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Phytochemical Screening and Anti Inflammatory Activity of Leaf Extracts of *Borreria hispida* by Membrane Stabilization Tests

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ABSTRACT

The aim of the present study was to investigate the phytochemical screening, anti-inflammatory activity from the leaf of *Borreria hispida* by membrane stabilization test. The leaves were collected from different regions of Tamil Nadu. The leaf extracts of *Borreria hispida* from five different solvents were investigated. The phytochemical analysis revealed the presence of active ingredients such as phenols, flavanoids, saponins, alkaloids and terpenoids. Total phenols, flavanoids, saponins, alkaloids and terpenoids were quantitatively estimated. The maximum total phenol (33.2 mg GAE/g), flavanoid content (18.27 mg QE/g), Saponin (19.7 mg/g), alkaloid content (83.2 mg/g) and total terpenoids (76.4 mg/g) were recorded from the ethanolic leaf extract of *Borreria hispida*. The leaf extracts were evaluated by membrane stabilization test. The maximum % of inhibition of haemolysis of *Borreria hispida* was found to be 81.9% at a dose of 12.5 mg/ml. The extract shows significant anti-inflammatory activity which supports the traditional medicinal utilization of the plant.

KEY WORDS: Phytochemical screening, Anti-inflammatory, Membrane stabilization test.

1. INTRODUCTION

Plants are the fundamental wellspring of drug and extraordinary forces of warming infections in cutting edge pharmaceutical herbs assume a vital part (Prakash, 2010). What's more, the leaf concentrates of numerous therapeutic plants have been accounted for to have pharmacological action including auti-incendiary movement (Owoyele, 2005; Sertie, 2005). Irritation is the organic reaction of vascular tissue to unsafe boosts including pathogens, aggravations or harmed cells (Denko, 1992). It is believed that present medications accessible, for example, opoids & non-steroidal mitigating medications are not helpful for provocative issue (Chellaram, 2009; Henson, 1989). It is essentially due to their reactions and intensity. As a consequence of this another option is vital (Ahmadiani, 1998). A wide assortment of chemicals novel mitigating operators could be found through the therapeutic plants. Research on the organic exercises of plants has handled various mixes for the improvement of present day drugs (Arivazhagan, 2000).

Borreria hispida, a weed is a procumbent, spread, shaggy or unpleasant herb 10 to 14 centimeters long. The branches are greenish or purplish, climbing, strong, 4-calculated. *Borreria hispida* is a perpetual hert being utilized as a part of different medicinal services frameworks for the treatment of assortment of disarranges including life undermining illness. This *Borreria hispida* is effectively accessible and developed as a support plant along home greenhouses all through India (Sampath, 2014). It is utilized as a grub furthermore devoured as vegetable in times of shortage (Chellaram, 2015). Along these lines the goal of present study with to assess Phenol, Flavanoids, Saponins, Alkaloids, Terpenoids substance and mitigating action by invitor HRBC membrance adjustment technique.

2. MATERIALS AND METHODS

Chemicals: 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 kanchipuramTamilnadu, India. The collected leaves were brought to the laboratory and maintained at Sathyabama University Chennai, Tamil Nadu, India.

Preparation of the plant extracts: Extraction of the plant tests was done by mix of techniques (Pizzale, 2002; Lu, 2001). The shade dried leaf (15 g each) of *Borreria hispida* were finely powdered with pestle and mortar and removed with 150 ml watery, ethanol, chloroform, $(CH_3)_2CO$ and petroleum ether independently for 1 minute utilizing a Ultra Turax blender (13,000 RPM) and splashed overnight at room temperature. The specimen was then separated through Whatman No.1 channel paper in a Buchner pipe. The sifted arrangement was dissipated under vacuum in a rotavator at 40ºC to a steady weight and afterward broke up in particular solvents. The concentrated concentrates were put away in hermetically sealed compartment in fridge underneath 10°C.

