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IN VITRO ANTIINFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF *HOPEA ODORATA* (ROXB.) LEAVES.

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ABSTRACT

Objective: To examine the anti-inflammatory effect of methanol extract of *Hopea odorata* (Roxb.) leaves. **Methods:** Leaves of *Hopea odorata* was extracted with pure methanol (MEHO), which is tested for *in vitro* anti-inflammatory activity found by protein denaturation. **Result:** The production of auto antigen in certain arthritic disease may be due to denaturation of protein, membrane lysis and proteinase action. The maximum percentage inhibition of protein denaturation membrane stabilization and proteinase inhibitory action were observed as *H. odorata* (80.13%, 75.33%, 67.43%, 52.83%, 38.31%, 22.29%) at 400, 200, 100, 50, 25, 12.5 µg/ml respectively. **Conclusion:** From our study, it can be concluded that *in vitro* anti-inflammatory activity of the leaves extract of *H. odorata* might be attributed to the presence of different phytochemicals. These experimental findings support the traditional use of this plant for the treatment of various ailments especially against pain and inflammatory conditions.

KEYWORDS: *Hopea odorata*, methanol extract, anti-inflammatory, protein denaturation.

INTRODUCTION

The majority population of the developing world relies on traditional herbal medicine as the primary source of treatment for illnesses (H. M. Ullah et al., 2014). The issue of compliance with herbal medicines varies according to local beliefs and socio-cultural status, and is less reliant on the efficacy of the traditional medicine. About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use of the 252 drugs considered as basic and essential by the World Health Organization (WHO), 11% are exclusively of plant origin and a significant number are synthetic drugs obtained from natural precursors. Modern social context and economic view of health services, the needs of the pharmaceutical market and the recognition that research on medicinal plants used in folk medicine represents a suitable approach for the development of new drugs (Calixto et al., 2000) have led to an increase in the number of publications in this field, and private and governmental institutions are now financially supporting research programs worldwide. Inflammation is a complex biological response of vascular tissues to harmful stimuli. It is also a protective attempt by the organism to remove the injurious stimuli and initiate the healing process (Ezugwu, Okonta, & Nwodo, 2004; Read, 1995). At the onset of an inflammation, the cells undergo activation and release inflammatory mediators. These mediators include histamine, serotonin, slow reacting substances of anaphylaxis (SRS-A), prostaglandins and some plasma enzyme systems such as the complement system, the clotting system, the fibrinolytic system and the kinin system. These mediator molecules work collectively to cause increased vasodilatation and permeability of blood vessels. Thus, leading to increased blood flow, exudation of plasma proteins and fluids, and migration of leukocytes, mainly neutrophils, outside the blood vessels into the injured tissues. Inflammation can be classified as either acute or chronic inflammation. Acute inflammation is the initial response of the body to injurious stimuli and is achieved by increased movement of plasma and leukocytes from the blood into the injured tissues. The process of acute inflammation is initiated by cells already present in the tissues. This is characterized by marked vascular changes, including vasodilatation and increased capillary permeability which are induced by the actions of the various inflammatory mediators. Chronic inflammation is a prolonged inflammatory response that leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissues from the inflammatory process. *Hopea odorata* (Roxb.) belongs to the Dipterocarpaceae family, locally known as Telsur (Bangladesh). The wood of *H. odorata* varies in color from a very pale yellow, or white to brown when first cut and

characteristically darkens to a brownish or yellowish-brown color after more or less prolonged exposure to the air. The dammar of this tree is said to have medicinal property used in treating sores and wounds (Hossain, Kabir, Chowdhury, Hasanat, & Chakrabarty, 2015). Phytochemical studies reported that the heartwood of *H. odorata* enclose with certain types of phenolic compounds (Coggon et al., 1964). These polyphenols are reported to be useful as antioxidants, anticarcinogens, scavengers of free radicals and therefore have implications in the prevention of pathologies such as cancer and cardiovascular disease (Kabir et al., 2015; Scalbert, Johnson, & Saltmarsh, 2005). The aim of the present research is to identify the anti-inflammatory activity of methanol extract of *Hopea odorata* leaves. Thus the plant may be a source of effective herbal drug.

METHODS AND MATERIALS

Plant collection & identification

Fresh leaves of *Hopea odorata* were collected from area of University of Chittagong, Chittagong, Bangladesh in the month of November 2014. It was authenticated by Dr. Shaikh Bokhtear Uddin, Professor, Department of Botany, University of Chittagong, Chittagong-4331, Bangladesh.

Chemicals and Reagents

All other chemicals and reagents were of analytical grade. Methanol was purchased from Merck (Germany). Ecospirin was purchased from Beacon Pharmaceuticals.

Extract preparation

The leaves were dried for a period of 10 days under shade and ground. The ground leaves (400 gm) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring then the whole mixture was filtered and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterlin Ltd, UK) to get a viscous mass. The viscous mass was kept at room temperature under a ceiling fan to get a dried extract (about 8%). The extract prepared was for pharmacological screening.

