ANTI-INFLAMMATORY ACTIVITIES OF EUCALYPTUS GLOBULUS PLANT EXTRACTS

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ABSTRACT: E. globulus is commonly used in traditional medicine because of its expectorant and balsamic activity. A systematic investigation was undertaken to screen the phytochemical and anti-inflammatory activity of Eucalyptus Globulus plant. In present study anti-inflammatory activity of acetonic extracts of Eucalyptus globules were investigated by disc diffusion assay against a panel of bacteria and fungi. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate of acetonic extract.

Key Words- Anti-inflammatory Activities, Acetonic Extract, Eucalyptus globules.

INTRODUCTION-

The ultimate goal of modern drug discovery is to identify a therapeutic agent that is effective against a disease. Although the process of drug discovery is a complex issue, it may be divided into three main steps; **i)** Development of relevant biological system for testing of the compounds *in vitro* and *in vivo*; **ii)** Identification of lead compounds for concept test in the biological assays; **iii)** Optimization of the lead structure to enhance the selectivity ratio, toxicity profile or pharmacokinetics, and ultimately furnish a candidate drug suitable for appropriate *in vivo* studies and further clinical evaluation [1,2,3,4].

In the last few years there has been an exponential growth in the field of herbal medicine and these drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. Many traditional medicines in use are derived from medicinal plants, minerals and organic matter [**5**]. A number of medicinal plants, traditionally used for over 1000 years named rasayana are present in herbal preparations of Indian traditional health care systems [**6]**. In Indian systems of medicine most practitioners formulate and dispense their own recipes [**7]**. The World Health Organization (WHO) has listed 21,000 plants, which are used for medicinal purposes around the world. Among these 2500 species are in India, out of which 150 species are used commercially on a fairly large scale. India is the largest producer of medicinal herbs and is called as botanical garden of the world. The current chapter focuses on herbal drug preparations and plants used in the treatment of diabetes mellitus, a major crippling disease in the world leading to huge economic losses.

Considering the vast area of potentiality of plants as sources for drugs and taking into account the local traditional uses, a systematic investigation was undertaken to screen the phytochemical and anti-inflammatory activity of Eucalyptus *Globulus plant* [8].

The essential oil of *E. globulus* is commonly used in traditional medicine because of its expectorant and balsamic activity. The leaves could also be a promising source of phenolic compounds, which can be used for possible applications in food, pharmaceutical and cosmetic industries [9].

MATERIALS AND METHODS

E. globulus leaves were collected since March a June of 2015 around Desh Bhagat University, Mandi Gobindgarh (Punjab), India. The plant material was air dried for 10 days and stored at ambient temperature (25±1ºC) without exposure to direct sunlight.

Extraction method

The collected plant material was shade dried at room temperature. And after proper dryness of plant material it converted into powder. Prepared powdered plant material (2g, 4g and 6 g respectively) was transferred to dark-coloured flasks and mixed with 40 ml of solvents with different polarities (water, methanol, acetone) respectively and stored at room temperature. After 24, infusions were filtered through Whatman No. 1 filter paper and residue was re-extracted with equal volume of solvents. After 48 h, the process was repeated. Combined supernatants were evaporated to dryness under vacuum at 40 ^{0}C using Rotatory evaporator. The obtained extracts were kept in sterile sample tubes and stored in a refrigerator at 4 ^{0}C.

Paper Disc Diffusion Technique

The sterilized medium [10] (autoclaved at 120 °C for 30 min) (40–50 °C) was inoculated (1 ml/100 ml of medium) with the suspension (105 cfu/ml) of the micro-organism (matched to 0.9 Mc Farland barium sulphate standard) and poured into a Petri dish to give a depth of 3–4 mm. The paper impregnated with the test extract was placed on the solidified medium. The plates were pre-incubated for 2 h at room temperature (24˚C) and incubated at 37–28 °C for 24 h for anti-inflammatory. Diclofenac sodium was used as standard for anti-inflammatory.

Minimum Inhibitory Concentration (MIC)

MIC [11] of the extract was determined by agar streak dilution method. A stock solution of the extract in DMSO was prepared and graded quantities of the test compounds incorporated in specified quantity of Muller Hinton agar for anti-bacterial activity and Sabouraud dextrose agar medium for anti-fungal activity. A specified quantity of the medium (40°C) containing the compound was poured into a Petri dish to give a depth of 3 mm and allowed to solidify.

Suspension of the micro-organism was prepared to contain approximately 105 cfu/ml and applied to plates with serially diluted compounds in DMSO to be tested and incubated at 30°C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth of bacteria or fungi on the plate.

RESULT AND DISCUSSION-

Phyto-chemical screening of Eucalyptus globules-

Results of the chemical screening	
Metabolites	***Eucalyptus globules***
Saponosides	+
Anthocyanins	+
Leuco-anthocyanins	-
Alkaloids	-
Flavonoids	+
Tannins	+

Determination of Total Phenols-

The determination of the content in total phenolics in the hydro-methanolique of Eucalyptus globulus extract is made by using the colorimetric method of Folin-Ciocalteux, this content has been reported in Gallic acid equivalent mg/g of dry plant material. The results show that alcoholic extracts of Eucalyptus species have a high content of total phenols (79, 35 and 105, 31 mg GAE/gDM).

