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# A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts

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ABSTRACT

*Aim of the study:* The present study was conducted to evaluate the antioxidant, anti-inflammatory and hepatoprotective potential of *Aegiceras corniculatum* Linn. Blanco (Aegiceratacеae).
*Methods and results:* The *n*-hexane, ethyl acetate and methanol extracts, derived from *Aegiceras corniculatum* stems, scavenged superoxide anions ($O_2^{\bullet -}$) and hydroxyl radicals ($^{\bullet}OH$) in nitro blue tetrazolium reduction and deoxyribose degradation assays, respectively. All the extracts inhibited the process of lipid peroxidation at its initiation step. Additionally, in rat liver microsomes *n*-hexane and ethyl acetate extracts also caused termination of radical chain reaction supporting their scavenging action towards lipid peroxy radicals ($LOO^{\bullet}$). Moreover, increased production of $O_2^{\bullet -}$ in human neutrophils, stimulated by phorbol-12-myristate-13-acetate (PMA) and/or opsonized zymosan were also suppressed ($IC_{50}$ ~3–20 μg/mL). Thereby, revealing the ability of plant extracts to antagonize the oxidative stress *via* interference with NADPH oxidase metabolic pathway. These *in vitro* results coincide with the reduction in the glucose oxidase-induced paw edema in mice in the presence of ethyl acetate and methanol extracts (10, 50, and 100 mg/kg, *i.p.*). Plant extracts (250, 500 and 1000 mg/kg, *p.o.*) also significantly protected the carbon tetrachloride (CCl4)-induced oxidative tissue injury in rat liver. This was reflected by a ~60% decline in the levels of serum aminotransferase enzymes.
*Conclusion: Aegiceras corniculatum* extracts found to possess pronounced antioxidant effect that may be at least in part related to its anti-inflammatory and hepatoprotective activities. This study provides a scientific basis for the ethnomedical claims that *Aegiceras corniculatum* is effective against inflammation and liver injury.



## 1. Introduction

Under normal circumstances, reactive oxygen species (ROS) such as $O_2^{\bullet -}$, $^{\bullet}OH$, $LOO^{\bullet}$ and $H_2O_2$ are detoxified by an efficient antioxidant system that includes enzymes such as superoxide dismutase, catalase and glutathione peroxidases. In case this defense system is inefficient, the cells experiences an oxidative stress which contributes in a variety of chronic inflammatory diseases such as arthritis and atherosclerosis as well as other ailments *viz.* cancer, diabetes, hepatitis, neurodegeneration and early aging (Halliwell, 1994; Aviram, 2000).

During inflammatory conditions NADPH oxidase residing in polymorphonuclear and mononuclear cells are activated and generates ROS. Besides their defensive role as microbicidal, ROS are among the most potent stimuli responsible for increasing the vascular permeability, enhancing the production of proinflammatory cytokines (TNF-α, IL-8, IL-1β), chemotactic factors (leukotriene $B_4$) and provoke lipid peroxidation (plasma membrane) and oxidation of DNA. Thus, ROS deregulate the cellular function and induce tissue damage, which in turn augments the state of inflammation (Salvemini et al., 2001; Nardi et al., 2007).

Likewise in liver injury, free radicals and lipid peroxidative metabolites also cause damages to hepatocytes leading to severe necrosis, sepsis or endotoxemia (Kono et al., 2003). Carbon tetrachloride ($CCl_4$) is a widely used hepatotoxin in rodents and its trichloromethyl radical ($^{\bullet}CCl_3$)-induced toxicity in rat liver closely resembles to human cirrhosis and hence is an acceptable animal model for analyzing hepatoprotective agents.

