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Effects of antioxidant and antibacterial activities of flavonoid extract of *Andrographis echioides*

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Abstract
The present study assessed the antioxidant and antibacterial activities of flavonoid extract of *Andrographis echioides* leaves. The antioxidant activity was determined by ABTS radical lipid peroxidation inhibition, superoxide scavenging and metal chelating assay and also antibacterial activity was evaluated by the disc diffusion method. The flavonoid extract of *A. echioides* showed high antioxidant activity ABTS (76.32%), lipid peroxidation inhibition (78.23), superoxide scavenging (71.23%) and had antibacterial activity against both Gram positive and Gram negative bacteria. Higher inhibition zone was detected against *S. aureus* (17.2 mm). The extract of this plant showed high phenolic, flavonoids contents and it can be concluded that these compounds may be responsible for antioxidant and antibacterial activity. The results indicated that the flavonoid extract of *Andrographis echioides* possessed strong antibacterial and antioxidant properties and could be an important source of natural compounds for development of new drugs.

Keywords: antioxidant; antibacterial; flavonoid extract and *Andrographis echioides*

Introduction
Plants have engage in recreation a momentous part in providing the human fight with remedies. Herbal performance chief roles in human lifespan not only as providers of oxygen but also as an essential source to withstand the human contest on this earthly plane. Plants also produce essential nutrition by changing energy from the sun during photosynthesis. In addition, plants have been used extensively in traditional remedy meanwhile time long-established. Therapeutic value of uncommon some plants have been stated but a decent number of floras still used by local folklore are yet to be discovered. In Indian medicine like Siddha and Ayurveda systems of medicine be responsible for good base for systematic consideration of remedially essential molecules from nature. Antioxidants play a significant part in hindering and scavenging free radicals, thus provided that defense to humans against contaminations and wasting diseases; however, fresh concern has been dominant concerning the possible damaging side effects of artificial additives in humans (Mercy *et al*., 2018) [9]. The possible of the antioxidant metabolites of plant materials for the conservation of health and defense from coronary heart disease and cancer is also levitation attention among experts and food manufacturers as regulars move near purposeful foods with specific health effects (Huang *et al*., 2011) [6]. The anti-oxidative outcome is mostly due to plant contained such a group of phenolic components, flavonoids, and anthocyanin. In recent times there has been an increase of attention in the beneficial capacities of medicinal plants as antioxidants in plummeting such free radical tempted tissue injury. Furthermore, information and solicitation of some probable antioxidant undertakings in reducing oxidative stresses *in vivo* has incited many detectives to search for strong and gainful antioxidants from various plant bases (Boulekbache *et al*., 2013) [2]. *Andrographis echioides* is an important medicinal plant and extensively used entire world. Which, belongs to the family Acanthaceae. *A. echioides* is used as a traditional herbal medicine in India, and it is well known uses for the treatment of snake bite, bug bite, diabetes, dysentery, fever. In the Siddha and Ayurvedic medicines, *A. echioides* is one of the frequently used plants. In current eras, profitable arrangements of this plant extracts are used in certain countries. Nevertheless, the arrangements up till now need to be standardized for their better effectiveness. The aerial part of *A. echioides* contain diterpenoids, diterpene glycosides, lactones, flavonoids, and flavonoid glycosides.

Materials and Methods
Plant materials
The leaves of *Andrographis echioides* were collected from Government siddha medical college, herbal garden, Arumbakkam, Chennai, Tamilnadu, during August 2021 and it was taxonomically identified and authenticated as leaves of *Andrographis echioides* by Dr. S. Sankaranarayanan, Head, Department of Medicinal Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106, Tamilnadu. A voucher specimen was deposited in the herbarium for future reference (Ref.No. MB/2021/Ceasal-365).

Preparation of extracts
The leaves of *A. echioides* was carefully cleaned, dried out under the shade and powdered. The extract of flavonoid was prepared according to a previously reported method Kumarappan *et al*. (2012).

Phytochemical screening
The Phytochemical screening exposed to the aqueous leaves extract of *A. echioides* to regulate and determine by using standard procedures (Harborne 1973) to find out secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973).

Thin layer chromatography

By using the standard procedures (Harborne 1973) Thin layer chromatography of flavonoid extract of *Andrographis echioides* was performed. The flavonoid extract was positioned cautiously in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The placed spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer.

ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical scavenging assay

ABTS radical scavenging activity of flavonoid extract of *Andrographis echioides* was followed by Re *et al*. (1999). ABTS radical was newly prepared by addition 5 ml of 4.9 mM potassium persulfate solution to 5 ml of 14 mM ABTS solution and kept for 16 h in dark. This solution was watered down with distilled water to produce an absorbance of 0.70 at 734 nm and the same was used for the antioxidant activity. The final solution of standard group was made up to 1 ml with 950 µl of ABTS solution and 50 µl of Ascorbic acid. Correspondingly, in the experiment group, 1 ml reaction mixture encompassed 950 µl of ABTS solution and 50 µl of different concentration of each extracts. The reaction mixture was vortexed for 10 s and after 6 min, absorbance was recorded at 734 nm against distilled water by using a Deep Vision (1371) UV–Vis Spectrophotometer and compared with the control ABTS solution.

Inhibition of lipid peroxidation activity

In the method of Ohkawa *et al* (1979) in the egg yolk Lipid peroxidation was induced by Fe^{2+}ascarbate system was assessed as thiobarbituric acid reacting substances (TBARS). The experimental mixture contained 0.1 ml of egg yolk (25% w/v) in Tris-HCl buffer and different concentrations of flavonoid extract of *Andrographis echioides* in a final volume of 0.5 ml. The experimental mixture was incubated at 37°C for 1 h. After the incubation period the absorbance of butanol-pyridine layer was recorded at 532 nm in Deep Vision (1371) UV–Vis Spectrophotometer) to quantify TBARS.

Superoxide radical scavenging assay

This assay was grounded on the capacity of the flavonoid extract of *Andrographis echioides* to inhibit the photochemical reduction of Nitroblue tetrazolium (NBT) in the presence of the riboflavin-light-NBT system (Tripathi and Pandey Ekta, 1999; Tripathi and Sharma, 1999) [16].

Nitric oxide radical scavenging activity

The flavonoid extract of *Andrographis echioides* treated with Nitric oxide scavenging ability was measured according to the method described by Olabinri *et al*. (2010) [12]. 0.1 ml of sodium nitroprusside (10 mM) in phosphate buffer (0.2 M, pH 7.8) was mixed with different concentration of extracts and incubated at room temperature for 150 min. After treated period, 0.2 ml of Griess reagent was added. The absorbance of the experimental sample was read at 546 nm against blank.

Metal chelating activity

The Activity of metal chelating capacity of flavonoid extract of *Andrographis echioides* was measured according to Iihami *et al*., (2003) [7]. 1 ml of different concentrations of flavonoid rich fraction was added to 0.05 ml of 2 mM ferric chloride solution. The reaction was began by the addition of 0.2 ml of 5 mM Ferrozine and the mixture was shaken vigorously. After 10 min, the absorbance was measured at 562 nm against blank. All readings were taken in triplicate and ascorbic acid was used as standard. The % inhibition of ferrozoine-Fe2+complex was calculated by following equation.

$$\% \text{ Inhibition of ferrozoine-Fe2+complex} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of control and A_1 was the absorbance of flavonoid rich fraction.

Culture collection and maintenance

The bacterial strains of *Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*. These standard strains were acquired from gene bank (MTCC); Institute of Microbial Technology, Chandigarh, India and Microbial Type Culture Collection.

Antibacterial activity

The evaluation of the antibacterial activities of the flavonoid extricate of *A. echioides* were proved using the disc diffusion method (Bajalan *et al*., 2017). On Mueller Hinton agar Bacteria were placed grown overnight plates, five colonies were suspended in 5 ml of sterile saline (0.9%) and the bacterial population in the suspension was adjusted to ~$3x10^8$ CFU/ml. The different concentration of flavonoid extricate of *A. echioides* was presented on to each plate and the control plate gotten as it were 7%. The plates were incubated at 37°C for 24 h and the restraint zone was measured and calculated.

Statistical analysis

All the tests were exhausted triplicate. The SPSS program adaptation 20 was utilized for information investigation. The results are communicated as mean values with standard deviation (±SD) from three experiments. The exploratory information gotten were analyzed for different comparisons utilizing one-way analysis of variance (ANOVA) and when the comes about were significant, Duncan's test was too utilized.

Result and Discussion

Phytochemical analysis

The phytochemical screening of aqueous leaves extract of *Andrographis echioides* studied presently showed the presence of alkaloids, flavonoids, polyphenol, terpenoids, and absence of glycosides and tannin (Table -1).

