Research Article

Evaluation of *in- vitro* Anti-inflammatory Potential of *Grevillea robusta A. Cunn, EX R.BR.* Leaves

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ABSTRACT

Synthetic Non-steroidal anti-inflammatory drugs (NASAIDs) are the choice of drug for inflammation. NASAIDs caused severe side effects like hyperacidity, gastric ulcer and so on. To avoid the side effects of NASAIDs, there is an urgent need for searching new molecule from natural origin. Present study is therefore aimed to explore *Grevillea robusta A. Cunn, ex R.Br.* Family *proteaceae* leaves for anti-inflammatory activity. Microscopic measurement (fibre length and width), Ash values and extractive values of *Grevillea robusta* leaves were determined to set the pharmacognostic standards. Chemical constituents were evaluated through chemical tests. The Ethanol extract of *Grevillea robusta* leaves (GRLE)) were subjected to evaluate i*n-vitro* anti-inflammatory activity through HRBC method and Heat induced haemolytic method. The leaves of *Grevillea robusta* showed significant anti-inflammatory activity. The Ethanol extract (GRLE) showed significant anti-inflammatory activities. GRLE was found to contain polyphenols as chemical constituents which was the basis of anti-inflammatory activity. On the basis of result we can conclude that Ethanol extract of leaves of *Grevillea robusta* has good anti-inflammatory activity. GRLE could be used for treatment of inflammation.

Keywords: *Grevillea robusta*, leaves, anti-inflammatory activity.

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INTRODUCTION

Inflammation is part of the complex biological response of tissues to harmful stimuli, such as, damaged cells or irritants. The classical sign of acute inflammation are pain, heat, redness, swelling, and loss of function. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function[1,2]. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process.

Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased gastric and duodenal ulcer and elevate the risk of blood clot resulting in heart attacks and strokes[3].Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Natural products are rich source for discovery of new drugs because of their chemical diversity. A natural product from medicinal plants plays a major role to cure many diseases associated with inflammation. The conventional drug available in the market to treat inflammation produces various side-effects.

Grevillea robusta timber was widely used for external window joinery, as it is resistant to wood rot. It has been used in the manufacture of furniture, cabinetry, and fences. In North Garo Hills, Meghalaya, NE India, *Grevillea robusta* bark and leaves used for headaches and dizziness[4]. In Kenya, natives of the Kakamega forest use the plant to treat sore throats, earache, chest problems, flu and toothache. Present study is therefore aimed to explore *Grevillea robusta* as traditionally it is used as anti-inflammatory agent. Therefore, the present study is aimed for pharmacological evaluation of *Grevillea robusta* leaves ethanolic extract for anti-inflammatory potential.

MATERIALS AND METHODS

Collection and authentication

Fresh leaves of *Grevelliea robusta* were collected from Dev Bhoomi Institute of Pharmacy and Research, Dehradun and were authenticated by Botanical Survey of India, Northern Regional Centre by matching the sample with BSD student herbarium (Acc. No. 112) vide letter number BSI/NRC…Tech./Herbs(Indent.)/2019-20. The leaves were dried in shade and powdered to a coarse form. The leaves were carefully checked for the presence of any foreign matter.

Quantitative Investigation

The ash values, loss on drying and extractive values of the powdered samples were performed to evaluate the samples quantitatively[5, 6, 7].

a. Determination of Ash values and loss on drying: Ash values (Evans, 1996) and Loss on drying (WHO Guide line)

of *Grevelliea robusta* leaves were also determined to check the purity of the sample.

b. Preparation and extract: Extracts were prepared by the cold maceration method for 48 h using petroleum ether, toluene, ethyl acetate and ethanol (Sample: Solvent ratio:: 1:10). The extracts were evaporated to dryness using rotary evaporator and corresponding extractive values were determined.

Phytochemical Evaluation

All extracts were subjected to phytochemical evaluation for flavonoids, alkaloids and terpenoids using the methods as described by Evans, 1996.