In vitro anti-inflammatory activity

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of extract so that final concentrations become 12.5, 25, 50, 100, 200, 400 µg/mL (H. A. Ullah et al., 2014). Similar volume of double-distilled water served as control. Then the mixtures were

incubated at (37°C ± 2) in a BOD incubator (Lab line Technologies) for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Ecospirin at the final concentration of (12.5, 25, 50, 100, 200, 400 µg/mL) was used as reference drug and treated similarly for determination of absorbance.

The percentage inhibition of protein denaturation was calculated by using the following formula.

$$\% \text{ Inhibition } = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

Calculation and Graphical Software

All results are expressed as mean. For data calculation, Excel 2007 software used. GraphPad Prism® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) used for graphical presentation.

RESULTS

***In vitro* anti-inflammatory activity**

The production of auto antigen in certain arthritic disease may be due to denaturation of protein, membrane lysis and proteinase action. The maximum percentage inhibition of protein denaturation membrane stabilization and proteinase inhibitory action were observed as *H. odorata* (80.13%, 75.33%, 67.43%, 52.83%, 38.31%, 22.29%) at 400, 200, 100, 50, 25, 12.5 µg/ml respectively as shown in Table 1. From the results of Figure 2, our study reveals that methanol extract of *H. odorata* are capable of controlling the production of auto antigen and inhibits denaturation of protein, membrane lysis and proteinase action in rheumatic disease.

Table 1: Anti-inflammatory activity of *H. odorata* compared with Ecospirin.

Concentration	Abs. of Control	Abs. of Test	% of protein inhibition of *H. odorata*	% of protein inhibition of Ecospirin
12.5	1.48	1.15	22.29	49
25	1.48	0.913	38.31	55
50	1.48	0.698	52.83	68
100	1.48	0.482	67.43	73
200	1.48	0.365	75.33	82
400	1.48	0.294	80.13	91

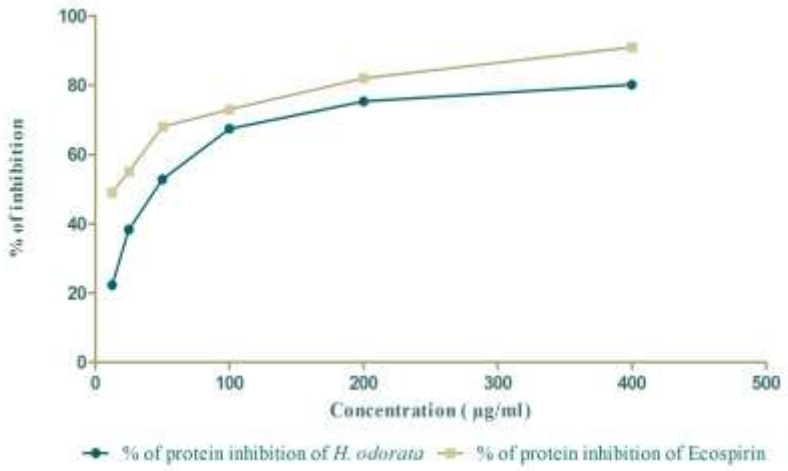

Figure 1: Anti-inflammatory activity of *H. odorata* compared with Ecospirin.

DISCUSSIONS

Denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So it may be said that tissue protein denaturation is a marker for inflammatory and arthritic diseases (Bhattacharya, Chandra, & Dey, 2013). Agents that can prevent protein denaturation, therefore, would be possible candidate for anti-inflammatory drug development (Hossain, Kabir, Hasanat, et al., 2015). With this idea in mind, the in vitro test was done as a preliminary screen to check presence of anti-inflammatory property before doing the *in vivo* test. In the present study, the protein denaturation bioassay was selected for in vitro assessment of anti-inflammatory property of methanol extract of *H. odorata* leaves with a wide range of dose concentrations.

The method of anti-denaturation of egg albumin was chosen to evaluate anti-inflammatory property of *H. odorata*. In anti-denaturation assay the denaturation of egg albumin is induced by heat treatment. The denatured protein expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulo-nephritis etc. (Ahmad, Khan, & Rasheed, 1992). Heat-denatured proteins are as effective as native proteins in provoking delayed hypersensitivity (Gell & Benacerraf, 1959). Moreover, it was already proved that conventional NSAID's like phenylbutazone and indomethazine do not act only by the inhibition of endogenous prostaglandins production by blocking COX enzyme but also by prevention of denaturation of proteins (Phillips, Szerenyi, Campos, Krueger, & McDonnell, 1993). Thus anti-denaturation assay is the convenient method to check the anti-inflammatory activity. From the result of the present study, the extract has shown considerable anti-inflammatory activity.

CONCLUSION

Based on the present investigation, it can be concluded that *in vitro* anti-inflammatory activity of the leaves extract of *H. odorata* might be attributed to the presence of the plant's various secondary metabolites like tannins, saponins, steroids, alkaloids, reducing sugars, terpenoids and flavonoids. These experimental findings support the traditional use of this plant for the treatment of various ailments especially against pain and inflammatory conditions. However, further investigations are required to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms of action responsible for the anti-inflammatory activity of the plant extract.

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