Table 1: Phytochemical screening of aqueous leaves extract of *Andrographis echioides*

Sl. No.	Phytochemical Constituents	Observation	Aqueous leaves extract *Andrographis echioides*
1	Alkaloids-Dragendorff's Test-Mayers test	Orange / red precipitate Yellow or white precipitate	- +

2.	Flavonoids-Alkalai Reagent-Lead acetate test	Intense yellow colour Precipitate formed	- +
3.	Glycosides Keller-Killiani test	Reddish brown colour ring formed	-
4.	Tannin-$FeCl_3$ test	Blue black coloration	-
5.	Saponins-Frothing test	Foam	+
6.	Terpenoids-*Salkowski test*	Dark reddish brown color in interface	-
7.	Polyphenols-Ferrozine test	Raddish blue	+
8.	Anthocyanin test Ammonia	Ammonia layer yellow in color	+

+ indicate positive result; -- Indicate negative result

Free radical-scavenging ability

The antiradical movement of clears out, as the agent of dietary food source, were surveyed in vitro by ABTS assay, as well as by assessment of potential to decoloration of ABTS. In table-2 the comes about of antioxidant activity obtained for tested, as well as Vitamin-C utilized as standard are appeared. It can be clearly seen that flavonoid extract of Andrographis echioides shown striking antioxidant movement 76.32%, altogether higher than Vitamin-C. Be that as it may, in show consider appeared that these exercises were basically due to nearness of flavonoid compounds. Indeed in spite of the fact that the ABTS radical has slight importance to organic frameworks and living beings, this procedure is broadly watched as uncovering of the capacity of plant extricates to rummage free radical, and will say to hydrogen particle or electron gift capacity, independently of any enzymatic action (Mileva *et al*., 2014).

Table 2: Free radical-scavenging ability by flavonoid extract of *Andrographis echioides*

Different concentration of extract	Percentage of ABTS radical activity	
	Flavonoid extract of *Andrographis echioides*	**Standard Vitamin-C**
25 µl/ml	18.34±1.37	16.34±1.78
50 µl/ml	34.56±1.69	31.25±1.56
75 µl/ml	52.34±2.56	48.37±2.45
100 µl/ml	76.32±1.34	71.32±1.34
EC_{50} value	61.34	65.34

[a]Results are expressed as percentage inhibit of ABTS ability with respect to control. Each value represents the mean+SD of three experiments

Inhibition of lipid peroxidation activity

Within the show think about, egg yolk was used as substrate for free radical intervened lipid peroxidation, which may be a non-enzymatic method. Flavonoid extricate of A. echioides clears out too repressed the lipid peroxidation induced by ferrous sulfate in egg yolk homogenates. Maximum inhibition was recorded in flavonoid extricate of A. echioides 78.23% with EC_{50} esteem 60.21 µl/ml and least inhibition percentage ascorbic acid 73.64% with EC50 63.32 (Table-3).

Ordinarily, the mechanism of flavonoid compounds for neutralizing lipid free radicals and anticipating deterioration of hydroperoxides into free radicals (Parejo *et al*., 2002).

Table 3: Inhibition of lipid peroxidation activity of flavonoid extract of *Andrographis echioides*

Different concentration of extract	Inhibition percentage of Lipid peroxidation	
	Flavonoid extract of *Andrographis echioides*	**Standard Vitamin-C**
25 µl/ml	22.34±0.89	20.31±2.34
50 µl/ml	44.32±1.23	39.32±0.25
75 µl/ml	61.34±1.34	58.32±1.45
100 µl/ml	78.23±1.45	73.64±0.23
EC_{50} value	60.21	63.32

[a] Results are expressed as percentage inhibit of lipid peroxidation with respect to control. Each value represents the mean+SD of three experiments.

Superoxide scavenging activity

Flavonoid extract of *A. echioides* clears out shown effective rummaging action for superoxide radicals in a concentration dependent process than positive control. Flavonoid extract of *A. echioides* appeared most noteworthy radical movement within the rate of 71.23% with EC_{50} esteem 62.31 µl/ml when compared to positive control 67.23% with EC_{50} Esteem 68.45 µl/ml (Table-4). One of the standard method to create Superoxide radicals is through photochemical decrease of nitro blue tetrazolium (NBT) within the nearness of a riboflavin-light-NBT framework. Superoxide radical is known to be a really destructive species to cellular components as an antecedent of more responsive specie. The superoxide radical is known to be created in vivo and can result within the arrangement of hydrogen peroxide by means of dismutation response (Liyana *et al*., 2006). The result clearly shows that the plant additional super oxide scavenging activity.