*Aegiceras corniculatum* L. (small tree or shrub) is a mangrove plant that exists in coastlines of tropical and subtropical regions. Mangrove plants have substantial medicinal values and are well

*Abbreviations:* ALT, alanine aminotransferase; AST, aspartate aminotransferase; $CCl_4$, carbon tetrachloride; $^{\bullet}OH$, hydroxyl radical; $H_2O_2$, hydrogen peroxide; $LOO^{\bullet}$, lipid peroxy radicals; TBARS, thiobarbituric acid reactive species; NDGA, nordihydroguaiaretic acid; OZ, opsonized zymosan; PKC, protein kinase C; PMA, phorbol-12-myristate-13-acetate; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide anion; SOD, superoxide dismutase.

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documented in Arab pharmacopoeia and in an Australian traditional medicinal system termed as Bush medicine. The plants belonging to the genus *Aegiceras* are used in the treatment of ulcers and liver injuries in the form of decoctions and macerates. *Aegiceras corniculatum* extracts derived from stems are used as anti-asthmatic, anti-diabetic, anti-rheumatic and anti-inflammatory by the local community residing near the coastal areas (Bandaranayake, 1998, 2002). Chemical investigation reports the presence of benzoquinones, hydroquinones, flavonoids, lignans, phenolic acids, saponins, sterols, tannins and triterpenes (Rao and Bose, 1961; Hensens and Lewis, 1966; Gomez et al., 1989; Xu et al., 2004; Zhang et al., 2005; Wang et al., 2006). *Aegiceras corniculatum* has been shown to possess cytotoxic and anti-fungal properties (Xu et al., 2004).

In the present investigation, antioxidant activity of *Aegiceras corniculatum* (stems) extracts was studied using *in vitro* and *in vivo* model systems. The anti-inflammatory and hepatoprotective effect of plant extracts have been correlated with its antioxidant and free radical scavenging potential.

## 2. Materials and methods

### 2.1. Chemicals

Bovine brain extract, 2-deoxy-2-ribose, diphenyleneiodinium, glucose oxidase, hypoxanthine, nitro blue tetrazolium salt NBT (di-*p*-nitrophenyl-5,5′-diphenyl-3-3-(3,3′-dimethyl-4,4′-biphenylene)-ditetrazolium chloride), nordihydroguaiaretic acid, phenidone, phorbol-12-myristate-13-acetate, quercetin, rutin, superoxide dismutase, xanthine oxidase, xanthine and zymosan A were purchased from Sigma–Aldrich, St. Louis, MO, USA. Allopurinol and staurosporin were acquired from Wako Pure Chemicals, Osaka, Japan and tetrazolium salt WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2*H*-5-tetrazolio]-1,3-benzene disulfonate) was purchased from Dojindo Laboratories, Japan.

### 2.2. Animals

Animal studies were performed in accordance with the declaration of Helsinki and the European Community guidelines for the ethical handling and the use of laboratory animals and through the clearance of institutional animal use committee. Wistar and Sprague–Dawley rats and albino mice of either sex from the animal house of International Center for Chemical and Biological Sciences, University of Karachi, Pakistan were used throughout the study.

### 2.3. Preparation of various extracts from Aegiceras corniculatum

Aerial parts of *Aegiceras corniculatum* were collected during June 2001 from Khuddi and Khi creek, Indus Delta, Sindh, Pakistan and identified by Dr. Surriya Khatoon, taxonomist of the Department of Botany, University of Karachi, Pakistan (voucher specimen labeled as KUH G.H.S. No. 68219). Air-dried powdered stems (9.0 kg) were soaked in 50 L of *n*-hexane for a week. After filtration and evaporation under vacuum, *n*-hexane extract (6.0 g or 0.07%, w/w) was obtained and its residue was soaked again in 50 L ethyl acetate for a period of 7 days. Similarly ethyl acetate extract (8.5 g or 0.09%, w/w) was acquired. Subsequently the residue was soaked in *n*-butanol and methanol and respective extracts of 11.0 g (0.12%, w/w) and 9.8 g (0.11%, w/w) were obtained. This extraction scheme in brief, yielded non-polar (*n*-hexane), less polar (ethyl acetate) and highly polar (methanol) extracts and the presence of different classes of secondary metabolites including flavones, flavonoids, phenolics, quinones, saponins, sterols, tannins and triterpenes were confirmed using conventional phytochemical tests.

