

Supplementary Materials and Methods

2.1. Fungal identification and isolation of *A. flavus* from nuts

Fungal species were identified by microscopic examination and according to various mycological keys as mentioned previously by (Visagie et al. 2014) and *A. flavus* was selected for further studies (Supplementary table S1). *A. flavus* was identified by molecular approach. DNA of *A. flavus* was recovered by SDS-Proteinase K-CTAB method. Universal fungal primers of 18S rDNA, NS1 (5'-GTAGTCATATGCTTGTCTC-3') and NS4 (5'-CTCCGTCAATTCCTTTAAG-3') used to amplify partial region of rDNA by PCR. The nucleotide sequences were determined by the sequencing of the DNA [GenBank: LC096956.1].

2.2. Extraction and purification of 5'-HA

The extraction was carried out using petroleum ether at room temperature. After evaporation till dryness, the extract was obtained in yields (w/w) of 5,55.

A sample from petroleum ether extract (15.25 g) was applied to a Sephadex LH-20 column equilibrated with: PE:MeOH:CHCl₃ (2:1:1). After comparison with TLC five fractions were obtained 1: (0.4076 g), 2: (1.66 g), 3: (4.25 g), 4: (0.734 g), 5: (0.0813) g. Fraction 3 (4 g) was applied to a Sephadex LH-20 column. After comparison with TLC, fractions were combined resulting in five new fraction: 1: 0.0055 g, 2: 0.0661 g, 3: 0.2234 g, 4: 0.5321g, 5: 0.7221g. From fractions 1-5 pure compound (85.66 mg) was obtained. The compound was identified as 7-(5-Hydroxy-3, 7-dimethylocta-2, 6-dienyloxy)-chromen-2-one depending on NMR data (Kurdelas et al. 2010, Ngadjui et al. 1989).

2.8. Measurement of antioxidant enzymes

Prior to determination of antioxidant enzymes, the antioxidant activity of 5'-HA was evaluated by measuring DPPH radical scavenging activity according to the method of (Choi et al. 2002). Ten 414 mg of ascorbic acid (SAP Chemical) were dissolved in 100

mL methanol (BRATACO Chemical) to 415 obtain a solution with a concentration of 100 ppm. Solution was then prepared into several final 416 concentrations of 0, 5, 15, 30, 45 and 60 ppm for being used subsequently in antioxidant activity assay. 417 The free radical scavenging activity of the different concentrations of 5'-HA was analyzed using 2,2- 418 diphenyl-1-picryl-hydrazyl (DPPH). The DPPH solution was prepared in methanol and subsequently 419 added to various concentrations of the 5'-HA (20 - 80 µg/ ml The absorbance changes were measured 420 at 517 nm. Ascorbic acid was used as standard. These measurements were performed in duplicate and percentage of inhibition (%I) was calculated using the following equation:

$\% = \frac{Ab - As}{Ab} \times 100$, Ab is the absorbance of control and As is the absorbance of the extract. The IC₅₀ values were calculated using linear regression analysis and used to indicate antioxidant capacity.

For catalase (CAT) and superoxide dismutase (SOD) measurements, cultures of *A. flavus* were performed on MEA medium and maintained in the dark place at 27 °C for 4 days. For CAT measurements, 200 mg of *A. flavus* mycelium were placed in 50 mM potassium-phosphate buffer (pH 7.0 containing 1 mM EDTA) while for SOD measurements, samples were placed in a 20 mM HEPES buffer (pH 7.2; 1 mM EGTA, 210 mM mannitol and 70 mM sucrose) (Caceres Isaura et al. 2016). Samples were centrifuged at 10,000 rpm for 15 min at 4° C and enzyme activities were measured in supernatants. Enzymatic activity was evaluated using commercial kits Catalase (CAT-707002) and Superoxide Dismutase (SOD-706002) (Interchim, Montluçon, France). Absorbance was measured at 540 and 450 nm for catalase and superoxidedismutase assays respectively, using an ELISA plate reader (Spectra thermo scan, Tecan, NC, USA).

References

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