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INHIBITORY EFFECT ON NITRIC OXIDE AND PROTEIN DENATURATION OF TWO TRADITIONAL PLANTS USED IN ARTHRITIS TREATMENT

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Abstract

Nitric oxide free radical scavenging assay and protein denaturation inhibition assay were investigated to assess the *in vitro* anti-inflammatory effect of *Plectranthus mollis* (PM) and *Salvia officinalis* (SA). Leaves were extracted with mixture of ethanol and water (7:3 ratios), both the plant extracts produced significant anti-inflammatory activity in concentration dependent manner. PM and SA extract at 100µg/ml produced 87.86 and 84.68% inhibition of nitric oxide, respectively; while at same concentration PM and SA extract exhibit 94.69 and 87.89% protection, respectively against the protein denaturation.

Keywords: *Plectranthus mollis (PM), Salvia officinalis (SM), In vitro, Anti-inflammatory, Leaves*

Introduction

Nitric oxide (NO) is a pleiotropic inhibitor required for several physiological processes such as smooth muscle relaxation, vasodilation immune response and blood pressure, neuronal signalling & inhibition of platelet aggregation [1]. But higher concentration of nitric oxide is implicated in several physiological conditions including cancer and inflammation [2]. Moreover, inflammation accelerates the appearance of neurodegenerative disorders like Parkinson and Alzeimer's diseases [3]. These insights provoke the interest of searching new anti-inflammatory therapeutic approaches to cancer and neurodegenerative diseases development. The plant kingdom always represent many sources of new compounds with significant anti-inflammatory actions. Among the plant species, Lamiaceae family members are most widely used as a source of many bioactive compounds such as antioxidants and several therapeutically active substances.

Plectranthus species are most widely used in the treatment of gastrointestinal, pain/fever and skin conditions [4]. Previously many phenolics and abietane diterpenoids have been reported in a variety of *Plectranthus* species [5]. *Plectranthus mollis* (PM) was acknowledged in different traditional and folkforic medicine for many medicinal purposes in Asia and Latin America. Another important genus is *Salvia*, which includes about 700 species spread throughout the world. *Salvia officinalis* (SA) is used for various disorders including seizure, ulcers, gout, rheumatism and hyperglycemia [6]. In our earlier studies, we have reported some of the polyphenolic compounds [7] which are of special interest due to their biological properties such as antioxidant and anti-inflammatory potential [8]. PM and SA were ethnobotanically claimed for the treatment of age-related cognitive disorders and inflammatory conditions in the throat and skin [9,10]. Owing to their much pharmacological properties, the present study aim to investigate the possible benefits of leaf extracts of PM and SA in the treatment of inflammation by evaluating their nitric oxide scavenging assay and protein denaturation assay respectively.

Methods

Drugs and chemicals

Reference standard Diclofenac sodium was procured from Cipla Ltd, Bangalore, India. Phosphate buffered saline (PBS) was obtained from E.Merck Ltd, Mumbai, India All other chemicals used were of analytical grade obtained commercially. Double distilled water from all-glass still was used throughout the study.

Preparation of extract

The dried and powdered leaves (100 g each) were defatted with petroleum ether at 60-80 °C followed by extraction with mixture of ethanol : water (7 : 3 ratios) by a Soxhlet apparatus 45 °C for 5 hours. The extracts obtained were concentrated in a rotary evaporator to yield gummy mass for *Plectranthus mollis* (PM) and *Salvia officinalis* (SA).

Nitric oxide scavenging activity

The scavenging activity of nitric oxide [11] by the extracts was determined by the method of Jaishree *et al.* with slight modification. A solution of sodium nitroprusside (10mM) prepared in phosphate buffered saline (PBS, pH 7.4) and the test samples at various concentrations (25, 50, 75 and 100µg/ml) was incubated at 25° C for 150 min. After incubation, 0.5ml of Griess reagent (1%w/v), sulfonilamide (2%v/v), orthophosphoric acid (2%v/v) and 1ml naphthylethylene diamine dihydrochloride (0.1% w/v) was added. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric acid, which reacts with oxygen to produce nitrite ions, which can be estimated at 540 nm.

