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Evaluation of Anti-inflammatory Activity of *Bryophyllum calycinum* (Crassulaceae) on Acute and Chronic Inflammation Models

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Abstract

The *Bryophyllum calycinum* (Crassulaceae) is an herbal plant and it is used as medicinal plant. The leaf juice of the plant were used for antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumor, hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, ant diabetic, astringent, antiseptic, antilithic and cough suppressant. The present study was to evaluate the Anti-Inflammatory potential of *Bryophyllum calycinum.* The Anti-inflammatory activity of ethanol, chloroform and n-hexane extracts of whole plant was evaluated through Carrageenan induced Paw Edema and Cotton Pellet induced Granuloma Model. They produced significant inhibition of rat paw edema induced by carrageenan on dose dependent. The ethanol, chloroform and n-hexane extracts at the dose level of 400 mg/kg shows the percentage inhibition of paw 91.80%, 88.37% and 85.81% respectively. The standard drug Diclofenac Sodium produced a significant Anti-inflammatory Activity (96.79% inhibition of paw edema) with respect to inflammation control. It was concluded that the 100, 200 and 400 mg/kg ethanol extract of whole plant of *Bryophyllum calycinum* possesses significant Anti-inflammatory.

Keywords: *Bryophyllum calycinum*, Anti-Inflammatory and Chemical Constituents

INTRODUCTION

The *Bryophyllum calycinum* (Crassulaceae) is an herbal plant and it is used as medicinal plant has extensive concentration for their medicinal properties [1]. Discover relevance in folk medicine, over and above in the contemporary medicine [2, 3]. The plant Bryophyllum calycinum is frequently known as air plant, love plant, miracle leaf, life plant, Zakham-e-hyat, panfutti and Ghayamari, canterbutury bells, Parnabija etc [4, 5]. It conventional as an herbal remedy in approximately all parts of the world [6, 7 and 8]. This plant is widely grown in hot and humid areas, around the dwelling place, along road sides and herbal garden and field etc. The plant *Bryophyllum calcynium* are widely used in folk medicine and it is easily found in India, Tropical Africa, Madagascar, China, Australia, pakisthan, Hawai and Tropical America [9,10 and 11]. This usually medicinal plant of leaves stems a roots and flowers portion that shows the chemical has high index in therapeutic value. Medicinal plants have been known for millennia and are extremely well-regarded all over the world as a prosperous resource of therapeutic agents for the anticipation of diseases and ailments [12, 13]. The leaves and leaf juice of the plant *(Bryophyllum calycinum)* were used like-antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumor, hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, ant diabetic, astringent, antiseptic, antilithic and cough suppressant [14,15,16,17,18,19,20 and 21].

The *Bryophyllum calycinum is* used as Medicinal plant, ornamental and crassulencent herb. It is cultivation in houses, herbal garden, and field. The plant that grows all over the India in hot, humid and moist areas. The plant height is about 1-1.5m in long and it consist of opposite leaves and 10-20cm long glabrous leaves. The lower leaf is usually simple and upper one 3-7 foliate and are long-petioled. They are freshly dark green color and trimmed in reddish purple and Leaves blade are pinnately compound with 3-7 leaflets. The stem is hallowing four angled and usually branched, the flowers are 2-3 cm long and color of plant is reddish purple. Fruits are membranous follicles enclosed in the persistent papery calyx and corolla, seed smooth and ellipsoid. The session of Bryophyllum calycinum plant flowers grow in November to March Fruit in April .it is astringent and sour in taste and sweet in the post digestive effect and it has hot potency [22].

The phytochemical screenings of *Bryophyllum calycinum* have yielded alkaloids, triterpenes, glycosides, flavonoids, steroids, butadienolides, lipids, and organic acid, Phenol and tennis, free amino acid and terpenoids [23, 24]. The *Bryophyllum calycinum* plants yields of arachidic acid, astragalin, behenic acid, beta amyrin, benzenoids, bersaldegenin, beta-sitsterol, bryophollenone, bryophollone, bryophyllin, caffeic acid, ferulic acid, quercetin, steroids, and taraxerol as well as the plant extracts yielded a potent cytotoxic bufadienolide orthoacetate. Bufadienolide has been reported to be poisonous and toxicity with digitalis-toxicity type cardiac effects (slowing of heart rate, heart blocks and potentially fatal ventricular arrhythmias for example bryotoxin A, B, C.Bryophillin A, a bufadienolide compounds and It has shown anti-tumor promoting activity [25,26]. The leaves of plants yielded malic acid and fractionation of an Ethyl acetate yielded seven kaempferol rhamnosides: kaempferol 3-O-α-L-(2-acetyl)rhamnopyranoside-7-O-α-L-rhamnopyranoside, kaempferol 3-O-α-L-(3-acetyl) rhamnopyranoside-7-O-α-L-rhamnopyranoside, kaempferol 3-O-α-L-(4-acetyl) rhamnopyranoside-7-O-α-L-rhamnopyranoside, kaempferol 3-O-α-D- glucopyranoside-7-O-α-L-rhamnopyranoside, afzelin, and α-rhamnoisorobin**.** *Bryophyllum calcynium* is contain Isocitric acid and citric acid. Phenols, Phenyl propanoids and Flavanoids like Syringic acid, caffeic acid, 4-hydroxy-3-methoxycinnamic acid, 4-hydroxybenzoic acid, para-hydroxy cinnamic acid, paracoumaric acid, ferulic acid, protocatechuic acid, phosphoenol pyruvate and protocatechuic acid. Triterpenoids and Steroids like α-amyrin, α-amyrinacetate, β-amyrin, β-amyrinacetate, bryophollenone, bryophollone, taraxerol, pseudo taraxasterol, 18-α-oleanane, friedelin and glutinol [27, 28, and 29].