### 2.4. Chemical and biological evaluation of antioxidant activity

#### 2.4.1. Deoxyribose degradation assay

The assay was adopted from a method described by Halliwell et al. (1987). In case of non-sites–pecific hydroxyl radical system, reaction mixture contained 28 mM 2-deoxy-2-ribose, 1 mM EDTA plus 200 μM $FeCl_3$ (1:1), 1 mM $H_2O_2$ and 1 mM ascorbic acid in 10 mM phosphate buffer solution (pH 7.4). In site-specific hydroxyl radical system, EDTA was replaced by the phosphate buffer. This reaction mixture was incubated at 37 °C for 1 h in the absence and presence of plant extracts or quercetin (reference compound). The generated OH radicals reacted with the deoxyribose forming malondialdehyde (MDA) which was measured spectrophotometrically (532 nm) by thiobarbituric acid (TBA) reaction.

#### 2.4.2. Nitro blue tetrazolium (NBT) reduction assay

Hypoxanthine–xanthine oxidase was used to generate superoxide anions (Guzmán et al., 2001). The reaction was performed in the absence and presence of plant extracts or allopurinol or superoxide dismutase (reference compounds), containing 1 mM EDTA, 100 μM hypoxanthine, 100 μM NBT with final volume adjusted to 1.2 mL by 50 mM phosphate buffer (pH 7.4). The $O_2^{\bullet -}$ generation was initiated by the addition of 0.066 U xanthine oxidase and detected by the NBT reduction/min spectrophotometrically at 560 nm.

#### 2.4.3. Xanthine oxidase activity

To rule out possible direct effect of the plant extracts on xanthine oxidase activity, this assay was conducted by the same procedure as described above however, hypoxanthine was replaced by 100 μM xanthine and subsequent rate of uric acid formation/min was noted as absorbance at 295 nm.

#### 2.4.4. Lipid peroxidation

The effect of plant extracts on lipid peroxidation was evaluated using three different assays (a) $Fe^{3+}$/ascorbate dependent non-enzymatic lipid peroxidation using bovine brain phospholipids, (b) $Fe^{3+}$/ADP/NADPH dependent enzymatic lipid peroxidation and (c) free radical chain reaction in rat liver microsomes:

(a) $Fe^{3+}$/ascorbate dependent non-enzymatic lipid peroxidation in bovine brain phospholipids was conducted according to Houghton et al. (1995). The reaction in the absence and presence of plant extracts or rutin (reference compound) containing 5 mg/mL of bovine brain phospholipids, 1 mM $FeCl_3$ and 1 mM ascorbic acid in 10 mM phosphate buffer with a final volume of 500 μL, was incubated at 37 °C for 1 h. The OH radicals generated in the reaction, initiated the lipid peroxidation resulting in MDA production that was measured by TBA-reaction.
(b) In $Fe^{3+}$/ADP/NADPH dependent enzymatic lipid peroxidation, rat liver microsomes were prepared as described by Kiso et al. (1984). Protein contents of rat liver microsomes were measured by the method of Lowry et al. (1951) using bovine serum albumin (10–50 μg/mL) as a standard. Rat liver microsomes were used for the enzymatic lipid peroxidation as described by Xiong et al. (1996) with slight modification. The reaction was performed in the absence and presence of plant extracts or rutin (reference compound), containing microsomal suspension (20 mg protein/mL), 1.7 mM ADP, 100 μM $FeCl_3$ and 0.4 mM NADPH in 37.2 mM Tris–HCl buffer (pH 8.0) to a final volume of 1 mL followed by an incubation at 37 °C for 30 min. After cooling on ice cold water the lipid peroxides were measured as described by Kiso et al. (1984).
(c) Free radical chain reaction was performed using the same procedure as described for enzymatic lipid peroxidation in rat liver microsomes however, the major difference was that the plant