

NOTE

Antioxidant Activity of *Acacia nilotica* Bark†

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Aqueous extract of the bark of *A. nilotica* showed strong antioxidant activity in nitroblue tetrazolium (NBT) photoreduction method. Bioactivity guided isolation of the extractives of the bark led to the identification of gallic acid as the source of antioxidant activity.

Key words: Antioxidant, activity, *Acacia nilotica* bark.

A. nilotica (L.) Willd.¹ (Family: Mimosaceae), commonly known as “Black Babool”, is a tree having alternate leaves with 15–25 pairs of leaflets. Flower heads globose, yellow in colour with axillary fascicles. Bark is dark brown in colour with fissures. In traditional medicine, a decoction of the bark was reported to be useful for relieving cough². The plant was established to possess molluscicidal activity^{3,4}, antiplatelet aggregatory activity⁵ and hypoglycemic activity⁶. Polyphenols⁷ and catechin gallates² were isolated earlier. We report herein the results of antioxidant activity studies on the extractives of the bark of *A. nilotica*.

Determination of superoxide scavenging activity: Superoxide scavenging activity of the various extracts and fractions was determined by the method of McCord and Fridovich⁸. The assay mixture contained different concentrations of extracts and EDTA (6 μM containing 3 μg NaCN), NBT (50 μM), riboflavin (2 μM) and phosphate buffer (58 μM , pH 7.8) to give a total volume of 3 mL. The tubes were uniformly illuminated for 15 min, and thereafter the optical density was measured at 560 nm. The percentage inhibition of the extracts of superoxide production was evaluated by comparing the absorbance values of the control and experimental tubes. Inhibitory concentration 50 (IC₅₀) was determined by plotting concentration against percentage inhibition.

Commercially available aqueous extract in the form of dry powder was supplied by Laila Impex (AE1, AR 5109). Milled stem bark of *A. nilotica* (100 g) was extracted in a soxhlet apparatus with hexane (HE, 650 mg), chloroform (CE, 870 mg), ethylacetate (EE, 1580 mg), methanol (ME, 5800 mg) and water (AE2,

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7500 mg). All these extracts were tested for antioxidant activity by NBT/riboflavin photoreduction method⁸. The ethyl acetate extract showed maximum antioxidant activity in NBT method, was fractionated further by preparative HPLC (reversed phase, supercosil PLC-18, 250 × 21.2 mm, 12μ acetonitrile : water with 0.1% phosphoric acid, 14 : 86, 10 mL/min, UV detection at 272 nm) into two fractions (Fraction-1, RRT: 7.35 min and Fraction-2, RRT: 8.87–20 min). The results of antioxidant activity studies were noted in Table-1.

TABLE-1
ANTIOXIDANT ACTIVITY OF *A. nilotica* BARK EXTRACT

Extract	AE-1	HE	CE	EE	ME	AE-2	F-1	F-2	GA ^b	GT ^c	GSE ^d	Vit. E
I.C. 50 ^a	4.7	80	33	5.4	13	10	2.2	95	2.2	3.8	4.8	> 1000

^a50% inhibitory concentration of extract in μg/mL; ^bGalic acid (Sigma); ^cGreen tea extract (catechins, 65%); ^dGrape seed extract (polyphenols, 90%). *Method*: NBT/riboflavin photoreduction method.

Identification of active constituents: Fraction-1 showed maximum antioxidant activity and it was identified as gallic acid. The identity was established further by IR, mass co-HPTLC and co-HPLC with authentic gallic acid.

The strong antioxidant activity exhibited by the extracts of the bark of *A. nilotica* is mainly due to the presence of gallic acid (0.15%).

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