



In vitro anti-lipid peroxidation and anti-arthritic activities of *Avicennia officinalis*

Anuya A. Rege, Parikshit R. Juvekar, Archana R. Juvekar*

Department of Pharmaceutical Sciences and Technology, Institute of Chemical Technology, Matunga, Mumbai-400019

Abstract

In the present study, aqueous extract of *Avicennia officinalis* was screened for lipid peroxidation inhibitory and anti-arthritic activities using standard *in vitro* procedures. It exhibited important free radical-scavenging activity towards the lipid peroxidation and DPPH radicals. *A. officinalis* also revealed moderate anti-arthritic activity. Folin-Ciocalteu reagent in terms of gallic acid equivalent achieved the total phenolic content which may be responsible for these activities.

1. Introduction

Lipid peroxidation is one of the mechanisms of oxidative damage caused by free radicals in biological system and free radicals have been implicated in etiology of several human diseases including rheumatoid arthritis [1]. Antioxidants are the compounds that prevent this oxidative damage by different mechanisms [2]. As synthetic antioxidants possess adverse effects [3]. Hence screening for safe, effective and economical antioxidants from natural sources especially plants is preferred.

Avicennia officinalis belongs to mangrove plants. Various traditional and medicinal uses of mangrove plants have been reported [4]. However, reports on anti-lipid peroxidation and anti-arthritic activities of *A. officinalis* are

sparse. Hence it has been subjected to investigate these activities using different *in vitro* models.

2. Materials and Methods

1.1 Collection of the material

Avicennia officinalis was collected from Mahim Creek, Mumbai. It was identified and authenticate by Dr.J.M.Pathak, Research Director (Pharmacognosy), Zandu Pharmaceuticals, Mumbai. The leaves were washed, shade-dried and powdered in a grinder mixer.

1.2 Preparation of Aqueous extract

One gram of material was added to 20 ml of distilled water and kept at RT for 24 hr. It was then filtered and evaporated to dryness on boiling

* Corresponding author
Email: arj04@rediffmail.com

water bath and the percentage yield of the extract was calculated which was found to be 20%. w/w.

1.3 Lipid peroxidation inhibitory activity

The protective effect of *A. officinalis* was evaluated against Fe²⁺-Ascorbic acid-induced lipid peroxidation using rat liver mitochondria as model system. The rat liver mitochondria were isolated by differential centrifugation method [5]. The protein was estimated by Lowry's method [6]. Protein concentration of mitochondrial samples was adjusted to 5 mg/ml. The effect of *A. officinalis* on lipid peroxidation in mitochondrial samples was estimated by thiobarbituric acid reactive substances (TBARS) method [7]. Butylated Hydroxyl Toluene (BHT) was used as a standard.

1.4 DPPH radical-scavenging assay

The free radical-scavenging activity of *A. officinalis* was measured by 1, 1 diphenyl-2-picryl hydrazyl (DPPH) assay as described earlier [8]. Ascorbic acid was used as a standard.

1.5 Proteinase inhibitory activity

Proteinase inhibitory activity of *A. officinalis* was estimated as described by Chatterjee *et al* [9]. Acetyl salicylic acid was included as a standard.

1.6 Phenolic content determination

The total phenolic compounds in *A. officinalis* were determined using Folin-Ciocalteu reagent acc as gallic acid equivalent (GAE) in milligrams per gram of dry sample.

Statistical analysis of the data was done by Student's *t-test* and P<0.05 was regarded as significant.

3. Results

Table -2: DPPH radical scavenging activity of *A.officinalis*

	Conc.(µg/ml)	% Inhibition (Mean ± SD)	IC ₅₀ (µg/ml)
A.officinalis	50	9.26 ± 1.44	162.2
	100	29.19 ± 0.47	
	150	46.11 ± 4.41	
	200	67.10 ± 0.61	
	250	81.66 ± 0.11	
	300	89.27 ± 0.15	
Ascorbic Acid	2	1.66 ± 2.13*	11.3
	4	11.01 ± 1.27	
	8	34.90 ± 3.98	
	12	52.15 ± 0.16	
	16	71.97 ± 0.82	
	20	95.18 ± 1.28	