Pharmacological Evaluation

The extracts of *Grevelliea robusta* leaves were evaluated [8, 9] for Anti- inflammatory activity by two methods:

a. The human red blood cell (HRBC) membrane stabilization method: The membrane stabilization with *Grevelliea robusta* leaves extracts were evaluated through their capacity to resist hypotonic solution-induced hemolysis of human erythrocytes following the method developed by Omale *et al*. (2008).

b. Heat induced hemolytic method: *Grevelliea robusta* leaves *extracts* were evaluated through their capacity to resist heat- induced hemolysis of human erythrocytes following the method prescribed by Omale *et al*. (2008)

RESULTS AND DISCUSSION

Quantitative Investigation

a. **Determination of Ash values and loss on drying** The percentage of total ash, water-soluble ash, acid-insoluble ash, and Sulfated ash values for *Grevillea robusta* leaves are listed in [Table 1].

b. **Preparation and extract:** Extractive values of the sample in petroleum ether (GRLPE), toluene (GRLT), ethyl acetate (GRLEa), ethanol (GRLT) and water (GRLW) are tabulated in [Table 2].

These data may be utilized for setting the pharmacognostic standard of *Grevillea robusta* leaves.

Table 1: Evaluation of Loss on drying and different ash values of the dried powdered Grevillea robust leaves

Evaluation parameters	Value (% w/w)* (Leaves)
Loss on drying	4.95±0.86
Total ash value	1.34±0.24
Water-soluble ash value	0.86±0.11
Acid-insoluble ash value	0.31 ± 0.09
Sulfated ash value	0.34 ± 0.07

*Mean value of five counts.

Table 2: Evaluation of different extractive values of dried powdered leaves of *Grevillea robusta* leaves

Evaluation parameters	Values (%w/w)*
Petroleum ether extractive value	1.7±0.69
Toluene extractive value	2.9±0.36
Ethyl acetate extractive value	16.9±0.58
Ethanol extractive value	45.6±0.32
Water extractive value	11.09±0.32

*Mean value of five counts.

Phytochemical Evaluation

The chemical constituent of extract was found to be maximum in case of GRLE and minimum in case of GRLT and GRLPE [Table 3]. GRLE and GRLEa contained good amount of Tannins, Flavonoids and Saponins. GRLW contain no flavonoid and saponins.

Table 3: Phytochemical screening of extracts of *Grevillea robusta* leaves.

S.N.	Test	Toluene extract (GRLT)	Ethyl acetate extract (GRLEa)	Ethanol extract (GRLE)	Water extract (GRLW)
1.	**Carbohydrates**	+	+	+	+
2.	**Alkaloids**	–	+	+	+
3.	**Tannins**	–	+	+	+
4.	**Flavonoids**	+	+	+	–
5.	**Saponins**	–	+	+	–
6.	**Glycosides**	+	–	–	–
7.	**Mucilage**	–	+	+	+

Pharmacological Evaluation

It was observed that GRLE and GRLEa at the concentration of 100 µg/ml exhibited 79.33% and 76.66% HRBC membrane stabilization activity respectively [Table 4]. Heat induced hemolytic inhibitions of GRLE and GRLEa at a concentration 100 µg/ml were 88.42% and 76.32% respectively [Table 5]. GRLW did neither produced any HRBC membrane stabilization activity nor Heat induce hemolytic inhibition which emphasize the fact flavonoid may be responsible chemical constituent for anti-inflammatory activity.

