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Original Article

EFFICACY OF ANTIOXIDATION AND ANTI- INFLAMMATION OF THE LEAF EXTRACTS OF *BORRERIA HISPIDA*

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ABSTRACT

Objective: The aim of the present study was to evaluate the total content of phenol, terpenoid and flavonoids from leaf extract of *Borreria hispida* which was collected from Tamilnadu regions. The leaf extract was also evaluated for antioxidant activity and *in vitro* anti-inflammatory activity.

Methods: In the present investigation, the best accession was screened out through antioxidant activity by qualitative and quantitative methods. The leaf extract was evaluated for antioxidant activity by 1,1–diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay. The *in vitro* anti-inflammatory activity was evaluated using albumin denaturation assay.

Results: Among the five solvents, maximum antioxidant activity was found in the ethanolic extract of Thanjavur accession (81.3±0.1 %). Total content of phenol, terpenoid and flavonoids were quantified as 33.21±0.06 mg Gallic Acid Equivalents, 76.4±0.02 and 18.27±0.08 mg QE/g respectively. The maximum inhibition rate of albumin denaturation of *Borreria hispida* was found to be 89.3±0.5% at a dose of 10 mg/ml.

Conclusion: It was concluded that the maximum percentage of antioxidant activity was found in Thanjavur accession which attributes the highest radical scavenging activity and the ethanolic leaf extract of *Borreria hispida* was exhibited a superior level of anti-inflammatory activity.

Keywords: Antioxidant activity, Anti-inflammatory activity, Phenol, Terpenoid and Flavonoids content

INTRODUCTION

Many higher plants are major sources of natural products used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives and pesticides (Balandrin and Klocke, 1988). The search for new plant metabolites should be a priority in current and future efforts toward sustainable conservation and utilization of biodiversity. Medicinal plants are widely used as alternative therapeutic tools for the prevention of many diseases [1, 2]. Herbal based drugs remain an important source because of the availability, relatively cheaper cost and no side effects when compared to modern medicine [3]. Plants are potential sources of natural antioxidants which protects the cells against the damaging effects of reactive oxygen species (ROS). Antioxidants from plant materials reduce the action of free radicals and protect our body from various disease [4, 5].

Inflammation is the response of the organism to invasion by a foreign body such as bacteria and viruses reaction to irritation, heat, swelling, loss of function and pain. Therefore the search of effective non-toxic natural compounds with antioxidative activity has been identified in recent years [6, 7]. Plants containing polysaccharides are the most potent in curing inflammatory diseases.

Borreria hispida (*Rubiaceae*) commonly known as "GATHIYU OR SHANKHLO" is perennial herb, easily available and grown as a hedge plant along home gardens throughout the India. It is used as a fodder and also consumed as a vegetable in times of scarcity. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine [8]. The present investigation was carried out to evaluate the quantification of total phenol, terpenoids, flavonoids content, antioxidant and anti-inflammatory activities of *borreria hispida* Our study showed the herbal usage of *borreria hispida* in various health care systems for the treatment of inflammation, including life threatening diseases.

MATERIALS AND METHODS

Chemicals and materials

Folin–Ciocalteau reagent, Aluminium chloride, Potassium acetate, Quercetin, Methanolic 1,1 diphenyl-2-picryl-hydrazyl DPPH, Butylated Hydroxy Toluene (BHT), phosphate buffered saline (PBS) were received from Himedia. The medicinal plant *Borreria hispida* were collected from Thanjavur, Tirunelveli, Cuddalore Dindigul and Kanchipuram Tamilnadu, India. The collected leaves were brought to the laboratory in the month of October 2014 and this plant is authenticated by Dr. N. Vijayakumar, Associate professor, Department of Botany, S. T. Hindu College, Nagercoil-629002 and maintained at Sathyabama University, Chennai, Tamil Nadu.

Preparation of the plant extracts

Extraction of the plant samples was done according to a combination of methods [9, 10]. The shade dried leaf (15 g each) of *Borreria Hispida* were finely powdered with pestle and mortar and extracted with 150 ml aqueous, ethanol, chloroform, acetone and petroleum ether separately for 1 minute using an Ultra Turax mixer (13,000 RPM) and soaked overnight at room temperature. The sample was then filtered through Whatman No.1 filter paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota evaporator at 40 °C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in the refrigerator below 10 °C.

Estimation of total phenol content

The Folin–Ciocalteau reagent method has been used for the estimation of total phenolic extracts quantities. Five concentrations of crude extracts of the plant have been prepared and then 100 µl have been taken from each concentration and mixed with 0.5 ml of Folin–Ciocalteau reagent (1/10 dilution) and 1.5 ml of Na_2CO_3 2% (w/v). The blend was incubated in the dark at room temperature for 15 min. The absorbance of the blue-colored solution of all samples was measured at 765 nm. The results were expressed in mg of gallic acid equivalent (GAE) per g of dry weight of plant powders.

