Available online at www.joac.info

ISSN: 2278-1862

Journal of Applicable Chemistry

2016, 5 (1): 179-184

(International Peer Reviewed Journal)

In Vitro Anti-inflammatory Activity of *Tagetes Erecta* Flower, *Cyano Dactylon* and *Curcuma Amada* Rhizome Extracts

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Accepted on 3rd December 2015

ABSTRACT

To evaluate the in vitro anti-inflammatory effect of Tagetes erecta flower, Cyanodon dactylon and Curcuma amada rhizome extract against the denaturation of protein. The extract at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance and viscosity to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the Tagetes erecta flower, Cyanodon dactylon and Curcuma amada rhizome extract. The effect of diclofenac sodium was found to be less when compared with the test extract. From the present study it can be concluded that Tagetes erecta flower, Cyanodon dactylon and Curcuma amada rhizome possessed marked in vitro anti-inflammatory effect. Among the different plant, Curcuma amada rhizome produced marked in-vitro anti-inflammatory activity than other plants.

Keywords: *In vitro*, *Tagetes erecta* flower, *Cyanodon dactylon* and *Curcuma amada* rhizome, protein denaturation.

INTRODUCTION

Inflammation is a bodily response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. It is triggered by the release of chemical mediators from injured tissue and migrating cells [1]. The commonly used drug for management of inflammatory conditions are non-steroidal anti-inflammatory drugs, which have several adverse effects especially gastric irritation leading to formation of gastric ulcers[2]. Natural products have contributed significantly towards the development of modern medicine. Recently traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The major merits of herbal medicine seem to be their perceived efficacy, low

incidence of serious adverse 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 [3]. For these reason, there is a need for ant-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. The objective of present work is to study the *in-vitro* anti-inflammatory activity of *Tagetes erecta* flower, *Cyano grass* and *Mango ginger* rhizome extract by protein denaturation method.

MATERIALS AND METHODS

Evaluation of *in vitro* anti-inflammatory activity: Anti-inflammatory activity of the *Tagetes erecta* flower, *Cyano grass* and *Mango ginger* rhizome extracts were evaluated by protein denaturation method as described by Padmanabhan and Jangle [4]. Diclofenac sodium, a powerful non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of *Tagetes erecta* flower, *Cyano grass* and *Mango ginger* rhizome extracts (100-500 µg/mL) or standard diclofenac sodium (100-500 µg mL^{-1}) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank. Each experiment was done in triplicate and the average was taken. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = \frac{At - Ac}{Ac} \times 100$$

Where, At=absorbance of test sample; Ac=absorbance of control

The plant concentration for 50% inhibition (IC_{50}) was determined by plotting percentage inhibition with respect to control against treatment concentration.

RESULTS AND DISCUSSION

To screen anti-inflammatory activity of extract of leaves of *Tagetes erecta flower, Cyano dactylon and Curcuma amada* rhizome extract by protein denaturation. The extracts at different concentrations was incubated with egg albumin in controlled experimental conditions and subjected to determination of absorbance to assess the anti-inflammatory property. Diclofenac sodium was used as the reference drug. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by the *Tagetes erecta flower, Cyano dactylon and Curcuma amada rhizome extracts*. The effect of *Shorea robusta* (500 µg/ml) was found to be close to diclofenac sodium. From the present study it can be concluded that *Tagetes erecta flower, Cyano dactylon and Curcuma amada rhizome extracts* showed marked *in vitro* anti Inflammatory effect against the denaturation of protein (Fig 1, Table 1). Among the different plant, *Curcuma amada rhizome* possess potential anti-inflammatory activity than other plants.

Table 1 *In vitro* anti-inflammatory activity *Tagetes erecta* flower, *Cyano grass* and *Mango ginger* rhizome extracts

Concentrations (µg mL^{-1})	*Cyano grass*	*Mango ginger* rhizome	*Tagetes erecta* flower	Standard (Diclofenac sodium)
100	16±1.15	21±2.8	17±1.08	23.25 ±1.53
200	26±1.75	39±3.2	31±2.1	46.45 ±2.64

300	36±1.8	61±4.2	44±2.45	62.78 ±4.8
400	64±4.55	82±5.6	61±2.8	78.25 ±5.8
500	71±4.9	85±5.95	79±5.6	91.89 ±6.32
IC_{50}	352	258.82	322.07	241.68

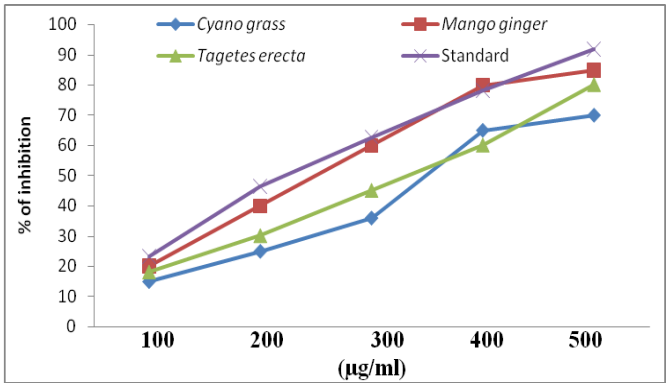

Fig 1 *In vitro* anti-inflammatory activity *Tagetes erecta* flower, *Cyano grass* and *Mango ginger* rhizome extracts

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair [5].It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations [6]. Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue [7]. However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis [8].

