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In Vitro Anti Inflammatory and Anti Arthritic Activity of *Commelina benghalensis* L. Leaves

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ABSTRACT

Introduction

Commelina benghalensis L. commonly known as **Benghal dayflower**, belongs to the family Commelinaceae. It is widely used for the treatment of wounds and skin diseases.

Aim

The current study focuses on the evaluation of in vitro anti-inflammatory and antiarthritic property of the leaf extracts of *Commelina benghalensis* L.

Methods

The hydroalcoholic extract (70%) of *Commelina benghalensis* L. (Leaf) was subjected to anti-inflammatory and anti arthritic activity by membrane stabilisation and inhibition of protein denaturation method were determined.

Results

The inhibitory concentration (IC₅₀) of HAECB in HRBC membrane stabilization study was found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml. It showed moderate anti-inflammatory activity. The inhibitory concentration (IC₅₀) of HAECB in protein denaturation was found to be 17µg/ml in comparison with diclofenac sodium 14µg/ml. It showed moderate anti-arthritic activity.

Conclusion

HAECB showed moderate anti-inflammatory activity which may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins and phenols. HAECB has shown moderate anti-arthritic activity which may be due to the phenolic constituent.

Keywords: Anti-inflammatory, Anti-arthritic, *Commelina benghalensis* L.

INTRODUCTION

Medicinal and culinary herbs are rich sources of anti-inflammatory compounds such as flavonoids.

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling

and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, fever, ischemic heart diseases and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vasculitis, celiac diseases, auto-immune diseases etc. Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For these reason, there is a need for anti-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is a lack of proper scientific evidence [1].

Leukocytes, the key players of inflammatory response, can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes. Destruction is caused by free radicals generated in activated leukocytes (neutrophils and monocytes) and lysosomal enzymes. Enzymes and reactive oxygen species may be released into the extracellular environment where it acts as mediators of inflammation. Such mediators are mainly arachidonic acid metabolites, generated through Cyclooxygenase and Lipoxygenase pathways. Most of the anti-inflammatory drugs are targeted on these pathways.

In a different approach, rather than blocking a particular mediator or its pathway, preventing the release of inflammatory mediators could be considered as a better option. The possibility of this approach is revealed in this research by studying the ability of the plant extract to prevent the lysosomal membrane destruction. An effective way to study this activity in vitro is to study the HRBC membrane stabilization activity of the plant extract. Lysosomal membrane and RBC membrane are similar in structure apart from the fact that luminal surface of the lysosomal membrane contains a glycoprotein coat which protects the membrane from digestion by lysosomal acid hydrolases. This method has been used in most preliminary anti-inflammatory screening procedures [2].

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation and cartilage destruction and commonly lead to significant disability. According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the diseases compared to males. It caused by no of proinflammatory molecules released by macrophages including reactive oxygen species and eicosanoids such as prostaglandins, leukotrienes and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like COX and LOX are the potential target for chronic inflammatory conditions. Even though various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, the potential side effects give a limitation for their use. Now it is a growing concern for the development of new safe, potent, less toxic antiarthritic drug. Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded [3].

Commelina benghalensis L. commonly known as **Benghal day flower**, belongs to the family Commelinaceae. *Commelina benghalensis* L. is a perennial herb native to tropical Asia and Africa.

Valaiyans of Piranmalai hills, Tamilnadu used the leaves for the treatment of rabies and wounds [4&5]. Bangladesh the kavirajes tribals used the young leaves for external poisoning [6].

The phytochemical screening of previous studies of *Commelina benghalensis* L. revealed the presence of tannins, phlobatannins, saponins, flavonoids, alkaloids, steroids and flavonoids, carbohydrates, phytosterol, terpenoids, quinon, volatile oil, anthraquinone [7-15]. GC-MS analysis of *Commelina benghalensis* L. revealed the presence of bioactive compounds such as 3-dodecene, 1-hexadecanol, 9-eicosene and tetratriacontane, Phenol 2,4 bis(1,1 dimethyl ethyl), hexadecen-1-ol trans-9, 9,10 anthracenedione, tetracosane, 1,4 benzene-dicarboxylic acid, bis (2ethylhexyl) ester, 13 docosenamide, tetracosane 11 decyl [16].

