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In vitro bioactivity-guided fractionation and characterization of polyphenolic inhibitory fractions from *Acacia nilotica* (L.) Willd. ex Del.

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Abstract

The present study was undertaken to evaluate antimutagenic and cytotoxic effects of different extracts/fractions of *Acacia nilotica* prepared by maceration method. The potency order of different extracts was more or less similar in Ames assay as well as in cytotoxic assay. Considering the maximum potential of acetone extract in both the assays, the studies were initiated to fractionate this extract. Two pure fractions, namely AN-1 and AN-2, were obtained from acetone extract, of which AN-2 was found to be of gallic acid and AN-1 fraction is still to be identified. In conclusion, the antimutagenic and cytotoxic activities exhibited by acetone extract may partially be ascribed to the presence of gallic acid and other polyphenols.

Keywords: Antimutagenicity; Cytotoxicity; Cancer cells; *Acacia nilotica*; *Salmonella typhimurium*

1. Introduction

Acacia nilotica (L.) Del. syn. *Acacia arabica* (Lam.) Willd. (Mimosaceae) is an important multipurpose tree that has been used extensively for the treatment of various diseases, e.g. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma (Ambasta, 1994). It also serves as a source of various products, including polyphenols (Kirtikar and Basu, 1975; Purseglove, 1998). The role of these natural products to the plant itself is not well understood, but for the human kind they can be of prime importance. Therefore, the bioprospection of naturally occurring polyphenolic compounds having ability to provide protection against certain types of mutagens and carcinogens is of great importance (Ferguson, 2001; Jiang et al., 2001; Yang et al., 2001). In the present study, the antimutagenic and cytotoxic activities of *Acacia nilotica* extracts have been examined using Ames assay and cell-lines, viz. S115 (mouse breast cancer), human osteosarcoma (HOS) and PC-3 (human prostate cancer), respectively.

2. Material and methods

2.1. Extraction/fractionation of bark components

The bark of *Acacia nilotica* was collected from a tree growing on the Guru Nanak Dev University Campus, Amritsar in the month of September. The plant was identified and submitted in the herbarium (voucher no. 1020). Dried powdered bark was subjected to sequential maceration with

Abbreviations: 2AF, 2-aminofluorene; DMSO, dimethylsulfoxide; NPD, 4-nitro-*o*-phenylenediamine

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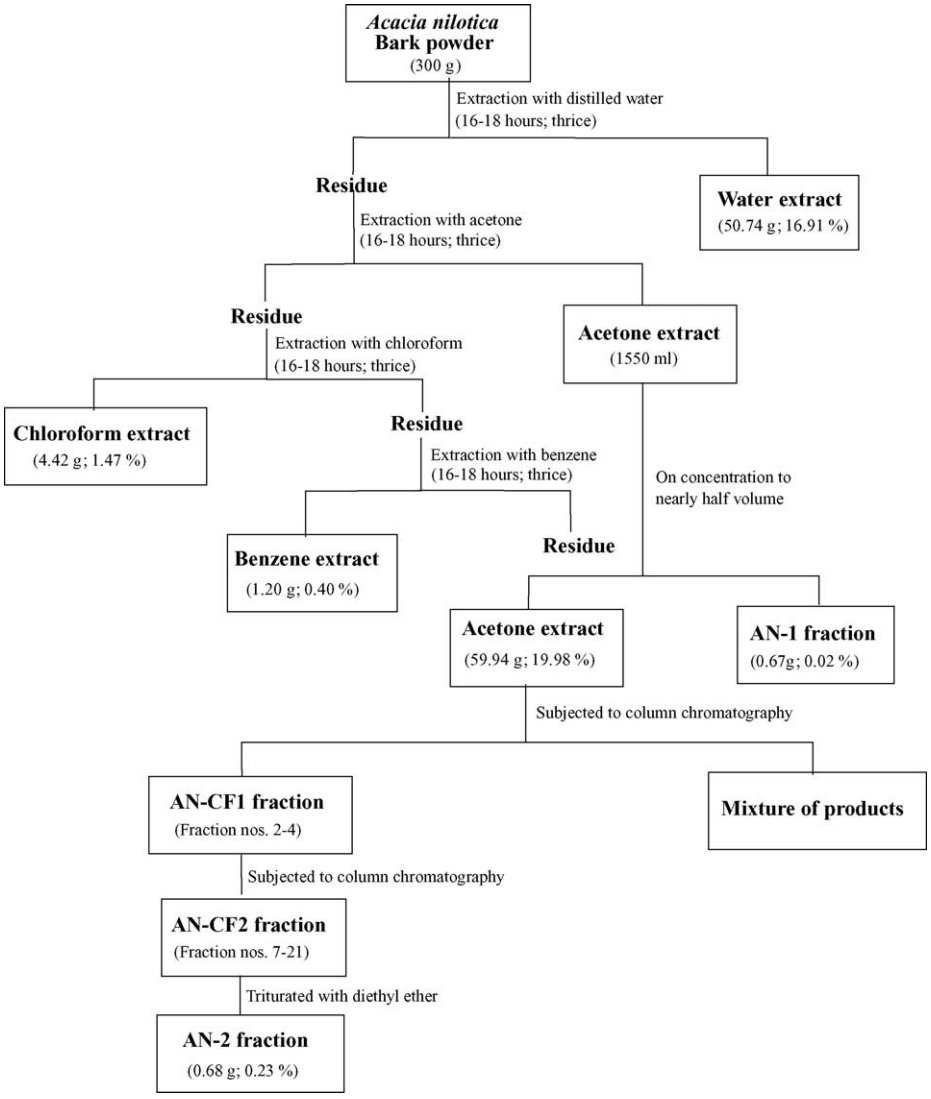

Fig. 1. Flow chart of the schematic representation of extraction of various extracts from *Acacia nilotica*.

water, acetone, chloroform and benzene at room temperature to get water, acetone, chloroform and benzene extract, respectively (Fig. 1). Acetone extract, on concentration to nearly half volume, gave a very small quantity of yellow-coloured precipitates that showed a single spot on TLC. This solid was separated out and designated as AN-1 fraction. The remaining acetone extract was completely dried and subjected to column chromatography on silica gel (60–120 mesh). The column was eluted with ethyl acetate and 10 fractions of 200 ml each were collected. On TLC observation, the fraction nos. 2–4 were combined and the solvent was distilled off to get AN-CF1 fraction that was again subjected to column chromatography on 60–120 mesh silica gel. The column was eluted with 50% ethyl acetate and hexane gradient, and 35 fractions of 50 ml each were collected. On TLC observation, the fraction nos. 7–21 containing gallic acid was combined and the solvent was distilled off to get solid residue AN-CF2. This residue was triturated with diethyl ether to get pure gallic acid and AN-2 fraction (Fig. 1). Retention factor (Rf) of AN-2 fraction was 0.48. By superimposable infrared (IR) spectrum, it was found to be comparable to standard gallic acid.

2.2. Antimutagenicity assay

Salmonella tester strains TA98 and TA100 were kindly supplied by Dr. B.N. Ames, University of California, Berkeley (USA) and Dr. Wagner Skip and Dr. Meena Rao, BioReliance Corporation, Rockville, MD (USA). Sodium azide, 4-nitro-*o*-phenylenediamine (NPD) and 2-aminofluorene (2AF) were procured from M/s Sigma Chemical Co., St. Louis, MO (USA). The *Salmonella* histidine point muta-