Significant (P<0.05) difference with control

*Non-Significant (P<0.05) difference with control

Table-1: Lipid Peroxidation Inhibitory Activity of *A. officinalis*

Plants	Conc. ($\mu\text{g/ml}$)	% Inhibition (Mean \pm SD)
<i>A.officinalis</i>	40	9.23 \pm 2.98*
	200	26.90 \pm 0.10
	400	45.92 \pm 0.70
BHT	40	83.78 \pm 1.45

Significant ($P < 0.05$) difference with control*Non-Significant ($P > 0.05$) difference with control**Table-3: Effect of *A.officinalis* on proteinase Inhibition**

	Conc. ($\mu\text{g/ml}$)	% Inhibition (Mean \pm SD)
<i>A.officinalis</i>	100	8.91 \pm 2.52*
	200	16.94 \pm 0.38
	400	24.40 \pm 0.28
	800	38.52 \pm 0.43
Acetyl salicylic Acid	800	16.19 \pm 5.93

Significant ($P < 0.05$) difference with control*Non-Significant ($P > 0.05$) difference with control

4.Discussion

Several herbs and spices have been reported to exhibit antioxidant activity [11]. Besides, medicinal plants, mangrove plants are also used in treatment of several ailments. Some of the biological properties of *A. officinalis* have been reported previously [12]. In the present *in vitro* study it has been evaluated for anti-lipid peroxidation and anti-arthritis activities.

Effect of *A. officinalis* on lipid peroxidation was assessed in rat liver mitochondria by inducing lipid peroxidation with Fe^{2+} -ascorbate system. According to Conforti *et al* [13], ascorbic acid

is a well-known antioxidant but it also has prooxidant properties in presence of certain transition metal ions like Fe or Cu. Iron can stimulate lipid peroxidation by the Fenton reaction and also accelerates peroxidation by different mechanisms [14]. Lipid peroxidation in biological system is known to produce cellular damage in brain, liver and kidney [15,16]. Thiobarbituric acid reactive substances (TBARS) assay which was originally developed for testing rancidity due to oxidized lipids in food material [17], is now widely used as an index for measuring lipid peroxidation. According to

Pandima Devi *et al* [18], during lipid peroxidation, malonaldehyde (MDA), is formed by oxidation of polyunsaturated fatty acids and react with two molecules of thiobarbituric acid (TBA) to give TBARS, a pinkish red chromagen that can be read at 532nm. In the present study, the *A. officinalis* extract is not as good as the standard BHT, however it showed dose-dependent prevention towards generation of lipid peroxides (Table-1).

The ability to scavenge the DPPH radicals is related to the inhibition of lipid peroxidation [19]. DPPH, a stable radical is widely used as a model system to investigate the scavenging activities of various natural compounds. Discoloration from purple to yellow indicates noticeable effect of test compound on scavenging of free radicals. In the present study, *A. officinalis* showed significant ($P<0.05$) scavenging effect on DPPH radicals, which increased with increasing concentration (Table-2). The inhibition of lipid peroxidation by *A. officinalis* may be due to its free radical-scavenging activities as its significant effect in scavenging of DPPH has shown in this study.

A. officinalis extract was also evaluated for its anti-arthritis activity. Rheumatoid arthritis still remains a formidable disease capable of producing functional disabilities [20]. It was

previously reported that proteinases have been implicated in arthritic reactions and significant level of protection was provided by proteinase inhibitors [21]. In the present study, aqueous extract of *A. officinalis* showed potent ($P<0.05$) proteinase inhibitory activity in concentration dependent manner. At 800 µg/ml, it also exhibited significant ($P<0.05$) inhibitory activity in comparison with the same dose of acetyl salicylic acid which was included as a standard (Table-3).

As evidenced, phenolic compounds contribute to antioxidative action through various mechanisms, viz., scavenging free radicals, by stabilizing lipid peroxidation, through redox properties and by chelating metals [22-24]. Hence the total phenolic content of *A. officinalis* was determined by Folin-Ciocalteu method and it was found to be 33.04 mg Gallic acid Equivalent/gm (Table-4).

A. officinalis was reported to have anti-HIV activity by 2 different mechanisms [12]. In the present study, it showed antioxidant potential which would help in decreasing damage caused by oxidative stress in AIDS.

Thus it can be concluded that *A. officinalis* has moderate anti-lipid peroxidation and anti-arthritis activities, which can be attributed to the presence of polyphenolic compounds.

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