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain [30, 31]. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. In the other term, inflammatory response is a complex process that includes activation of white blood cells, the release of immune system chemicals. Cyclooxygenase (COX) is the key enzymes in the synthesis of prostaglandins, prostacyclins and thromboxanes which are involved in inflammation, pain and platelet aggregation. Inflammation is mainly of two types Acute, Chronic [32].The drugs used to reduce inflammation are NSAIDs. These drugs block COX-1 and COX-2 enzyme activity. COX enzymes assist with prostaglandin production. NSAIDs, steroidal anti-inflammatory drugs are being used till now, As a result long term

uses of these drugs cause adverse side effects and damage human biological system such as liver, gastrointestinal tract, etc. As a result of adverse side effects, like gastric lesions, cardiovascular, renal failure [33] and gastrointestinal damage caused by NSAIDs [34, 35].

EXPERIMENTAL

Plants Materials

The plant of *Bryophyllum Calycinum* was collected from around the dwelling place, along road sides and herbal garden and field. The plant material was taxonomically identified and authenticated by Dr. (Mrs.) Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, Delhi (RHMD) CSIR-NISCAIR, India. A reference no NISCAIR/ RHMD/ Consult/ 2014/ 2516/95India, for future reference.

Preparations of Extracts

Whole plant part of *Bryophyllum calycinum* were taken and grounded, and about 500g of the plant material was consecutively macerated for seven days each in n-hexane, chloroform and ethanol, respectively. On basis of the preliminary phytochemical tests conducted, the ethanol extract was found to be rich in terms of chemical constituents and therefore was selected for the anti-inflammatory activity. The ethanol was removed under reduced pressure to obtain a semisolid mass. The Ethanol extracts of *Bryophyllum calycinum* was then stored in vacuum desiccators until used. The plant extract was subjected to screening for various phytochemical tests [36].

Animal used

All the experiments were carried out using Swiss albino mice (20-25 g) and Wistar albino rats (150-200 g), were used for analgesic activity and anti-inflammatory respectively. Which were obtained from the animal house (Reg No: 955/A/06/CPCSEA) of M.M.College of Pharmacy Mullana (Ambala), India. They were kept in polyacrylic cages with paddy husk as bedding. The animals were housed under standard housing condition (room temperature 24-27ºc and humidity 60-65% with 12:12 light: dark cycles) and fed with standard diet. The animals were fasted overnight before the experiment and given water ad libitum. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (MMCP/IAEC/15/08) and were in accordance with the guidelines of the CPCSEA, Ministry of Forests and Environment, Government of India.

Dose Preparation

The ethanol, chloroform and n-hexane extracts of dried whole plant powder of *Bryophyllum calycinum* were prepared in distilled water at three divided dose (100mg/kg, 200mg/kg and 400mg/kg) and Diclofenac sodium (25mg/kg) used as a standard.

Phytochemical Screening

The plant extracts were subjected to screening for various phytochemical employing standard protocols for determining the presence of steroids, alkaloids, tannins, flavonoids, glycosides, Phenol, free amino acid and terpenoids etc [37].

Carrageenan-induced rat paw oedema

Carrageenan-induced hind paw oedema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent for the choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effect [38]. Moreover, the model exhibits a high degree of reproducibility. The probable mechanism of action of carrageenan-induced inflammation is bi-phasic; the first phase is attributed to the release of prostaglandin and lysosome enzymes 1, 2 and 3 hours. Acute inflammation was produced by sub plantar injection of 0.1ml of 1% suspension of carrageenan in normal saline in the right hind paw of the rats 60 minutes after the oral administration of extracts and vehicles. The paw volume was measured by digital Plethysmometer at 0 minutes, 30 minutes, 60 minutes, and 120 minutes after the induction of inflammation on the same day. The percentage inhibition of oedema compound with that of control was taken as anti-inflammatory activity. The percentage inhibition of oedema was calculated by the formula;

Percentage inhibition of oedema = (A-B)/A X 100. Where, A represents the paw volume of control group and B represents the paw volume of the test drug treated group.

