

## Abstract

*Nicotiana glauca* Graham (Solanaceae), or tree tobacco, is found in dry arid climates of North America, Africa and Europe. It has been reported to have both toxic and medicinal properties. The main aim of this study was to analyze the phytochemical screening and quantitative estimation of polyphenols, flavonoids and flavonols and identification of the main chemical compounds by LC-ESI-MS/MS of crude extracts from the leaves of *N. glauca* Graham to evaluate its in vitro antioxidant properties.

Three different solvents were used to extract bioactive compounds from powdered leaves of *N. glauca* : dichloromethane (DCM), ethyl acetate (AE) and n-butanol (*n*-BuOH). The three extracts were then subjected to qualitative phytochemical screening using standard procedures. Total phenolics, total flavonoids and total flavonols contents of the extracts were measured by Folin Ciocalteu and Aluminium chloride methods respectively. The three extracts were then subjected to qualitative phytochemical screening using standard procedures. These methods showed the presence of polyphenols and coumarins in all extracts. Moreover, flavonoids, tannins, steroids and quinones were reported in the AE and *n*-BuOH extracts.

In addition, alkaloids were seen to be present in DCM extract, while saponins and phlobatannins were absent in all extracts.

The chromatographic identification by LC-ESI-MS/MS spectrometry, conducted on the DCM, AE and *n*-BuOH extracts, provided tentative identification of 16 phenolic compounds, the majority of which were detected for the first time in this species in the present work, including 5 alkaloids, 2 coumarins, 2 flavonoids, 2 monoterpenes, 4 hydroxycinnamic acid derivatives and one homoserine lactone.

Furthermore, The antioxidant capacity was performed using the DPPH, ABTS, DMSO alkaline, Phenanthroline, FRAP and CUPRAC methods. Results using the DPPH method showed strong free radical scavenging activity for three extracts. This activity decreased with increasing concentration in the following order : *n*-BuOH > AE > DCM.

In other assays, all extracts showed good antioxidant activity which decreased with increasing concentration in the following order : AE > *n*-BuOH > DCM. Extracts were compared with standards : BHT, BHA, Tannic acid and  $\alpha$ -Tocopherol. The antioxidant of these extracts is probably related to polyphenols content (351,55 $\pm$ 0,07, 284,98 $\pm$ 0,08 and 133,8 $\pm$ 0,06 mg/g), flavonoids (105,97 $\pm$ 0,04, 164,44 $\pm$ 0,07 and 1,18 $\pm$ 0,005 mg/g) and flavonols (22,41 $\pm$ 0,24, 18,75 $\pm$ 0,46 mg/g) in AE and *n*-BuOH, respectively.

From all these results, it was concluded that the leaves of *N. glauca* AE and *n*-BuOH extracts were the most efficient ; therefore, they were selected for the separation of bioactive molecules. This separation using column chromatography (CC). The compound separated was elucidated by the various methods spectral : NMR (1H, 13C), UV and comparison with literature values.

In parallel, the antioxidant potential of the majority compound separated P\* Rutin, was evaluated by the same six methods used previously, showed a very significant antioxidant activity (DPPH : IC<sub>50</sub> = 7,19 $\pm$ 0,11  $\mu$ g/mL ; ABTS : IC<sub>50</sub> = 10,12 $\pm$ 0,14  $\mu$ g/mL ; DMSO alkaline : A<sub>0,5</sub> = 3,09 $\pm$ 0,05  $\mu$ g/mL ; phenanthroline : A<sub>0,5</sub> = 33,88 $\pm$ 0,23  $\mu$ g/mL ; FRAP : A<sub>0,5</sub> = 56,27 $\pm$ 1,56  $\mu$ g/mL et CUPRAC : A<sub>0,5</sub> = 7,55 $\pm$ 0,18  $\mu$ g/mL).

**Key words:** *Nicotiana glauca* Graham, LC-ESI-MS/MS, antioxidant activity, screening