The plant exhibited various pharmacological activities such as anti inflammatory activity [9], 15-lipoxygenase inhibition, anticoagulant activity [13&14], antibacterial activity [15,19&20], antimicrobial activity [17&18], antiplasmodial activity, thrombolytic and cytotoxic activity and antidiarrhoeal & anthelmintic activity [21-23].

The current study focuses on the evaluation of in vitro anti-inflammatory and antiarthritic property of the leaf extracts of *Commelina benghalensis* L.

MATERIALS AND METHODS

Plant Collection & Authentication

Fresh leaf of *Commelina benghalensis* L. were collected from Madurai Medical College, Madurai (DT), during the month of August- 2017 and was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Assistant professor, Department of Botany, American College, Madurai-20. The herbarium of this specimen was kept in the department for further reference.

Preparation of Hydroalcoholic Extract of *Commelina Benghalensis* L.

Procedure

The leaves were collected, shade dried was powdered coarsely and was defatted with petroleum ether (60-80°C). The residue was dried and extracted with hydroalcohol (70%) by maceration until the complete extraction of the powder was filtered and concentrated under reduced pressure to obtain a solid residue (dark brown). (HAECB)

Invitro Anti-Inflammatory Activity Screening By Membrane Stabilization Study

Hydroalcoholic extract was subjected to in vitro anti inflammatory and anti arthritic activity as per Sadique et al., Oyedapo et al., [24-27]

PROCEDURE

Preparation of HRBC suspension in isosaline

The human erythrocytes suspension was used for the *in-vitro* membrane stabilization assay. Blood was collected from the healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. The blood was mixed with equal volume of Alsever's solution (2% dextrose, 8.0% sodium citrate, 0.5% citric acid, 0.42% sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% v/v erythrocyte suspension in isosaline was prepared.

Membrane Stabilization Study

The assay mixture was prepared with 2 ml of hypoosaline and 1 ml of phosphate buffer and varying volumes (0.1 to 0.5 ml) of HAECB extract at different concentration (10, 20, 30, 40, 50 µg/ml) and 0.5 ml HRBC suspension in isosaline, then the final volume were made upto 4.5 ml with isosaline. The control was prepared as mentioned above without the test extract, while product control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56°C for 30 min in a water bath, then the tube was cooled under running water and the absorbance of the released haemoglobin was measured at 560 nm. Diclofenac sodium was used as a reference standard. The percentage membrane stabilization activity of the compounds were determined by the formula

$$\% \text{ Membrane stabilization} = \frac{[A_{\text{control}} - (A_{\text{test}} - A_{\text{product control}})]}{A_{\text{control}}} \times 100$$

Where

- A_{control} - Absorbance in control
 A_{test} - Absorbance in test
 $A_{\text{product control}}$ - Absorbance in product control

The results are displayed in (Figure I) and tabulated in (Table I)

Invitro Antiarthritic Activity By Protein Denaturation Method

Hydroalcoholic extract was subjected to in antiarthritic activity ed by inhibition of protein denaturation method as per Lavanya et al., and Shravan Kumar et al.,[28&29].

Experimental Protocol

The following four solutions were prepared

Test solution (0.5 ml)

The test solution consists of 0.45 ml bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of

HAECB (10, 20, 30, 40, and 50 µg/ml concentration).

Test control solution (0.5 ml)

The test control solution consists of 0.45 ml bovine serum albumin and 0.05 ml distilled water.

Product control (0.5 ml)

The product control consists of 0.45 ml of distilled water and 0.05 ml of HAECB (10, 20, 30, 40, and 50 µg/ml concentration).

Standard solution

Standard solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of Diclofenac sodium solution.