Calculation of Final Paw Volume

Final paw volume (ml) = Mean paw volume after 3 hours of induction – Mean paw volume at the time of induction.\

Calculation of % inhibition

The percentage (%) inhibition of edema is calculated using the formula

$$\% \text{ inhibition} = To - Tt / T_0 \times 100$$

Where T_t is the thickness of paw of rats given test extract at corresponding time and T_o is the paw thickness of rats of control group at the same time.

Cotton Pellet Induced Granuloma

In this study Wistar rats were divided into five groups having six animals in each group and treatments were given as per carrageenan-induced paw edema method. The animals were administered with vehicle standard drug and test drug thirty min prior to the cotton pellets implantation [39]. After thirty min of first dosing 10 ± 0.5 mg of sterile cotton pellet was inserted one near each axilla region by making small subcutaneous incision in anaesthetized animals. Vehicle, standard drug and test drug (100mg, 200mg and 400mg) were administered for seven consecutive days. On the eighth day, the animals were sacrificed by excessive anaesthesia and the cotton pellets were removed surgically. Pellets were separated from extraneous tissue and weighed immediately for wet weight and then dried at 60^0 C until the weight become constant. The percent inhibition increase in the weight of the cotton pellets was calculated by:

% Inhibition = [Wc – Wd / Wc] X 100

Wc = Difference in pellet weight of the control group

Wd = Difference in pellet weight of the drug treated group

Statistical Analysis

All values were expressed as Mean ± S.D. The differences between control and treatment groups were tested for significance using ANOVA followed by Dunnet's t test. $P<0.05$ were considered significant.

RESULTS

Anti-inflammatory Effect of Ethanol, Chloroform and n-hexane Extracts of *Bryophyllum calycinum* on Carrageenan-induced paws oedema in rats.

The anti-inflammatory effect of Ethanol, Chloroform and n-hexane Extracts of *Bryophyllum calycinum* were evaluated by Carrageenan Induced Paw Oedema. Administration of Carrageenan in sub plantar region of left hind paw significantly increased the paw volume. Ethanol Extract, Chloroform Extract and n-hexane Extract were administered orally 60 minutes before the Carrageenan injection and produced significant inhibitory effect in rats by reducing paw volume. The results showed that Ethanol, Chloroform and n-hexane Extracts of *Bryophyllum*

calycinum produced statistically significant inhibition of Oedema induced by Carrageenan at doses of 100, 200 and 400 mg/kg at 1st hr, 2nd hr and 3rd hr. when compared with inflammation control and produced anti-inflammatory effect at 1st hr, 2nd hr and 3rd hr. Treatment with Ethanol Extract the percentage inhibition of paw oedema was found 78.71%, 98.52% and 91.80% at 100, 200 and 400 mg/kg. Diclofenac Sodium produced a significant anti-inflammatory activity (99.93% inhibition of paw edema) with respect to inflammation control. Treatment with Chloroform Extract at 100, 200 and 400 mg/kg dose produced significant anti-inflammatory activity on comparison with inflammation control .The percentage inhibition of Paw Oedema was found 85.23%,87.60% and 88.37% at 100 ,200 and 400mg/kg, when treatment with Ethanol Extract. Diclofenac Sodium produced a significant anti-inflammatory activity (98.82% inhibition of paw edema) with respect to inflammation control. Treatment with n-hexane extract at 100, 200 and 400 mg/kg dose produced significant anti-inflammatory activity on comparison with inflammation control .The percentage inhibition of paw edema was found 82.37%,83.22% and 85.81% at 100 ,200 and 400mg/kg, when treatment with Ethanol Extract. Diclofenac sodium produced a significant anti-inflammatory activity (96.79% inhibition of paw edema) with respect to inflammation control.

Treatment with Ethanol Extract at 100, 200 and 400 mg/kg dose produced significant anti-inflammatory activity on comparison with inflammation control. The percentage inhibition of dry weight of cotton was found 5.25, 10.25 and 12.04% at 100, 200 and 400mg/kg, when treatment with Ethanol Extract and Diclofenac Sodium produced a significant anti-inflammatory activity (13.77% inhibition of cotton) with respect to inflammation control. Wet Weight of Cotton was found 47.83, 53.38 and 56.83% at 100, 200 and 400 mg/kg, when treatment with Ethanol Extract and Diclofenac Sodium produced a significant anti-inflammatory activity (61.78% inhibition of Weight of Cotton) with respect to inflammation control.

