

Phytochemical Compositions and *in-vitro* Anti-inflammatory Activity of *Plectranthus mollis*

Ushir Yogesh V.*, Morankar Pravin G., Dhake Avinash S

S.M.B.T. College of Pharmacy, Dhamangaon, Nashik, India

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ABSTRACT

The objective of study was to isolate phytochemicals from *Plectranthus mollis* and evaluate for anti-inflammatory activity. First time for *Plectranthus mollis* the study are carried out here for the mentioned activity. The constituents are PMC-1 and PMC-2 were isolated from *Plectranthus mollis*. The identification of isolated compounds was carried out by various physical, chemical and instrumental techniques. The PMC-1 is identified as β - sitosterol and PMC-2 is identified as stigmasterol in *Plectranthus mollis*. *In-vitro* anti-inflammatory models were studied; as inhibition of protein denaturation, effect of membrane stabilization of human RBC. We conclude that, the chloroform fraction of *Plectranthus mollis* shows strong anti-inflammatory activity at different concentration. The chloroform fraction showed dose dependent activity, when compare to aspirin and, hydrocortisone as a standard.

Keywords: Anti- inflammatory activity, *Plectranthus mollis*, constituents, protein denaturation, membrane stabilization.

INTRODUCTION

The mechanism of inflammation injury is attributed, in part, to release of Reactive Oxygen Species (ROS) from activated neutrophil and macrophages. Thus free radicals are important mediators that provoke or sustain inflammatory processes and consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation (Catherine et al., 2006). The inflammation leads to Rheumatoid arthritis which is a major ailment among rheumatic disorders (Jadhav and Kadam, 2009).

The *Plectranthus species* ethanobotanically claimed for the treatment of RA (Anonymous 2003). So, the efforts begin to check the efficient fraction for the anti-inflammatory activity & constituents responsible for it.

MATERIALS AND METHODS

Plant material Collection

Place Jyotiba (Maharashtra; India) where *Plectranthus*

mollis grown widely. Plant collection was carried out two times, monsoon & autumn season, to accurately reflect the chemical composition of the respective plant.

Preparation of plant extracts

The dried powdered of aerial parts (Stem, leaves, flowers and seeds) of the *Plectranthus mollis* (PMA) was extracted with chloroform using Soxhlet apparatus for 5 hrs. It gives 10.38% chloroform extract (PMACE). The crude extract is stored in desiccators along with calcium carbonate for use in research work.

Chemicals and reagents

All applied chemicals were of the great purity available and supplied by the Sigma-Aldrich Chemical Company. Hydrocortisone sodium Succinate, equivalent to 100 mg hydrocortisone (Primacort™ Macleods Pharmaceutical Ltd., Daman, India), obtained from shoppers shop, Nashik, India.

Chromatographic Separation of Fractions

The PMACE (1.5g) was separated on a silica gel (60-120#) column by gradiently with a hexane/EtOAc gradient with reference to polarity index (Wellsow et al., 2006; Rao et al., 2009). The loaded PMACE was yield six fractions (F1-F6). Fraction number F3 (31.5 mg) was recrystallised by ethanol to afford PMC-1.

Address for correspondence

Dr. Ushir Yogesh V.
Associate Professor,
Dept. of Pharmacognosy and Phytochemistry, S. M. B. T. College of Pharmacy, Dhamangaon, Tal. Igatpuri, Nashik, India
E-mail: ushir29@gmail.com

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Further in continuation of elution of PMACE yielded five fractions (F16–F21) was rechromatographed over a silica gel G preparative TLC in $\text{CHCl}_3/\text{MeOH}$ (90/6) solvent system. From this mixture one pure compound was obtained as; PMC-2.

Characterization of isolated compounds

The isolated compounds were characterized with help of physical, chemical and instrumental techniques.

Characterization of compound PMC-1

Is a Pale yellowish needles, soluble in Chloroform, methanol, ethyl acetate and ethanol, gives positive Salkovaski test, mp 132-134°C. UV (CHCl_3) λ_{max} : 250nm; IR (Zn Selenoide-3275 Hydraulic Press Tech): 3434 (O-H stretch), 2964.35 (C-H stretch), 2872.21 (C-H stretch), 2861.85 (C-H stretch), 1574 (C-H bend), 1460 (C=C stretch), 1231 (O-H bend), 1000-900 (C-C stretch) cm^{-1} ; LC-ESI-MS (m/e): 414, 383, 274, 247, 219, 180, 161, 158; $^1\text{H-NMR}$ (CDCl_3 400MHz): δ 4.34 (tdd), 5.23 (1H, m), 1.23 (3H, s), 1.39 (3H,s), 1.06 (3H,s), 1.29 (3H,d), 1.29 (3H,d), 1.25 (3H,s), 1.23 (3H,s), 0.88 (3H,s).