PROCEDURE

All the above test samples was adjusted to p^H 6.3 using a small amount of 1N hydrochloric acid. They were incubated at 37° C for 20 minutes and heated at 57° C for 3 minutes. Allow to cool and about 2.5 ml of phosphate buffer (p^H 6.3) was added to all the above solution. The absorbance was measured using UV spectrophotometer at 416 nm. The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Percentage inhibition} = 100 - \frac{\text{OD of test solution} - \text{OD of product control}}{\text{OD of test control}} \times 100$$

The control represents 100% protein denaturation. The results were compared with the standard drug diclofenac sodium treated sample. The results are displayed in (Figure II) and tabulated in (Table II)

RESULTS AND DISCUSSION

Anti-Inflammatory Activity

The inhibitory concentration (IC₅₀) of HAECB in HRBC membrane stabilization study is found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml.

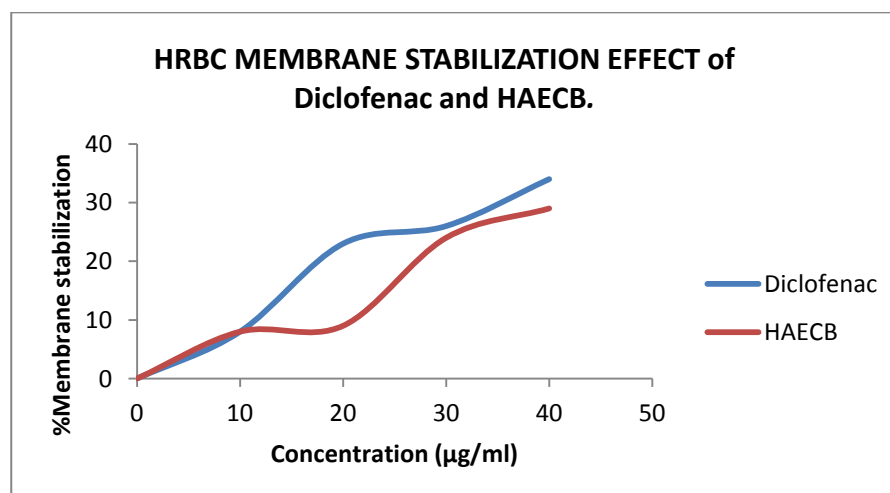


Figure I: Percentage of Membrane Stabilization By Diclofenac Sodium And HAECB

Table I: Percentage of Membrane Stabilization By Diclofenac Sodium and HAECB

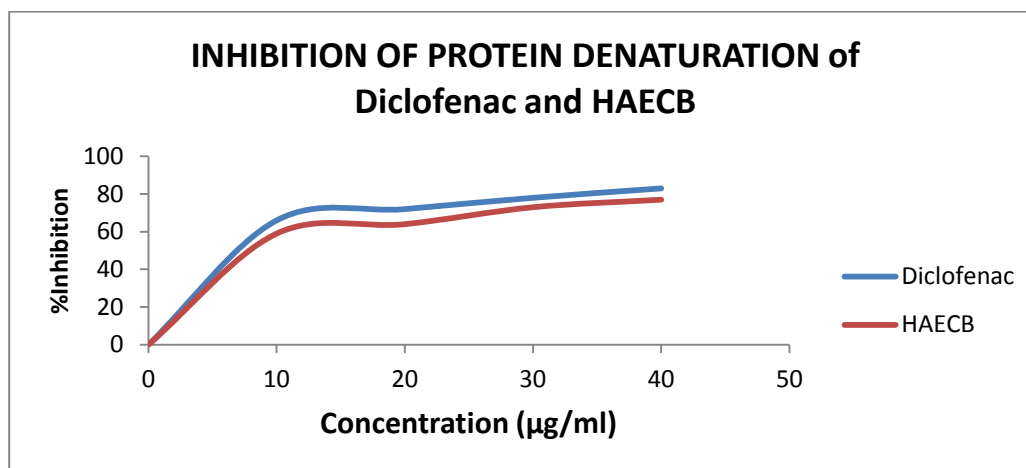
S.NO	Concentration($\mu\text{g/mL}$)		Percentage Membrane stabilization of Diclofenac	Percentage Membrane stabilization of HAECB
	Diclofenac	HAECB	Mean \pm SEM*	Mean \pm SEM*
1	10	10	8.14 \pm 0.0088	7.7 \pm 0.0577
2	20	20	22.75 \pm 0.0088	9 \pm 0.0577
3	30	30	25.9 \pm 0.0088	23.5 \pm 0.1453
4	40	40	34.4 \pm 0.3333	29.3 \pm 0.0882
		IC ₅₀	57 $\mu\text{g/mL}$	69 $\mu\text{g/mL}$

*Mean of three readings \pm SEM

The hydroalcoholic extract of *Commelina benghalensis* L. showed mild anti-inflammatory activity in comparison with diclofenac used as reference..