Table 1. Anti-inflammatory Activity of Ethanol Extract of *Bryophyllum Calycinum* by Carrageenan Induced Rat Paw Oedema Methods in Rats.

Treatment	Dose mg/kg	%of inhibition after 3hr
Control	0.4ml	-
Diclofenac Sodium	25mg/kg	96.79
Ethanol Extract	100mg/kg	78.71
Ethanol Extract	200mg/kg	89.52
Ethanol Extract	400mg/kg	91.80

The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant.

Table 2. Anti-inflammatory Activity of Chloroform Extract of *Bryophyllum Calycinum* by Carrageenan Induced Rat Paw Oedema Methods in Rats.

Treatment	Dose mg/kg	%of inhibition after 3hr
Control	0.4ml	-
Diclofenac Sodium	25 mg/kg	96.79
Chloroform Extract	100 mg/kg	85.23
Chloroform Extract	200 mg/kg	87.60
Chloroform Extract	400 mg/kg	88.37

The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant.

Table 3. Anti-inflammatory Activity of n-Hexane Extract of *Bryophyllum Calycinum* by Carrageenan Induced Rat Paw Oedema Methods in Rats.

Treatment	Dose mg/kg	% of inhibition after 3hr
Control	0.4ml	-
Diclofenac Sodium	25 mg/kg	96.79
n-hexane Extract	100 mg/kg	82.31
n-hexane Extract	200 mg/kg	83.22
n-hexane Extract	400 mg/kg	85.81

The data obtained from various groups were statistically analyzed using one-way ANOVA followed by Tukey's Multiple Range Test. $p<0.05$, is considered statistically significant.

Table 4. Anti-inflammatory Activity of Ethanol Extract of *Bryophyllum calycinum* by Cotton Pellet Induced Granuloma in Rats.

Treatment	Dose	Wet weight of Cotton Pellet (mg)	% Inhibition	Dry Weight of Cotton Pellet (mg)	% Inhibition
Control	0.01ml	119.3±2.16	-	57.31±1.53	-
Diclofenac Sodium	10mg/kg	68.61±1.26	61.78	24.95±1.77	13.77
Ethanol Extract	100mg/kg	85.26±1.35	47.83	29.83±1.03	5.25
Ethanol Extract	200mg/kg	78.61±1.92	53.38	26.97±1.82	10.25
Ethanol Extract	400mg/kg	74.52±1.26	56.83	25.94±1.96	12.04

DISCUSSION

The preliminary phytochemical screening of the ethanol extract of whole of plant *Bryophyllum calycinum* revealed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, Phenol, free amino acid and terpenoids. The presence of the various phytochemical constituents in the plant namely steroids, alkaloids, tannins, flavonoids, glycosides, Phenol, free amino acid and terpenoids showed the plant to be a potential source of crude drug that can positively serve as source of modern drugs. Flavonoids of medicinal plant origin were found to possessed significant pharmacological activities, analgesic and anti-inflammatory among others in the animal body systems.

Carrageenan Induced hind paw oedema is the standard experimental model of acute inflammation. Carrageenan is the

phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover the experimental model exhibits a high degree of reproducibility. Carrageenan induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substance which peak at 3 hrs. The *Bryophyllum calycinum* n-hexane, chloroform and ethanol extract of peel were evaluated by carrageenan induced rat paw edema. They produced significant inhibition of rat paw edema induced by carrageenan on dose dependent. The inhibition was however, less than that of standard drug Diclofenac Sodium. From these overall results, we can conclude that the 100, 200 and 400 mg/kg of Ethanol Extract of peels of *Bryophyllum calycinum* possesses significant anti-inflammatory.

This can be useful for the treatment of acute pain and local inflammation. Preliminary phytochemical investigation of this plant showed the presence, phytosterols, flavonoids, which might be in part responsible for analgesic and anti-inflammatory effects. It could be concluded and confirmed that the ethanol extracts of plant of *Bryophyllum calycinum* has maximum anti-inflammatory effects comparable with other extracts, Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the anti-inflammatory effects activity and study its pharmacological actions.

As discussed in the introduction section, the flavonoids hesperidin and naringin are found in *Bryophyllum calycinum.* The flavonoids and their respective aglycones viz. hesperetin and naringenin may be shown potent anti-inflammatory activity against different *in vitro* and *in vivo* models as discussed earlier. The plant *Bryophyllum calycinum* contains high quantity of flavonoids of proven anti-inflammatory potential. Therefore, it can be concluded that due to the presence of the flavonoids *Bryophyllum calycinum* can act as potent anti-inflammatory agents.

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