70	11 ±0.77	13 ±0.91	0

Values are expressed as Mean ± SD for triplicate.

Table 7. Antibacterial activity of *Indoneesiella echioides* (L) Nees plant leaves against *Pseudomonas aeruginosa* bacteria at different concentrations.

Samples	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Chloramphenicol for bacteria) (mm)	Control (solvent)
Crude extract	8 ±0.56	9 ±0.63	10 ±0.70	15 ±1.05	0
Ethanolic extract	8 ±0.56	14 ±0.33	18 ±0.57	11 ±0.77	0
Methanolic extract	5 ±0.35	6 ±0.42	8 ±0.56	13 ±0.91	0

Values are expressed as Mean ± SD for triplicate.

Table 8. Antibacterial activity of *Indoneesiella echioides* (L) Nees plant leaves against *Proteus vulgaris* bacteria at different concentrations.

Samples	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Chloramphenicol for bacteria) (mm)	Control (solvent)
Crude extract	9 ±0.63	10 ±0.70	12 ±0.84	15 ±1.05	0
Ethanolic extract	8 ±0.56	12 ±0.84	15 ±1.05	11 ±0.77	0
Methanolic extract	5 ±0.35	6 ±0.42	8 ±0.56	13 ±0.91	0

Values are expressed as Mean ± SD for triplicate.

Table 9. Anti – fungal activity of *Indoneesiella echioides* (L) Nees plant leaves against *Candida albicans* fungus at different concentrations.

Samples	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Nystatin for fungi) (mm)	Control (solvent)
Crude extract	6±0.42	7±0.49	8±0.56	12±0.84	0
Ethanolic extract	6±0.42	9±0.63	12±0.84	15±1.05	0
Methanolic extract	5±0.35	7±0.49	9±0.63	12±0.84	0

Values are expressed as Mean ± SD for triplicate.

Table 10. Anti – fungal activity of *Indoneesiella echioides* (L) Nees plant leaves against *Aspergillus flavus* fungus at different concentrations.

Samples	50 µL (mm)	100 µL (mm)	150 µL (mm)	Standard (Nystatin for fungi) (mm)	Control (solvent)
Crude extract	5 ±0.35	6 ±0.42	9 ±0.63	13 ±0.91	0
Ethanolic extract	5 ±0.35	7 ±0.49	12 ±0.84	14 ±0.98	0
Methanolic extract	-	6 ±0.42	8 ±0.56	11 ±0.77	0

Values are expressed as Mean ± SD for triplicate.

3. RESULT

A. The ethanolic extract of the *Indoneesiella echioides* (L) Nees revealed the presence of alkaloids, terpenes, triterpenoids saponins, saponins, phenolic compound, steroids, and aminoacids. These phytochemicals were found to be dihydroechioidinin, along with four unknown flavones, echioidinin, echioidin, skullcapflavone I 2'-*O*-methyl ester and skullcap flavone I 2'-*O*-glucoside [19].

B. From the observation of DPPH radical scavenging activity of ethanol, methanol and crude extracts of *Indoneesiella echioides* (L) Nees is a concentration dependent manner and the result was found to be highest % scavenging activity of ethanol, methanol and crude extracts is 72.77%, 64.5%, and 86.36 % and IC_{50} value of the extracts were found to be 54.74, 64.45 and 47.06 and that of ascorbic acid was 35.03. This study clearly indicates that the leaves of *Indoneesiella echioides* (L) Nees extracts was higher in antioxidant potential in DPPH assay method. On the basis of our results, *Indoneesiella echioides* (L) Nees appears to have potential for treatment of oxidative stress related diseases.

C. Result obtained in the present study the antimicrobial activity of the *Indoneesiella echioides* (L) Nees plant leaves extracts are shown table 5 to 10. The result shows *Indoneesiella echioides* (L) Nees plant extract was effective against both antibacterial and anti-fungal activities.

For antibacterial activity the highest activity was recorded as the crude and methanol extracts of *Indoneesiella echioides* against *Escherichia coli* at diameter of zone inhibition by 10 mm at 150 µL, for *Staphylococcus aureus* bacteria the highest zone of inhibition was observed by 12 mm at 150 µL for methanolic extract, and *Pseudomonas aeruginosa* bacteria the highest zone of inhibition was observed by 18 mm at 150 µL for ethanolic extract, and *Proteus vulgaris* bacteria the highest zone of inhibition was demonstrated by 15 mm at 150 µL for ethanolic extract, when compared with chloramphenicol as standard.

For anti-fungal activity the highest activity was demonstrated by the ethanolic extracts of *Indoneesiella echioides* against *Candida albicans* at diameter zone of inhibition 12 mm at 150 µL, and *Aspergillus flavus* fungi, the highest activity was observed by 12 mm at 150 µL for ethanolic extract respectively, when compared with nystatin as standard.

4. CONCLUSION

In the present study ethanol, methanol and crude extract of *Indoneesiella echioides* (L) Nees leaves indicate that maximum phytochemicals were observed. Such as carbohydrates, glycosides, phenolic compounds, sterols, tannins, flavonoids and cumarins. With a wide spectrum of inhibition against both gram positive and negative bacteria and higher antioxidant potential in DPPH assay. Hence the plant leaves of *Indoneesiella echioides* (L) Nees are worthy for further investigation as used as a natural drugs developments.

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