ANTI-ARTHRITIC ACTIVITY

The inhibitory concentration (IC₅₀) of HAECB in Protein denaturation is found to be 17 $\mu\text{g/mL}$ in comparison with diclofenac sodium 14 $\mu\text{g/mL}$.

**Figure 2 Inhibition of Protein Denaturation of *Commelina benghalensis* L.****Table II: Effect of HAECB And Diclofenac Sodium on Inhibition of Protein Denaturation**

S.NO	Concentration ($\mu\text{g/mL}$)		Percentage Inhibition of Diclofenac	Percentage Inhibition of HAECB
	Diclofenac	HAECB	Mean \pm SEM*	Mean \pm SEM*
1	10	10	65.6 \pm 0.3464	59.4 \pm 0.2309
2	20	20	71.9 \pm 0.5774	64.1 \pm 0.0882
3	30	30	78.1 \pm 0.0882	73.4 \pm 0.2309
4	40	40	82.8 \pm 0.5774	76.6 \pm 0.1732
		IC ₅₀	14 $\mu\text{g/mL}$	17 $\mu\text{g/mL}$

*Mean of three readings \pm SEM

The hydroalcoholic extract of *Commelina benghalensis* L. showed mild anti-arthritic activity in comparison with diclofenac used as reference..

CONCLUSION

At the site of inflammation, HAECB may possibly inhibit the release of lysosomal content of neutrophils (bactericidal enzymes and proteinases) which upon extracellular release cause further tissue inflammation and damage [30]. In the present study, results indicate that the HAECB possesses significant anti-inflammatory properties which may be due to the strong occurrence of

polyphenolic compounds such as flavonoids, tannins and phenols. HAECB showed significant antiarthritic activity by inhibition of protein denaturation. HAECB had shown significant anti-arthritic activity and the phenolic constituent may be responsible for this activity.

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REFERENCES

- [1]. Habibur Rahman, Chinna Eswaraiah M and Dutta AM. In-vitro Anti-inflammatory and Anti-arthritic Activity of *Oryza sativa* Var. Joha Rice (An Aromatic Indigenous Rice of Assam). *American-Eurasian J. Agric. & Environ. Sci.* 15(1), 2015, 115-121.
- [2]. Anilkumar M, and Jibin Johny. Evaluation of In Vitro Anti-Inflammatory Activity of the Methanolic Extract of *Litsea quinqueflora* (Dennst.) Suresh. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*. 10(2), 2015, 32-36.
- [3]. Kore KJ*, Shete RV, Desai NV. Anti-Arthritic activity of Hydroalcoholic extract of *Lawsonia Innermis*. *International Journal of Drug Development & Research*. 3(4), 2011, 217-224.
- [4]. Sandhya B, Thomas S, Isabel W and Shenbagarathai R. Ethnomedicinal Plants used by the Valaiyan Community of Piranmalai Hills (Reserved Forest), Tamilnadu, India. - A Pilot Study. *Afr. J. Trad. CAM*. 3(1), 2006, 101-114.
- [5]. Gupta A*, Nagariya AK, Mishra AK, Bansal P, Kumar S, Gupta V, Singh AK. Ethno-potential of medicinal herbs in skin diseases: An overview. *Journal of Pharmacy Research*. 3(3), 2010, 435-441.
- [6]. Mahabub Nawaz AH Md., Maruf Hossain, Masud Karim, Mujib Khan, Rownak Jahan, Mohammed Rahmatullah. An ethnobotanical survey of Rajshahi district in Rajshahi division, Bangladesh. *American-Eurasian Journal of Sustainable Agriculture*. 3(2), 2009, 143-150.
- [7]. Udaya Prakash NK, Jahnavi B, Abhinaya K, Gulbsy Rajalin A, Sekar Babu H, Prathap Kumar M, Upendra Reddy K, Dushyanth K Reddy, Sundraraman G, Elumalai K, Devipriya S, Kannan V, Sriraman V, Kalaivani R.A, Thanmathi M, Kathiravan Gand Bhuvaneswari S. Phytochemical Analysis of Common Weeds of Northern Districts in Tamil Nadu. *Intl. J. of Appl. Biol.* 2(1), 2011, 25-28.
- [8]. Dhole JA, Dhole NA, Lone KD and Bodke SS. Preliminary Phytochemical Analysis of Weeds in Marathwada Region. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 3(4), 2012, 764-767.
- [9]. Sanjeev Kumar Tiwari, Mangala Lahkar, Suvakanta Dash, Pavan Kumar Samudrala, Jaya Mary Thomas, Bibin Baby Augustine. Preliminary phytochemical, toxicity and anti-inflammatory evaluation of *Commelina benghalensis*. *International Journal of Green Pharmacy*. 2013, 201-205.
- [10]. Prakash Rao S, Venkata Rami Reddy K and Prayaga Murty P*. Preliminary Phytochemical Screening of Some Weed Species of Kadapa District, Andhra Pradesh, India; Research and Reviews: *Journal of Botanical Sciences*. 3(1), 2014, 19-22.
- [11]. Kharade Amit S, Jadhav Sangita S, Jadhav SN, Thite SV and Aparadh VT. Phytochemical investigation in *Commelina benghalensis* & *Cyanotis cristata*. *International Research Journal of Pharmaceutical and Applied Science*. 3(1), 2013, 46-48.