Estimation of total flavonoid content

Total flavonoids content in the ethanolic leaf extracts was determined by the Aluminium chloride colorimetric method [11]. 0.5 ml of leaf extracts of *Borreria Hispida* at a concentration of 1

mg/ml were taken and the volume was made up to 3 ml with methanol. Then 0.1 ml $AlCl_3$ (10%), 0.1 ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 min of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.

Determination of terpenoids

100 g of plant powder were taken separately and soaked in alcohol for 24 h. Then filtered, the filtrate was extracted with petroleum ether and the ether extract was treated as total Terpenoids [12]

Qualitative analysis of antioxidant activity of *Borreria Hispida*

The antioxidant activity of the extracts was determined by the following method as described by [13]. Ethanolic leaf extract of 50 µl was taken in the microtiter plate. Methanolic DPPH (100 µl of 0.1%) was added to the samples and incubated for 30 min in dark condition. The samples were then observed for discoloration from purple to yellow and pale pink were considered as strong and weak positive respectively.

Quantitative analysis of free radical scavenging activity of *Borreria Hispida*

The antioxidant activity was determined using DPPH, (Sigma-Aldrich) as a free radical. Sample extracts of 100 µl was mixed with 2.7 ml of methanol and 200 µl of 0.1 % methanolic DPPH. The suspension was incubated for 30 min in dark condition. Blank without the sample containing the same amount of methanol and DPPH solution was prepared and measured as control [14]. Subsequently, at every 5 min interval, the absorption of the solution was measured using a UV double beam spectra scan (Chemito, India) at 517 nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate; free radical scavenging activity was calculated by the following formula

% DPPH radical-scavenging = [(Absorbance of control-Absorbance of test Sample)/(Absorbance Of control)] x 100.

Inhibition of albumin denaturation

The reaction mixture (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg) 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of the test extract. A similar volume of double-distilled water served as control. Then the mixtures were incubated at 37±2ºC in an incubator for 15 min and then heated at 50ºC for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using the vehicle as blank. Diclofenac sodium was used as reference drug and treated similarly for determination of absorbance [15,16]. The percentage inhibition of protein denaturation was calculated by using the following formula.

% Inhibition of protein denaturation = 100 x [Vc–Vt/Vc]

Where, Vt = absorbance of the test sample, Vc = absorbance of control.

The extract/drug concentration for 50% inhibition (IC_{50}) was determined from the dose response curve by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION

Leaf extracts of *Borreria Hispida* are subjected for antioxidant activities by DPPH (1, 1-Diphenyl-2-picryl-hydrazyl) radical scavenging assay. DPPH scavenging ability of leaf extracts was screened in acetone, ethanol (75%), petroleum ether, chloroform and aqueous extract.100 µl of extracts were estimated for free radical scavenging activity using DPPH assay. The samples were observed for the colour change from purple to yellow and pale pink were considered as strong positive and positive respectively (table 1). The quantitative antioxidant activity of ethanolic leaf extract of *Borreria hispida* shown in table 2. Among the five different accessions and five different solvent extracts of *Borreria Hispida*, the ethanolic extract of Thanjavur accession recorded the highest radical scavenging activity (81.3±0.1 %) and the Kanchipuram accession recorded as lower radical scavenging activity (46.8±0.3%). In each case, ethanolic leaf extracts recorded a higher percentage of free radical scavenging activity than aqueous extractions followed by acetone, petroleum ether and chloroform extract. The antioxidant activity has been attributed to the phenolic compounds contained in the plants [17]. Scavenging activity of free radicals of DPPH (1,1-diphenyl-2-picryl hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources [18-20].

Estimation of total phenol content shows the sufficient amount of phenol present in the test samples of this study. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [21]. Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, the antioxidant capacity of a sample can be attributed mainly to its phenolic compounds [22-24]. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The mechanisms of action of flavonoids are through scavenging or chelating process [25,26]. The results showed that the terpenoids have been used for anti-cancer properties [27].

Table 1: Qualitative analysis of antioxidant activity from leaf extract of *Borreria hispida*

S. No.	Extractions	*B. hispida* (Thanjavur)	*B. hispida* (Tirunelveli)	*B. hispida* (Cuddalore)	*B. hispida* (Dindigul)	*B. hispida* (Kanchipuram)
	BHT (standard)	+++	+++	+++	+++	+++
S1	Ethanol	+++	+	++	+	++
S2	Aqueous	++	+	+	+	-
S3	Petroleum ether	-	-	-	-	-
S4	Acetone	+	+	-	+	-
S5	Chloroform	-	-	-	-	-

+++very strong positive++strong positive +positive -negative

Table 2: Quantitative analysis of antioxidant activity from leaf extract of *Borreria hispida*