Inhibition of protein denaturation

The extracts were screened for anti-inflammatory activity by inhibition of albumin denaturation method [12] studied according to Muzushima and Kobayashi with slight modification. The test sample (1ml) containing various concentrations (25, 50, 75 and 100µg/ml) of extracts were mixed with 1ml of 1% albumin solution in phosphate buffer saline (PBS, pH 7.4) and incubated at 37±2 °C in a BOD incubator (Labline technologies) for 15 min. Then the reaction mixture was heated at 60 °C in a water bath for 15 min to induce denaturation. After cooling, their absorbance was measured at 660 nm (Shimadzu UV, 1800) by using vehicle as blank. Percentage inhibition was calculated from control where no drug was added. Diclofenac sodium was used as reference drug. The percentage inhibition of protein denaturation was calculated by the formula

% Inhibition of denaturation = Vt /Vc - 1 x 100

where, Vt = absorbance of sample, Vc = absorbance of control.

Results

Earlier we have reported total phenol content and total flavanoid content which showed strong correlation with antioxidant activity [7]. In the present study, both the extracts at 100 µg/ml concentration exhibited 87.86 and 84.68 % inhibition of NO for PM and SA respectively. It was found that the capability to scavenge NO free radicals is in a concentration dependant manner (Table 1).

The anti-inflammatory potential were evaluated against the denaturation of egg albumin. The results are summarized in table 2. A concentration dependent inhibition of protein (albumin) denaturation was observed with both the extracts and the reference standard Diclofenac sodium. The effect of extracts were found to be comparable with the standard with better effect by PM. At 100 µg/ml concentration, both the extracts exhibit 94.69 and 87.89 % protection for PM and SA respectively against the protein denaturation

This was further confirmed by comparing their IC_{50} values. Extracts exhibited IC_{50} values of 13.62 µg/ml for PM, 26.55 µg/ml for SA, whereas that of diclofenac sodium was found to be 12.83 µg/ml respectively. Results indicates that the anti-inflammatory activity of the extracts from the leaves of PM and SA are due to its antioxidants. These results are in accordance with the other reports in the literature, which showed strong correlation between the antioxidant activitiy and anti - inflammatory potential of phenolic compounds [13].

Discussion

The NO radical scavenging activity of both the plants were proved. Denaturation of proteins in the tissue is one of the major cause of inflammatory and arthritic diseases. Production of auto antigens in arthritic diseases may be due to denaturation of proteins [14,15]. The polyphenols present in both the plants are able to inhibit denaturation of proteins by inhibiting and stabilizing the ROS. The major anti-inflammatory constituents of both the plants are β-sitosterol, stigmasterol, caffeic acid and quercetin [7]. This could evidence the anti-inflammatory activity of the extracts. The observed activity is due to synergistic effect rather than the single constituent. It can be suggested that both the plants have a role in inhibiting free radical mediated chain reactions in inflammatory related disorders.

The extracts were capable of controlling the production of auto antigens which are responsible for denaturation of proteins in inflammatory and rheumatic diseases by preventing the synthesis of free radicals. Nitric oxide scavenging efficacy and the protein denaturation inhibition activity of the extracts may be responsible for the anti-inflammatory and anti-arthritic activity. The inventions of anti-inflammatory potential of the selected plants which were acknowledged in traditional medicine as anti-arthritic harmonize with antioxidants and its free radical scavenging efficacy. However, further studies are required to isolate the phytochemicals and to evaluate the mechanisms of anti-inflammatory actions in these plants

The result of the present study shows that the ethanol extracts from the two selected plants possess anti-inflammatory activities. It can be concluded that the active compounds present in both the plants can be used as lead molecule for designing a potent anti-inflammatory and anti-arthritic drug which is useful in treating the said conditions.

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Table 1: Nitric oxide radical scavenging effect of ethanol extracts of plant leaves

Samples	Percentage Inhibition (n=3, mean ± SD)				IC_{50} (µg)
	100 µg	50 µg	25 µg	12.5 µg	
P.mollis (PM)	87.865±0.132	59.969±0.080	44.087±0.320	32.123±0.261	38.245±0.241
S.officinalis (SA)	84.683±0.490	53.597±0.120	36.316±0.399	30.191±0.511	41.786±0.311

Table 2: Effect of ethanol extracts of plant leaves on *in vitro* protein denaturation

Samples	*Percentage Inhibition (n=3, mean ± SD)*				*IC_{50} (µg)*
	100 µg	*50 µg*	*25 µg*	*12.5 µg*	
Diclofenac sodium	*93.802± 2.390*	*70.631 ± 2.252*	*58.754 ± 2.067*	*52.739 ± 1.621*	*12.831 ± 7.569*
P.mollis (PM)	*94.695 ± 1.745*	*70.707 ± 0.994*	*58.724 ± 0.651*	*52.740 ± 0.506*	*13.623 ± 1.888*
S.officinalis (SA)	*87.894 ± 0.185*	*66.055 ± 0.192*	*55.188 ± 0.122*	*49.602 ± 0.299*	*26.553 ± 0.995*