- [12]. Udaya Prakash NK*, Bhuvaneswari S, Balamurugan A, Radhika B, Bhagya R, Sripriya N, Prameela L, Sarojini S, Vigneshwari R, Chandran M and Arokiyaraj S. Studies on Phytochemistry of 100 Plants in Chennai, India. *British Journal of Pharmaceutical Research*. 3(3), 2013, 407-419.
- [13]. Cean Socorro M. Alaba, Christine L. Chichioco-Hernandez*. 15-Lipoxygenase inhibition of *Commelina benghalensis*, *Tradescantia fluminensis*, *Tradescantia zebrina*. *Asian Pacific Journal of Tropical Biomedicine*. 4(3), 2014, 184-188.
- [14]. Rathnakar Reddi K.V.N, Suman Joshi DSD, Satya Prasad M, Krishna Satya A. Studies on Efficacy of Medicinal Plants Against The Lethality of *Naja Naja* Snake Envenomation. *Journal of Pharmaceutical and Scientific Innovation*. 5(5), 2016, 168-173.
- [15]. Sumithra D* and Sumithra Purushothaman. Phytochemical screening and Antibacterial Activity of Leaf Extract of *Commelina Benghalensis* L. *European Journal of Biomedical and Pharmaceutical sciences.ejbps*, 4(6), 2017, 309-313.
- [16]. Sumithra D* and Sumithra Purushothaman. Phytochemical profiling of ethanolic leaves extract of *Commelina benghalensis* L. *World Journal of Pharmaceutical Research*. 6(2), 2017, 1101-1107.
- [17]. Mukesh Chandra Sharma and Smita Sharma. Preliminary Phytochemical and Antimicrobial Investigations of the Aqueous extract of *Ixora Coccinea* Linn and *Commelina benghalensis* L. on Gram-positive and Gram Negative Microorganisms. *Middle-East Journal of Scientific Research*. 6(5), 2010, 436-439.
- [18]. Gothandam KM*, Aishwarya R, Karthikeyan S. Preliminary Screening Of Antimicrobial Properties of Few Medicinal Plants. *Journal of Phytology*. 2(4), 2010, 01-06.
- [19]. Rajesh F Udgirkar *, Parvin Kadam, Nikhil Kale. Antibacterial Activity of Some Indian Medicinal Plant: A Review. *International Journal of Universal Pharmacy and Bio Sciences*. 1(1), 2012, 01-08.
- [20]. Joy Prabu H and Johnson I. Antibacterial activity of silver nanoparticles synthesized from plant leaf extract of *Cycas circinalis*, *Ficus amplissima*, *Commelina benghalensis* and *Lippia nodiflora* leaves. *Journal of Chemical and Pharmaceutical Research*. 7(9), 2015, 443-449.
- [21]. Njan Nloga AM, Ngo Yebga J, Ngo Bum E. Antiplasmodial Effect of *Commelina benghalensis*/*Steganotaenia araliacea* Plants Extract on the Human Population in Ngaoundere (Cameroon). *Journal of Medical Sciences*. 14(2), 2014, 68-74.