S. No.	Accessions	Solvents extractions					
		BHT	Ethanol	Aqueous	Petroleum ether	Acetone	Choloroform
1	Tanjavur	98.3±0.1	81.3±0.1	57.1±0.17	22.8±0.2	38.5±0.2	21.4±0.2
2	Thirunelveli	98.3±0.1	62.8±0.17	47.4±0.2	11.4±0.26	21.7±0.3	18.4±0.2
3	Cuddalore	98.3±0.1	63.8±0.26	31.3±0.2	20.0±0.2	27.5±0.2	12.4±0.1
4	Dindigul	98.3±0.1	42.6±0.2	32.3±0.2	12.9±0.17	22.8±0.1	14.3±0.2
5	Kanchipuram	98.3±0.1	46.8±0.3	33.4±0.26	17.1±0.26	22.6±0.2	20.0±0.2

Table 3: Estimation of total phenol and flavonoid, terpenoids content from ethanolic leaf extract of *Borreria hispida*

S. No.	Plant sample	Total phenol content(mg/GAE/g)	Total flavanoid content (mg/QA/g)	Total terpenoid content (mg/g)
S1	*Borreria Hispida*–Thanjavur	33.21±0.06	18.27±0.08	76.4±0.02
S2	*Borreria Hispida*–Tirunelveli	27.45±0.05	15.43±0.04	50.1±0.07
S3	*Borreria Hispida*–Cuddalore	12.76±0.13	11.19±0.12	43.2±0.17
S4	*Borreria Hispida*–Dindugal	11.91±0.09	10.53±0.11	36.7±0.10
S5	*Borreria Hispida*–Kanchipuram	9.46±0.14	8.71±0.16	24.2±0.02

Each result represents mean±SD for three replicates.

The result of the present study showed that the quantification of phenol, flavonoid and terpenoid content of the ethanolic leaf extracts of *Borreria Hispida*. The maximum amount of phenol (33.21±0.06 mg GAE/g), flavonoid (18.27±0.08 mg QE/g) and terpenoid (76.4±0.02 mg/g) content was found in *Borreria Hispida* (Thanjavur accession) followed by other accessions as shown in table 3.

Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat [28-30]. Most biological proteins loses their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation [31]. In the present study the *in vitro* anti-inflammatory activity was evaluated using albumin denaturation assay at different concentrations of leaf extract say (0.625, 1.25, 2.5, 5.0,10 mg/ml) and for the standard diclofenac sodium (0.078, 0.156, 0.312, 0.625, 1.250 mg/ml). The maximum albumin denaturation of Borreria Hispida was found to be 89.3±0.5% at a dosage of 10 mg/ml whereas in diclofenac sodium it was found to be 95.2±0.7% at a dosage of 1.250 mg/ml as indicated in the (table 3,4) fig. 2,3. In Both *Borreria hispida* leaf extract and diclofenac sodium the inhibition concentration (IC_{50}) value was found to be 3.64±0.7, 0.098±0.05 mg/ml respectively (table 6).

Table 4: Influence of *Borreria hispida* leaf extract against protein denaturation

S. No.	Concentration (mg/ml)	% Inhibition of protein denaturation
1	0.625	14.9±0.7
2	1.25	31.6±1.1
3	2.5	47.2±1.0
4	5.0	69.5±0.8
5	10.0	89.3±0.5

Each result represent mean±SD for three replicates.

Table 5: Influence of diclofenac sodium against protein denaturation

S. No.	Concentration (mg/ml)	% Inhibition of protein denaturation
1	0.078	39.4±1.2
2	0.156	57.1±1.4
3	0.312	64.8±0.5
4	0.625	72.5±0.4
5	1.250	95.2±0.7

Each result represents mean±SD for three replicates.

Table 6: IC_{50} values of *Borreria hispida* leaf extract leaf and diclofenac sodium against protein denaturation

S. No.	Treatments	IC_{50} values (mg/ml)
1	Diclofenac Sodium	0.098±0.05
2	*Borreria hispida* leaf extract	3.64±0.7

Each result represents mean+SD for three replicates.

In our study the leaf extract of *Borreria Hispida* shown maximum antioxidant and anti-inflammatory activity, which initiates the isolation of active compound and the herbal usage of *borreria hispida*. Similar studies have been shown by Sadique et. al., and Olugbenga et. al., Maloma et. al., suggested that higher percentage inhibition of protein denaturation of extract has potential to protect the membrane from tissue damage as well as the radical damage [32, 33].

CONCLUSION

Plants have played a significant role in human health care. Traditional plants exert a great role in the discovery of new drugs. The present study reported the antioxidant activity, total phenolic, terpenoids, and flavonoids content of leaf extracts of *Borreria Hispida*. The presence of phenolic content strongly revealed the antioxidant activity. The result obtained from the experiment it is concluded that the ethanolic leaf extract of *Borreria hispida* having good anti-inflammatory activity as determined by protein denaturation assay. Our study motivates the further isolation of active compound for herbal usage.

CONFLICT OF INTERESTS

Declared none

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