Table 4: HRBC membrane stabilization with different extract of *Grevillea robusta* leaves

S. N.	Type of extract	Concentration (µg/ml)	% Inhibition
1	Control	-	-
2	GRLT	50	43.33 ± 0.60
3	GRLT	100	52.66 ± 0.37
4	GRLEa	50	54.66 ± 0.25
5	GRLEa	100	76.66** ± 0.33
6	GRLE	50	66.66 ± 0.21
7	GRLE	100	79.33 ** ± 0.14
8	GRLW	50	-
9	GRLW	100	-
10	Diclofenac sodium	100	97.66 ± 0.16

Table 5: Heat induce hemolytic inhibition of different extract of *Grevillea robusta* leaves

S. N.	Type of extract	Concentration (µg/ml)	% Inhibition
1	Control	–	–
2	GRLT	50	46.84 ± 0.44
3	GRLT	100	51.63 ± 0.34
4	GRLEa	50	55.26 ± 0.21
5	GRLEa	100	76.32**± 0.19
6	GRLE	50	47.89 ± 0.17
7	GRLE	100	88.42**± 0.21
8	GRLW	50	–
9	GRLW	100	–
10	Diclofenac Sodium	100	98.95 ± 0.10

GLRE produced significant anti-inflammatory activity compared to the control in concentration depended on fashion and activity is almost comparable to well known synthetic NSAID Diclofenac Sodium (100µg). This result is in conformation with result of Ullah *et al*, 2014. Flavonoid present in the extract may be responsible chemical constituents for anti-inflammatory potential of GLRE.

CONCLUSION

In present study, the anti-inflammatory potential of various extract of *Grevillea robusta* leaves was studied. GRLE was found to be more potent than other extracts. GRLW was devoid of anti-inflammatory potential. GRLE possessed flavonoid and saponins as additional phytoconstituents than Water extract. On basis of the result, it was concluded that leaves of *Grevillea robusta* had significant anti-inflammatory activity. The activity may be attributed through flavonoids. Isolation of the chemical constituents responsible for above activities and semi synthetic modification for target specific anti-inflammatory activity may be the next research area. Safe and Economical anti-inflammatory agents could be formulated from *Grevillea robusta* leaves.

REFERENCES

1. Harsh Mohan. Inflammation and healing, Textbook of pathology; 5th ed. Vol. 6. New Delhi: Jaypee Brothers Medical Publishers (P) ltd. 2005;133–134
2. Peskar, B. A., Steffens, Ch., Peskar, B. M.: Radioimmunoassay of 6-keto-prostaglandin $F_{1\alpha}$ in biological material. In: Radioimmunoassay of drugs and hormones in cardiovascular medicine (A. Albertini, M. Da Prada, B. A. Peskar, eds.), Amsterdam: Elsevier-North Holland Publ. Co. 1979; 239–250.
3. Inger L. Meek, Mart A.F.J. van de Laar, and Harald E. Vonkeman. Non-Steroidal Anti-Inflammatory Drugs: An Overview of Cardiovascular Risks. Pharmaceuticals (Basel). 2010; 3(7): 2146–2162.
4. Sharma M, Sharma CL and Marak PN. Indigenous uses of medicinal plants in North Garo Hills, Meghalaya, NE India. Res. J. Recent. Sci. 2014;3(ISC-2013): 137-146
5. Evans WC. Trease and Evans' Pharmacognosy. 14th ed. London: WB Sounders Company Ltd.1996; 545–546.
6. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi, India: Vallabh Prakashan. 1994; 112–120.
7. Quality Control Methods for Medicinal Plant Materials World Health Organization (Geneva) Delhi: AITBS Publishers and Distributor.2004; 28–30.
8. Omale J and Okafor PN. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. African Journal of Biotechnology. 2008; 7 (17):3129-3133.
9. Ullah MS, Sikder MAA, Sharmin T and Rashid MA. Pharmacological Activities of Grevillea robusta, a Medicinal Plant of Bangladesh. Bangladesh Pharmaceutical Journal. 2014; 17(2): 135-137.

10. Rashida Ginwala, Raina Bhavsar, DeGaulle I. Chigbu, Pooja Jain and Zafar K. Khan. Potential Role of Flavonoids in Treating Chronic Inflammatory Diseases with a Special Focus on the Anti-Inflammatory Activity of Apigenin. Antioxidants. 2019;8(35):1-30.

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