TOTAL PHENOLIC

Hydro-methanolic extract	Yield	Total Phenolics
Eucalyptus globules	18%	105.31 mg GAE/gDM

Pharmacology

The extracted compounds were evaluated for anti-inflammatory activities. The extract compounds and the standard drugs were administered in the form of a suspension (1% carboxy methyl cellulose as a vehicle) by oral route of administration for anti-inflammatory. Each group consisted of six animals. The animals were maintained in colony cages at 25 ± 2 °C, relative humidity of 45–55%, under a 12 h light and dark cycle; they were fed standard animal feed. All the animals were acclimatized for a week before use [12].

Anti-inflammatory Activity

Anti-inflammatory activity was evaluated by carrageenan-induced paw oedema test in rats [13]. The anti-inflammatory activity data indicated that all extract compounds protected from carrageenan-induced inflammation. The compound **AE3** showed more potent anti-inflammatory activity and the compound **AE2** showed equipotent anti-inflammatory activity when compared to the reference standard diclofenac sodium.

Percent Anti-inflammatory Activity of Test Compounds

Compound	Dose (mg/kg)	Percent Protection			
		30 min	1 h	2 h	3 h
AE1	10	35 ± 1.33*	46 ± 1.29*	35 ± 1.12*	26 ± 1.23*
	20	38 ± 1.23*	42 ± 1.71**	45 ± 1.72**	36 ± 1.60*
AE2	10	36± 1.38*	32 ± 1.90*	39 ± 1.81*	32 ± 1.23*
	20	47 ± 1.27*	52 ± 1.56***	56 ± 1.36***	36 ± 1.82*
AE3	10	35 ± 1.96*	41 ± 1.06*	45 ± 1.23*	33 ± 1.22*
	20	45 ± 1.92**	58 ± 1.28***	60 ± 1.72***	41 ± 1.76*
AE4	10	29 ± 1.02*	33 ± 1.09*	36 ± 1.27*	27 ± 1.22*
	20	32 ± 1.19*	42 ± 1.42*	42 ± 1.51*	35 ± 1.70*
AE5	10	31 ± 1.52*	35 ± 1.47*	38 ± 1.72*	24 ± 1.41*
	20	38 ± 1.71*	42 ± 1.82*	46 ± 1.32**	33 ± 1.71*
AE6	10	28 ± 1.23*	33 ± 1.23*	35 ± 1.22*	26 ± 1.36*
	20	36 ± 1.72*	34 ± 1.26*	45 ± 1.30*	35 ± 1.55*
AE7	10	27 ± 1.72*	30 ± 1.53*	31 ± 1.90*	22 ± 1.27*
	20	34 ± 1.90*	36 ± 1.82*	39 ± 1.23*	30 ± 1.80*
AE8	10	28 ± 1.27*	32 ± 1.22*	35 ± 1.22*	29 ± 1.82*
	20	33 ± 1.91*	40 ± 1.97*	44 ± 1.81*	26 ± 1.06*
AE9	10	21 ± 1.08*	28 ± 1.19*	26 ± 1.08*	20 ± 1.22*
	20	29 ± 1.13*	32 ± 1.17*	35 ± 1.26*	23 ± 1.81*
AE10	10	26 ± 1.46*	28 ± 1.84*	34 ± 1.91*	23 ± 1.47*
	20	25 ± 1.84*	33 ± 1.92*	37 ± 1.87*	25 ± 1.26*
AE11	10	27 ± 1.29*	29 ± 1.93*	34 ± 1.27*	22 ± 1.32*
	20	32 ± 1.34*	33 ± 1.28*	36 ± 1.82*	30 ± 1.26*
AE12	10	25 ± 1.26*	28 ± 1.71*	34 ± 1.26*	20 ± 1.18*
	20	37 ± 1.17*	38 ± 1.26*	36 ± 1.23*	35 ± 1.71*
AE13	10	25 ± 1.27*	34 ± 1.80*	32 ± 1.22*	24 ± 1.06*
	20	33 ± 1.83*	37 ± 1.23*	35 ± 1.08*	38 ± 1.25*

AE14	**10**	**34 ± 1.08***	**35 ± 1.93***	**33 ± 1.41***	**28 ± 1.07***
	20	**32 ± 1.42***	**39 ± 1.26***	**46 ± 1.17***	**30 ± 1.25***
AE15	**10**	**26 ± 1.29***	**33 ± 1.73**	**34 ± 1.25***	**25 ± 1.18***
	20	**38 ± 1.35***	**38 ± 1.08****	**43 ± 1.92***	**38 ± 1.15***
Control		**5.4 ± 0.29**	**6.5 ± 0.27**	**5.8 ± 0.32**	**3.3 ± 0.93**
Diclofenac	**10**	**32 ± 0.63***	**37 ± 1.58***	**39 ± 1.97***	**37 ± 0.93***
	20	**48 ± 1.61****	**54 ± 0.92*****	**66 ± 1.52*****	**44 ± 1.36****

[a]Each value represents the means ± SD (n = 6). Significance levels $^{*}p < 0.5$, $^{**}p < 0.01$ and $^{***}p < 0.001$ as compared with the respective control; AE- Acetone Extract.

CONCLUSION-

Most previous studies of Eucalyptus *globules* have reported on the anti-inflammatory activity of oils [14-16] with variable results. This study uses acetone extracts to overcome the problems associated with the insolubility of oil components in agar gels. The broad range of microbial susceptibilities indicates the potential of these extracts as a surface disinfectant as well as for medicinal purposes and possibly as food additives to inhibit spoilage. However, further studies are needed before these extracts can be applied to these purposes. In particular, toxicity studies are needed to determine the suitability of these extracts for the use as antiseptic agents [16].

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