

ANTIOXIDANT ACTIVITIES OF NATURAL PHENOLIC COMPOUNDS FROM
ACACIA CONCURRENS BARK

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Abstract

The present study showed that the ethanolic extracts from the bark of *Acacia concurrens* exhibited a strong antioxidant activity. Among all the fractions from ethanolic extracts of bark, the EtOAc soluble fraction exhibited the best antioxidant performance. Furthermore, the amounts of total phenolic compound were determined from the ethanolic extracts. Therefore, *Acacia concurrens* could be considered as a potential source of natural antioxidant.

Keywords: Bark; Antioxidant activity; Free radical; Phenolic compounds.

Introduction

The importance of the reactive oxygen species (ROS) has attracted increasing attention over the last decade. Reactive oxygen species include free radicals such as super oxide anion radicals, hydroxyl radicals, non free radicals such as hydrogen peroxide and singlet oxygen, along with various forms of activated oxygen¹ and they are involved in various physicochemical processes and disease such as aging, cancer² and activity atherosclerosis³. Thus, recent studies have investigated the potential of plant products to serve as antioxidants against various diseases induced by free radicals⁴. Additionally, it has been determined that

the antioxidant effect of plant products is mainly due to phenolic compounds, such as flavonoids, phenolic acids, tannins and phenolic diterpenes^{5,6}. There have been numerous studies on the biological activities of phenolics, which are potent antioxidants and free radical scavengers^{7,8}.

Acacia concurrens is a vigorously growing, deciduous or evergreen tree, possibly attaining 10 m height. It belongs to the family Mimosaceae, and commonly known as Australian babul. Traditionally, in India the bark and wood are used for carving and bark infusion for coughs & colds. However, the potential health benefits of the bark extracts of A.

concurrans have not been studied in detail to date. The objective of the present study is to investigate the in vitro antioxidant activity of the bark of *Acacia concurrans* belongs to the family Mimosaceae.

Material & Methods

Chemicals :-

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, 3,4,5-trihydroxybenzoic acid, benzoic acid, and quercetin were all purchased from Sigma Chemical Co. (St. Louis, MO). All other unlabelled chemicals and reagents were of analytical grade.

Plant materials and extraction :-

The plant material were collected from the cultivated species in the local area of Gondia district in Maharashtra. The plant materials were identified and authenticated by experts of the region, whereas a voucher No. 5967/R has been deposited at Department of Botany, Nagpur University, Nagpur. The dried samples were cut into small pieces (5.50 kg) and soaked in 70% ethanol at ambient temperature for 7 days. The extracts were decanted, filtered under vacuum, concentrated in a rotary evaporator and then lyophilized. The resulting powder extracts were then extracted successively with *n*-hexane (72.4 g), EtOAc (325.6 g), *n*-butanol (573.7 g), and water (467.2 g). After removing the solvents used for the

successive extractions, four soluble fractions were obtained.

DPPH (1,1-Diphenyl-2-picrylhydrazyl) free radical scavenging assay :-

The DPPH free radical scavenging activity of *Acacia concurrans* bark extracts was examined⁹. First, 50 µ of test sample in methanol (final concentrations were 1, 5, 10, 50 µg/ml, respectively) were mixed with 450 µl of 50 mM Tris–HCl buffer (pH 7.4) and 1500 µl of 0.1 mM DPPH-ethanol solution. After 30 min of incubation at ambient temperature, the reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. Quercetin was used as a positive control. The inhibition ratio (percent) was calculated according to the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100.

Determination of total phenolics :-

Total phenolic contents were determined according to the Folin–Ciocalteu method¹⁰, using gallic acid as a standard. The test samples were dissolved in 5 ml of methanol/water (50/50). The sample solution (500 µl) was mixed with 500 µl of 1N Folin–Ciocalteu reagent. The mixture was allowed to stand for 5 min, which was followed by the addition of 1 ml of 20% Na₂CO₃. After 10 min of incubation at ambient temperature, the mixture was centrifuged for 8 min

(12,000g), and the absorbance of the supernatant was measured at 730 nm. The total phenolic contents were expressed as gallic acid equivalents (GAE) in milligrams per gram sample.