Phytochemical Screening from leaf extracts of *Borreria hispida*: The powdered examples were tried for the nearness of different phytochemicals. Plant concentrates were brought with five distinct solvents, for example, $(CH_3)_2CO$, ethanol, petroleum ether, chloroform and water. The plant concentrate was subjected for subjective phytochemical screening for the nearness of bioactive mixes terpenoids, alkaloids, glycosides and heart glycosides, steroids, quinines, coumarines, phenols, tannins, flavonoids, saponins, anthocyanin and betacyanin by the standard techniques (Yadav, 2011; Tiwari, 2011).

Estimation of Total Phenol Content: The Folin–Ciocalteau reagent strategy has been utilized for the estimation of aggregate phenolic extricates amounts .Five convergences of rough concentrates of the plant have been readied and afterward 100 µL have been taken from every focus and blended with 0.5 mL of Folin–Ciocalteau reagent (1/10 weakening) and 1.5 mL of Na_2CO_3 2% (w/v). The mix was brooded oblivious at room temperature for 15 min. The absorbance of blue-hued arrangement of all examples was measured at 765 nm. The outcomes were communicated in mg of gallic corrosive proportionate (GAE) per g of dry weight of plant powders.

Estimation of Total Flavanoid Content: Total flavonoids content in the ethanolic leaf extracts was determined by the aluminium chloride colorimetric method (Mervat, 2009). 0.5 ml of leaf extracts of *Borreria hispida* at a concentration of 1mg/ ml were taken and the volume was made up to 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml 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 minutes of incubation. A standard calibration plot was generated at 415nm 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.

Estimation of Saponin Content: The determination of aggregate saponin was done by technique utilized with minor changes. 1 g of powdered plant has been added to 100 ml of 20% watery ethanol and kept in a jar on stirrer for half hour and after that warmed over a for 4 h at 45 °C with blending. The blend was separated by utilizing channel paper whatman and the deposit again extricated with another 100 ml of 25% fluid ethanol. The consolidated concentrates were concentrated by utilizing rotating evaporator as a part of 40 °C to gets 40 ml around. The mass was moved into separator pipe and removed twice with 20 ml diethyl ether. The ether layer was disposed of while the fluid layer was kept and afterward re-removed with 30 ml n-butanol was included. The n-butanol concentrates were washed twice with 10 ml of 5% fluid sodium chloride. The rest of the arrangement was dissipated. After vanishing, the examples were dried in the broiler at 40°C to a steady weight and the saponin substance was figured.

Determination of Total alkaloids: The evaluation technique for alkaloids determinations has been utilized with a few alterations. 100 ml of 10% acidic corrosive in ethanol was added to 1 gram of dry powdered plant and after that the concentrates were secured and permitted to remain for 4 h. After that, the concentrates have been filtrated and focused on a water shower to 25 ml of its unique volume. The beads of concentrated ammonium hydroxide were added to the concentrate until the precipitation the entire arrangement was permitted to settle, and after that the hastens were washed with weaken ammonium hydroxide and afterward separated utilizing channel paper whatman. The deposit was dried in the broiler at 40°C and weighed. The alkaloid substance was resolved utilizing the accompanying equation (1):

$$\% \text{ alkaloid} = [\text{final weight of the sample} / \text{initial weight of the extract}] \times 100 \quad (1)$$

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

Evalution of anti-inflammatory activity by Membrane stabilizing test: Drug used as Standard: Acetylsalicylic acid available in the commercial name of Ecosprinʀ-75 marketed by USV Limited, Mumbai, Maharashtra, was used as a source of Acetylsalicylic acid.

Human Blood: The blood was gathered from a sound human volunteer who had not taken any NSAIDS for 2 weeks preceding the test and gathered in heparinzedvacutainer. The blood was washed three times with 0.9% saline and centrifuged all the while for 10 minutes at 3000 rpm. The pressed cells were washed with 0.9% saline and a 40% v/v suspension made utilizing isotonic phosphate support which was made out of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 utilized as Stock erythrocyte or RBC suspension.