- [22]. Tanvir Ahmad Chowdhury, Abul Hasanat*, Jakaria Md, Mostofa Kamal ATM, Mohammad Shah Hafez Kabir, Shakhawat Hossain Md, Arafatul Mamur, Mohammed Munawar Hossain. Thrombolytic and Cytotoxic Activity of Methanolic Extract of *Commelina benghalensis* (Family: Commelinaceae) Leaves. *Journal of Scientific and Innovative Research*. 4(2), 2015, 100-104.
- [23]. Mohammad Shah Hafez Kabir, Abul Hasanat, Tanvir Ahmad Chowdhury, Mohammad Mamun Ur Rashid*, Mohammed Munawar Hossain and Shabbir Ahmed. Study of antidiarrheal and anthelmintic activity methanol extract of *Commelina benghalensis* leaves. *African Journal of Pharmacy and Pharmacology*. 10(32), 2016, 657-664.
- [24]. Sadique J, Al-Rqobahs WA, Bughaith MF, El-Gindi AR. The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia*, 60, 1989, 525-532.
- [25]. Amujoyegbe OO, Agbedahunsi JM., Akinpelu BA, Oyedapo OO. *In vitro* evaluation of membrane stabilizing activities of leaf and root extracts of *Calliandra portoricensis* (JACQ) benth on sickle and normal human erythrocytes. *International Research Journal of Pharmacy and Pharmacology*. 2(8), 2012, 198-203.
- [26]. Akinpelu, Bolajoko Ayinke, Makinde, Amos Morakinyo, Amujoyegbe, Olayinka Olajumoke, Isa, Musibau Olalekan, Onwobiko, Victor Chinemerem, Akinwotu, Akintunde Oluwadare, Oladimeji, Ezekiel Seun, Agbedahunsi, Joseph Morunfolu, Oyedapo, Oluokun Oluboade. Evaluation of Anti-inflammatory and Antisickling Potentials of *Archidium ohioense* (Schimp. ex Mull) Extracts. *IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)*. 12(1), 2017, 18-26.
- [27]. Oyedapo, Oluokun Oluboade*, Makinde, Amos Morakinyo, Ilesanmi, Gbemisola Monijesu, Abimbola, Eunice Olasunmbo, Akinwunmi, Kemi Feyisayo, and Akinpelu, Bolajoko Ayinke. Biological Activities (Anti-Inflammatory and Anti-Oxidant) of Fractions and Methanolic Extract of *Philonotis Hastata* (Duby Wijk & Margadant). 12(4), 2015, 50-55.

- [28]. Lavanya R*, Uma Maheshwari S, Harish G, Bharath Raj J, Kamali S, Hemamalani D, Bharath Varma J, Umamaheswara Reddy C. *In-vitro* anti-oxidant, anti-inflammatory and anti-arthritic activities in the leaves of *Coldenia procumbens* Linn. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 1(4), 2010, 753-762.
- [29]. Shravan kumar N, Kishore G, Sivakumar G, Sindhu priya ES. *In-vitro* Anti-inflammatory and Anti-arthritic activity of leaves of *Physalis angulata* L. *Int. J. Pharm & Ind. Res*. 01(03), 2011, 211-213.
- [30]. Chou CT. The anti-inflammatory effect of *Tripterygium wilfordii* Hook F on adjuvant induced paw edema in rats and inflammatory mediators release. *Phytother Res*. 11, 1997, 152-154.