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. During inflammation, lysosomal hydrolytic enzymes are released into the sites which cause damages of the surrounding organelles and tissues with attendance variety of disorders [9]. Various methods were employed to screen and study drugs, chemicals, herbal reparations that exhibit anti-inflammatory properties or potentials. These techniques include uncoupling of oxidative phosphorylation (ATP biogenesis linked to respiration), inhibition of denaturation of protein, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation [10].

It is believed that current drugs available such as opoids and non –steroidal anti-inflammatory drugs (NSAIDS) are not useful in all cases of inflammatory disorders, because of their side effects and potency [11] .As a result, a search for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs [12]. Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs [13].

In the present study the protein denaturation bioassay was selected for *in vitro* assessment of antiinflammatory activity of ethanolic extract of *Tagetes erecta* flower, *Cyanodon dactylon*, *Curcuma amada* rhizome. Denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain rheumatic diseases may be due to in vivo denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding [14] Agents that can prevent protein denaturation therefore, would be worthwhile for antiarthritic drug development. The increments in absorbance of test samples with respect to control indicated stabilization of protein i.e. inhibition of heat-induced protein (albumin) denaturation by *Tagetes erecta* flower, *Cyanodon dactylon*, *Curcuma amada* rhizome and reference drug diclofenac sodiuma [15]. *Tagetes erecta* flower, *Cyanodon dactylon*, *Curcuma amada* rhizome extract contains flavonoids, tannins and a phenolic acid are known to promote anti-inflammatory activity [16]. From the present study it can be concluded that *Tagetes erecta flower, Cyano dactylon and Curcuma amada rhizome extracts* showed marked *in vitro* anti Inflammatory effect against the denaturation of protein.

Divya Singh *et al*[17] examined Anti-inflammatory and Anti-arthritic Activity of seed extract of *Pongamia pinnata* (L.) by *in vitro* model. The anti-arthritic and anti-inflammatory activity of *P. pinnata* hydroalcoholic extract was done by Inhibition of protein denaturation and Human red blood cell membrane stabilization (HRBC) in vitro methods. The hydroalcoholic extract of *P. pinnata* was subjected to in vitro Inhibition of protein denaturation in various concentrations i.e. 10, 50, 100, 200, 400, 800, 1000 and 2000µg/ml. HRBC method was also used for the estimation of anti-inflammatory activity from in various concentrations 100, 200, 400, 800 and 1600 µg mL^{-1}. *P. pinnata* hydroalcoholic extract exhibited a concentration dependent inhibition of protein (albumin) denaturation. The stabilization of HRBC membrane showed a concentration dependent anti-inflammatory activity, and the protection percent increased with increase in the concentration of the *P. pinnata* hydroalcoholic extract. The present study is support to the isolation and use of phytoconstituents from seed of *P. pinnata* in treatment of inflammation and arthritis.

Amar *et al* [18] reported that the *in vitro* anti-arthritic activity of *Cassia tora* Linn.leaves is using effect of membrane stabilization and protein denaturation using different concentration. The results are compared with standard drug. The aqueous extract of the selected medicinal plant showed significant activity. Anti-arthritic effect of *Cassia tora* Linn.leaves was studied by testing various in vitro studies. The effect of the selected plant on inhibition of protein denaturation and effect of membrane stabilization was 87.22 % and 87.25% respectively for the aqueous extract of the selected plant leaves. He concluded that *Cassia tora* possessed marked *in vitro* anti-inflammatory effect against the denaturation of protein

Susmitha Sudevan *et al*(2015) proved the anti-inflammatory activity of *Acmella Oleracea*. Anti-inflam matory agent is present in *Acmella Oleracea. Acmella Oleracea* can be considered as a resource for potential antiinflammatory and antimicrobial agents.

Sridevi *et al*[19] evaluated the anti-inflammatory effect of ethanolic extract of *Pergularia Daemai* (PD) by *in vitro* method by using membrane stabilization test and protein denaturation test. Membrane stabilization test was done by using human red blood cells (HRBCs). Protein denaturation test was done by using bovine serum albumin (BSA). The results revealed that PD extract was capable of rendering membrane stabilization by inhibiting the hypotonically-induced hemolysis of HRBCs in dose-dependent manner (50, 100, 200, 300, 400, 500 and 1000 µg/mL). In lesser concentration (50 µg mL^{-1}), the % inhibition of hemolysis was less (26.80%) and in higher concentration (1000 µg mL^{-1}), the % inhibition of hemolysis was more (76.30%), which was comparable with that of standard anti-inflammatory drug viz. diclofenac sodium (200 µg mL^{-1} – 80.60%). The PD extract was also capable of inhibiting BSA denaturation in dose-dependent manner (50µg mL^{-1} – 20.40%, 1000 µg mL^{-1} – 83.60%) which was comparable to that of Diclofenac sodium (200 µg mL^{-1}– 86.60%). This finding confirms the potentiality of PD extract as an anti-

inflammatory agent and justifies the recommendation of PD extract for the treatment of painful inflammatory conditions.

APPLICATIONS

In the present study the *Tagetes erecta* flower, *Cyanodon dactylon*, *Curcuma amada* rhizome were screened for their *in vitro* anti-inflammatory activity, which are promising as active pharmacophore. Further studies are undergoing to explore the *in vivo* activities.

CONCLUSIONS

The results of our study suggest that *Tagetes erecta* flower, *Cyanodon dactylon*, *Curcuma amada* rhizome are rich in phenolic compoundsand have a good antioxidant activity. It can be used as a natural source of antioxidants to prevent the progression of many diseases. Among the different plant, *Curcuma amada rhizome* produced marked *in-vitro* anti-inflammatory activity than other plants. This result justifies that its use in traditional system of medicine in India and other Asian countries.

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