Hypotonic solution –induced haemolysis or membrane stabilizing activity: This test was done by technique portrayed (Shinde, 1999) with slight changes. The test comprised of stock erythrocyte (RBC) suspension 0.030 ml blended with 5ml of hypotonic arrangement (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing test separate with different fixations. The control test comprised of 0.030ml RBC suspension blended with hypotonic supported arrangement alone. The standard medication acetylsalicylic was dealt with like test at different fixations. The trial was completed in triplicate (Dey, 2011; Chandra, 2012). The blends were brooded at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation (2).

$$\% \text{ Inhibition of haemolysis} = 100 \times [A\ 1 - A\ 2 / A\ 1] \quad (2)$$

Where: A 1 = Absorbance of hypotonic buffered solution alone, A 2 = Absorbance of test /standard sample in hypotonic solution.

3. RESULTS AND DISCUSSION

The preparatory phytochemical screening of leaf concentrates were evaluated by standard technique. Phytochemical screening was finished on the leaf removes using particular solvents to recognize the critical trademark manufactured get-togethers, for instance, Phenols, Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Glycosides, Cardiac Glycosides, Coumarins and Steroids. General reactions in these examinations revealed the proximity or nonattendance of these blends in the leaf isolates attempted. In the present study phytochemical screening was performed with three unmistakable solvents sush as chloroform, petroleum ether, $(CH_3)_2CO$ ethanol and watery leaf concentrates of *Borreria hispida*.

Among the three wild promotions and five distinct concentrates of *Borreria hispida*, the ethanol leaf concentrate of *Borreria hispida* collected from (Tamil Nadu-Thanjavur Accession)- rich in Phenols, Alkaloids, Flavonoids, Saponins, Tannins, Terpenoids, Glycosides, Cardiac Glycosides, Coumarins and Steroids took after by different increases (Table 1-3). This shows abnormal state of its conceivable restorative qualities (Dey, 2011). Phytochemical screening demonstrated the nearness of Phenolics & Flavonoids Saponins which may be in charge of auti-inflammtory activity (Anoop, 2015). Consequently the preparatory screening tests might be helpful in the location of the bioactive standards, prompting drug revelation and advancement (Kyenge, 2010; Doss, 2009).

Table.1. Phytochemical screening from leaf extracts of *Borreria hispida* (Thanjavur Accession)

Phytochemicals Tested	Leaf extracts of *Borreria hispida*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	+	++	-	-	++
Saponins	+	+	-	-	-
Quinones	+	++	-	-	++
Terpenoids	+	++	-	+	+
Steroids	+	++	+	+	+
Flavonoids	-	+	-	-	+
Phenol	+	++	+	+	++
Alkaloids	-	++	-	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	-	+	-	-	-
Coumarins	-	+	-	-	-
Anthocyanin	-	-	-	-	-
Beta cyanin	-	+	-	-	+

++ = strong positive; + = positive; - = negative

Table.2. Phytochemical screening from leaf extracts of *Borreria hispida* (Tirunelveli Accession)

Phytochemicals Tested	Leaf extracts of *Borreriahispida*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	-	+	+	+	-
Saponins	+	-	-	-	-
Quinones	+	+	-	-	+
Terpenoids	+	+	-	-	+
Steroids	+	+	-	-	+
Flavonoids	-	+	-	-	-
Phenol	+	+	-	-	+
Alkaloids	+	+	-	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	+	-	+	-
Coumarins	+	+	-	-	-
Anthocyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

++ = strong positive; + = positive; - = negative

Table 3. Phytochemical screening from leaf extracts of *Borreriahispida*(Kanchipuram Accession)

Phytochemicals Tested	Leaf extracts of *Borreriahispida*				
	Aqueous	Ethanol	Chloroform	Petroleum ether	Acetone
Tannins	+	+	-	-	+
Saponins	-	-	-	-	-
Quinones	+	+	+	+	+
Terpenoids	+	++	+	+	+

Steroids	+	+	+	+	+
Flavonoids	++	++	-	-	-
Phenol	++	++	+	+	+
Alkaloids	+	+	-	-	+
Glycosides	-	-	-	-	-
Cardiac glycosides	+	++	-	-	+
Coumarins	+	+	-	-	-
Anthocyanin	-	-	-	-	-
Beta cyanin	+	+	-	-	-