Results and discussion:

Free radical scavenging activity of bark extracts of *A. concurrens* :-

DPPH is one of the compounds that has a proton free radical with a characteristic absorption, which decreases significantly on exposure to proton radical scavengers¹¹. It is well accepted that the DPPH radical scavenging by antioxidants is attributable to their hydrogen donating ability¹². In this study, the free radical scavenging activity of its derived fractions of *A. concurrens* was assessed by DPPH assay. As shown in Table 1, except for the *n*-hexane fraction, all the test samples showed a significant inhibitory activity against the DPPH radical. Among them, EtOAc and BuOH fractions were the best. The complete inhibition of DPPH radical by test samples was observed at a range of 5–10 µg/ml. In addition, the IC₅₀ values (the concentration required to inhibit radical formation by 50%) of the crude extract, *n*-hexane fraction, EtOAc fraction, BuOH fraction, and water fraction were 3.5, 3.2, 3.6, and 4.4 µg/ml, respectively.

The IC₅₀ value of a well-known antioxidant compound used as a reference in this study, quercetin, is 1.6 µg/ml.

Compared with pycnogenol from the bark of the French maritime pine (*Pinus maritima*), a commercial product, the IC₅₀ value is approximately 5µg/ml¹³. Thus, the bark extracts of *A. concurrens* might be a good candidate to be developed into a commercial product.

Plant phenolics, in general, are highly effective free radical scavengers and antioxidants. The content of total phenolics in each fraction was determined spectrometrically according to the Folin-Ciocateu method and calculated as gallic acid equivalents (GAE). Accordingly, the total phenolic content of the EtOAc fraction (505.9 mg/g) was significantly higher than that of the BuOH fraction (323.3 mg/g), the water fraction (401.8 mg/g), and the *n*-hexane fraction (0.0 mg/g). These results indicated that the free radical scavenging activity of ethanolic extracts of *A. concurrens* bark could be effectively enriched in the EtOAc fraction. Thus, the EtOAc fraction was further investigated for its phytochemical characteristics and in vitro antioxidant activity.

Antioxidant activity of phenolic compounds:-

Presently, information on the distribution of phenolic acids within plants is very limited. The major phenolic compounds were found in the EtOAc fraction from *A. concurrens* bark. To

examine the structure antioxidant activity relationships, two other analogues including benzoic acid and 3,4,5-trihydroxybenzoic acid were selected and their antioxidant activity compared with the other phenolic compounds^{14,15}. The scavenging effects of these compounds are shown in **Fig. 1**. This sequence indicated that the free radical scavenging activity of the test compounds was due to their hydrogen-donating ability. These results also suggested that catechol skeletons had better antioxidant activity.

Conclusion

In this study, it was demonstrated for the first time that the ethanolic extracts from *A. concurrens* bark possessed an excellent antioxidant activity based on the DPPH free radical assay. Some of the compounds in *A. concurrens* bark exhibited the strongest radical scavenging activity. Future studies should focus on the employment of modern medical chemical techniques to modify the structures of certain purified plant ingredients into better agents with high efficacy and activity. In addition, in vivo pharmacological studies also should be made.

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Table 1: IC₅₀ values of ethanolic extracts from the bark of *A. concurrens* against DPPH free radical

Sr. no.	Specimens	µg/ml
1	Crude extract	3.5
2	<i>n</i> -Hexane fraction	>100
3	EtOAc fraction	3.2
4	BuOH fraction	3.6
5	Water fraction	4.4
6	Quercetin	1.6

Fig. 1. Free radical scavenging activity of various benzoic acids measured by the DPPH assay marked by different strikes. Results are mean ± SD (n=3).