Characterization of compound PMC-1

Is a off white color powder, Soluble in Chloroform, methanol, ethyl acetate and ethanol, gives positive Salkovaski test, mp 175-177°C. UV(CHCl_3) λ_{max} : 250nm; IR (Zn Selenoide-3275 Hydraulic Press Tech): 3458 (O-H stretch), 2935 (C-H stretch), 2890 (C-H stretch), 2862.87 (C-H stretch), 1665 (C-H bending), 1459 (C=C stretch), 1241.55 (O-H bend), 1000-900 (C-C stretch) cm^{-1} ; LC-ESI-MS (m/e): 412, 367, 329, 301, 275, 246, 179, 160, 157; $^1\text{H-NMR}$ (CDCl_3 400MHz): δ 5.02 (tdd), 5.35 (1H, m), 1.17 (3H, s), 1.24 (3H,s), 1.17 (3H,s), 5.34 (1H,m), 5.34 (1H,m), 1.02 (3H,s), 1.01 (3H,s), 0.69 (3H,s).

In- Vitro Anti-inflammatory Activity

The anti-inflammatory activity tested for fractions rich in PMC-1 and PMC-2. The two *in vitro* models are used as;

HRBC Membrane Stabilization

The test mixtures (4.5 mL) consisted of 2 mL hypotonic saline (0.25% NaCl), 1 ml 0.15 M phosphate buffer (pH 7.4) and 1 mL test solution (50, 100, 200,

400, 600 and 800 $\mu\text{g mL}^{-1}$ of final volume) in normal saline. 0.5 mL of 10% Human RBC (HRBC) in normal saline was added. Hydrocortisone sodium was used as the reference drug. For control tests, 1 mL of isotonic saline was used instead of test solution while product control tests lacked red blood cells. The mixtures were incubated at 56 °C for 30 minutes. The tubes were cooled under running tap water for 20 minutes. The mixtures were centrifuged and the absorbance of the supernatants read at 560 nm (Okoli and Akah, 2004; Aher et al., 2009). Percent membrane stabilizing activity was calculated as follows; % Protection = $100 - (\text{Optical density of drug treated sample} / \text{Optical density of control} \times 100)$

Inhibition of protein denaturation

The test mixture (0.5 mL) consisted of 0.45 mL bovine serum albumin (5% BSA aqueous solution) and 0.05 mL of test sample (50, 100, 200, 400, 600 and 800 $\mu\text{g mL}^{-1}$ of final volume). pH was adjusted at 6.3 using a small amount of 1 N HCl. Aspirin was used as the reference drug. The samples were incubated at 37° C for 20 min and then heated at 57° C for 3min. After cooling the samples, 2.5 mL phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was measured spectrophotometrically at 660 nm (Grant et al., 1990). For control tests 0.05 mL distilled water was used instead of sample while product control tests lacked BSA. The percentage inhibition of protein denaturation was calculated as follows; % inhibition = $(\text{Absorbance of Control} - \text{Absorbance of Sample}) / \text{Absorbance of Control} \times 100$

Statistical Analysis

All the results of pharmacological study are reported as Mean \pm Standard deviation (S.D.)

RESULTS

Isolation of Phytoconstituents

The isolated constituents were characterized with help of instrumental techniques as UV spectrophotometry, IR spectrophotometry, mass spectrometry and NMR spectroscopy. Co-TLC of the isolated constituents with authentic compounds was

also used as criteria for their identification. PMA offered two compounds; PMC-1 (β -sitosterol) and PMC-2 (stigmasterol) from CHCl_3 extract fractions. Structures of isolate compound are given in Figure 1.

In-Vitro Anti-inflammatory Activity

Membrane Stabilization of HRBC

Maximum inhibition, 97.67% was observed at 800 $\mu\text{g/ml}$ of PMC-1 and 97.53% was observed at 800 $\mu\text{g/mL}^{-1}$ of PMC-2. Hydrocortisone, a standard anti-

inflammatory drug showed the maximum inhibition, 98.29% at the concentration of 800 $\mu\text{g/mL}^{-1}$.

Inhibition of Protein Denaturation

Maximum inhibition, 82.38% was observed at 800 $\mu\text{g/mL}^{-1}$ of PMC-1 and 81.87 % was observed at 800 $\mu\text{g/ml}$ of PMC-2. Aspirin, a standard anti-inflammatory drug showed the maximum inhibition, 84.75% at the concentration of 800 $\mu\text{g/mL}^{-1}$.