++ = strong positive; + = positive; - = negative

The result of the present study recorded highest yield of total Phenol, Alkaloid, Saponin, –Terpenoid, Flavanoid contents was found to be 33.21mg GAE/g, 83.2mg/g, 19.7 mg/g, 76.4mg/g & 18.27 mg QE/g respectively in the ethanolic leaf extract of *Borreria hispida* (Thanjavur accession) table.4. On the basis of these result it could be inferred that the presence of the Phenols, Alkanoid, Terpenoids, Flavanoids, Saponins from ethanol extracts should significant anti-inflammatory activity who has reported from the ethanolic & aqueous extracts of *wrightiatirictoria* (Rajalakshmi, 2012).

Table.4. Estimation of Phenol, saponin, alkaloid, terpenoid content from leaf extract of *Borreria hispida*

Samples	Phenol content (mg/GAE/g)	Alkaloid content (mg/g)	Saponin content (mg/g)	Terpenoid content (mg/g)	Flavanoid content (mg QE/g)
***Borreria hispida*- Leaf- Thanjavur**	33.21	83.2	19.7	76.4	18.27

Agony is a side effect of numerous maladies requiring treatment with analgesics. Torment can be inspired by aggravation (James, 2011). A large portion of the non-steroidal calming specialists have pain relieving action. Some plant dynamic constituents additionally have calming movement (Manohara, 2009). Film adjustment is a procedure of keeping up the honesty of natural layers, for example, erythrocyte and Iysosomal layers against osmotic and warmth actuated Iyses. The film balancing out movement of red platelet layer showed by some medication, serves as a helpful *in vitro* technique for intensifies the calming action of different compounds. In the present examination mitigating action have been performed by *invitro* HRBC layer adjustment strategy at various grouping of leaf concentrate say (0.78, 1.56, 3.125, 6.25, 12.5 mg/mL) and for the standard Acetylsalicylic corrosive (0.078, 1.56, 0.312, 0.625, 1.25 mg/mL). The most extreme % of hindrance of Haemolysis of *Borreria hispida* was observed to be 81.9% at a measurement of 12.5 mg/mL, though in Acetylsalicylic corrosive it was observed to be 97.2% at a dose of 1.250 mg/mL (Table.5, 6). These outcomes may credits because of the nearness of high phenolic, Flavanoid substance (Amujoyegbe, 2012). In both *Borreria hispida* leaf concentrate and Acetylsalicylic corrosive the hindrance fixation (IC50) worth was observed to be 5.762, 0.1918 mg/mL individually Table.7. The layer settling movement of ethanolic leaf concentrate of *Borreria hispida* obtained in this study contrasted positively and standard medication Acetylsalicylic corrosive at all focuses utilized. The discoveries of this study bolstered the conventional utilized of the plant for treatment and administration of provocative related infection.

Table.5. Influence of *Borreria hispida* leaf Extract against Membrane Stabilization

S.No	Concentration (mg/mL)	%Inhibition of Haemolysis
1	0.78	12.8
2	1.56	26.5
3	3.125	40.7
4	6.25	62.5
5	12.5	81.9

Table.6. Influence of Acetylsalicylic acid against Membrane Stabilization

S.No	Concentration (mg/mL)	% Inhibition of Haemolysis
1	0.078	35.7
2	0.156	48.5
3	0.312	64.1
4	0.625	74.4
5	1.250	97.2

Table.7. IC_{50} Values of *Borreria hispida* leaf and Acetylsalicylic Acid against Membrane Stabilization

S.No	Treatments	IC50 values (mg/mL)
1	Acetylsalicylic acid	0.1918
2	*Borreria hispida*- Leaf extract	5.762

4. CONCLUSION

From the above findings it can be concluded that the ethanolic leaf extract of *Borreria hispida* possesses promising anti-inflammatory activity. The presence of Phenol, Flavanoids, Saponins, Alkaloids, Terpenoids in the ethanolic leaf extract of the plant under study may be respossible for their activities. It indicates that the plant material become an important source of natural drug compounds.

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