Table 4: Superoxide scavenging activity of flavonoid extract of *A. echioides*

Different concentration of extract	Percentage of Superoxide scavenging activity	
	Flavonoid extract of *A. echioides*	**Standard Vitamin-C**
25 µl/ml	19.32±0.89	17.34±2.34
50 µl/ml	34.56±1.56	31.23±1.48

75 µl/ml	56.37±2.45	52.34±2.34
100 µl/ml	71.23±1.34	67.23±1.48
EC_{50} value	62.31	68.45

[a] Results are expressed as percentage of Superoxide scavenging activity with respect to control. Each value represents the mean+SD of three

Metal chelating activity

The metal chelating property of flavonoid extract of *A. echioides* takes off was shown as per Table-6. Flavonoid extract of *A. echioides* were assessed for their capacity to compete with ferrozine for ferrous iron within the solution. In this assessment, the flavonoid extract of *A. echioides* ruined the arrangement of ferrous and ferrozine complex, meaning that they have chelating action and are able of capturing ferrous iron somtime recently ferrozine. The flavonoid extract of A. echioides decreased the greenish blue color complex instantly and appeared the most elevated chelating activity 74.35% With EC_{50} Esteem 59.32 µl/ml than positive control Vitamin-C 71.34% with EC_{50} value 61.47 µl/ml. Within the nearness of chelating agents the complex formation is dis-rupted, coming about in adiminish within the red colour of the complex. Estimation of colour lessening, hence, permits assessing the metal chelating action of the coexisting chelator (Elmasta*et al*., 2006).

Table 5: Metal chelating activity of flavonoid extract of *A. echioides*

Different concentration of extract	Percentage of Metal chelating activity	
	Flavonoid extract of *A. echioides*	Standard Vitamin-C
25 µl/ml	20.13±2.71	17.34±2.34
50 µl/ml	34.56±1.78	32.64±0.89
75 µl/ml	51.24±2.48	48.32±2.34
100 µl/ml	74.35±1.48	71.34±1.78
EC_{50} value	59.32	61.47

[a]Results are expressed as percentage of Metal chelating activity with respect to control. Each value represents the mean+SD of three experiments.

Antibacterial by disc diffusion method

Antibacterial movement of flavonoid extract of *A. echioides* tested against *Klebsiella pneumoniae, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa* were screened and tested as restraint zones within the agar plates (Table-6). Upon testing all the microscopic organisms tested were found to be sensitive to the flavonoid extract of *A. echioides*. Moreover, the zone of hindrance consider uncovered that the polyphenol extract had antibacterial action in extent to concentration slope ranges 25-100 µl/ml against the tested organisms. Among the microscopic organisms considered, *Staphylococcus aureus* and *Escherichia coli*\ was analyzed to be\ profoundly vulnerable taken after by *Pseudomonas aeruginosa* and *Enterococcus faecalis.*

This may affirm the antibacterial property of flavonoid extract of *A. echioides*. Cowan (1999) [3] detailed that phenolic compounds and particularly flavonoids can act as antimicrobial agents by means of a few distinctive instruments, counting inhibition of nucleic acid synthesis, which can result in cell pulverization, and weakening of pathogenicity.

Table 6: The antibacterial activity of the flavonoid extract of *A. echioides* by disc diffusion method

Pathogenic organism	Different concentrations flavonoid extract (µl/ml)			
	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ ml
Pseudomonas aeruginosa	7.8±1.3	9.2±0.6	10.2±1.6	13.6±1.4
Staphylococcus aureus	10.2±0.4	14.3±0.9	14.8±1.1	17.2±0.5
Escherichia coli	9.3±1.3	12.3±0.3	13.7±0.6	14.3±0.7
Enterococcus faecalis	7.2±0.5	10.1±0.6	12.1±0.7	15.4±0.8

[*]The inhibitory Zone size measured included the 6.0 mm size of the well by means of caliper. All the assays were duplicated, and the mean values were recorded.

Conclusions

The show think about affirmed that the flavonoid extract of *A. echioides* clears out had strong antioxidant and antibacterial belonging. This extract appeared high phenolic, content. It might be take a chance that phenolic compounds may be dependable for its antioxidant and antibacterial action. With regard to the results, the flavonoid extract of *A. echioides* may be a vital source of characteristic compounds with antioxidant capacity and antibacterial properties for improvement of modern drugs. Further considers are required to confine the bioactive compounds from the extract and to explain the precise mechanism of activity of the free radical rummaging impact and the antibacterial movement.

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