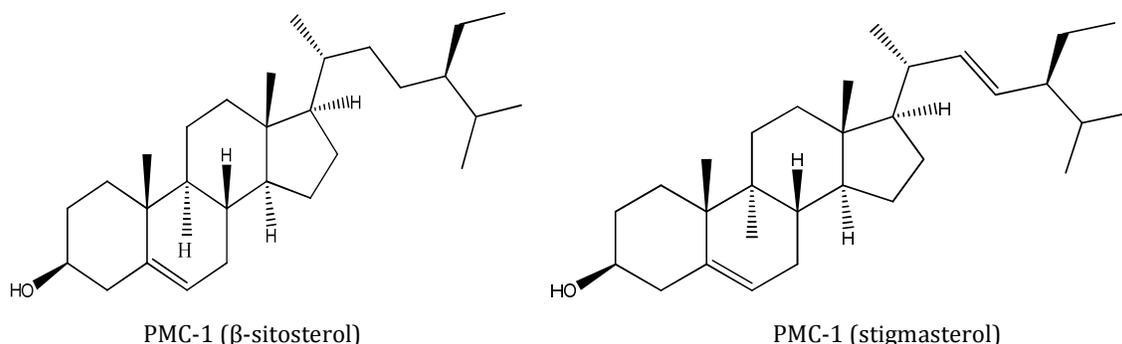


Figure 1: Structures of isolated compounds

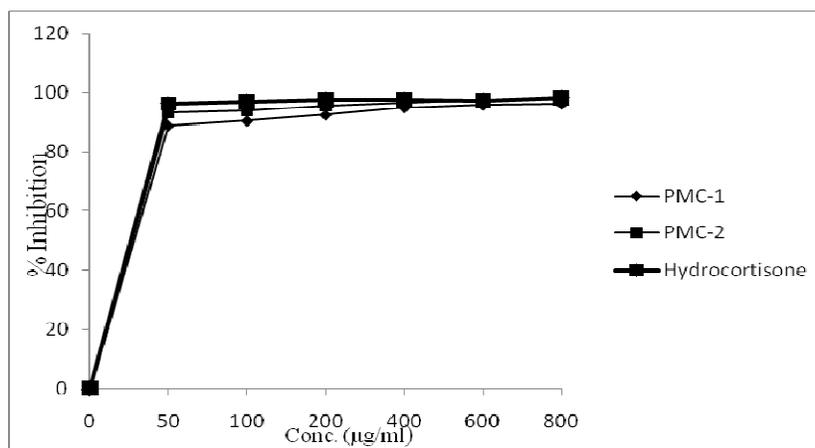


Figure 2: Effect of PMC-1 and PMC-2 on Membrane Stabilization of RBC

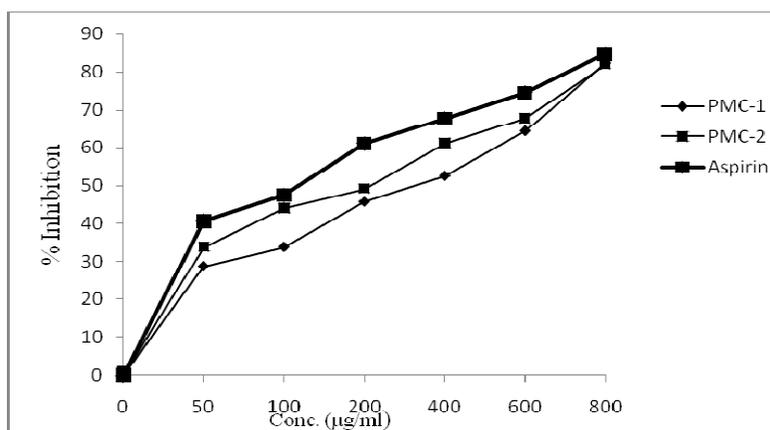


Figure 3: % inhibition of Effect of PMC-1 and PMC-2 in albumin denaturation model

DISCUSSION

The over anabolism of ROS leads to cell injury by damaging the lipid peroxidation of membranes and macromolecules. In addition, ROS propagate inflammation by stimulating the release of the cytokines such as IL-1, TNF- α , and IF- γ , which stimulate precipitation of extra neutrophil and macrophages. Most clinically important medicine belongs to cyclo-per-hydro-phenanthrene compounds or NSAIDs anti-inflammatory chemical therapeutics for treatment of inflammation related diseases. Though these have great activity and long term administration is required for treatments of long term diseases. Furthermore, these drugs have various and severe side effects. Therefore, medicinal plant agents with very less side effects are desirable to replacement to chemical therapeutics (Luo et al., 2002).

The ROS propagate inflammation by enhancing the inflammatory mediators, so its stabilization or inhibition is necessary. The presence of β -sitosterol and stigmasterol in *Plectranthus mollis* stabilize ROS present. Most of the investigators have reported that denaturation of the protein is one of the cause of rheumatoid arthritis (QiuHong et al., 2013). 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. The β -sitosterol and stigmasterol present in PMACE able to inhibit the denaturation of protein by inhibiting and stabilizing the ROS. Recent studies have shown that many phytosterol contribute significantly to the anti-inflammatory activity of many plants (Vadivu and Lakshmi, 2008). The lysosomal enzymes released during inflammation produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The steroidal drugs act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane (Wellsow et al., 2006). Since HRBC

membrane are similar to cell lysosome membrane the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drugs. We can say that, the β -sitosterol and stigmasterol in *Plectranthus mollis* are act as an anti-inflammatory agent by neutralizing lysosomal enzymes or by stabilizing the lysosomal membrane.

In both models as *Plectranthus mollis* shows promising effects. So, from the results of the present study it can be stated that *Plectranthus mollis* is capable of controlling the production of auto-antigens due to denaturation of proteins in rheumatic diseases either by preventing synthesis of free radicals. The presence of constituent's β -sitosterol and stigmasterol are responsible for stated activity.

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