EXTRACTION, PHYTOCHEMICAL ASSAY AND BIOLOGICAL ACTIVITIES OF PHENOLIC COMPOUNDS FROM A MEDICINAL PLANT: *EUCALYPTUS GLOBULUS*

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Résumé :

Extract of *Eucalyptus globulus* used in phytotherapy was screened *in vitro* for phenolic compounds content, antioxidant and antibacterial activities. Polyphenols were extracted from trunk bark with methanol (99 %). The proportion of total phenolics was 256 mg CE (catechin equivalents) /g of dry weight (dw). Antioxidant activity of the *Eucalyptus globulus* trunk bark extract (EGTBE) was evaluated using two methods, the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and hydrogen peroxide scavenging. The extract revealed a concentration – dependent anti-radical activity by inhibiting DPPH radical with 65,17 % for 50 µg / ml. In addition, the result obtained indicates that EGTBE had strong hydrogen peroxide scavenging activity by decrease in absorbance after 20 min. Antibacterial activity of the EGTBE was examined by means of disk-diffusion methods with six bacterial species (*Proteus vulgaris, Proteus mirabilis, Escherichia coli, Citrobacter freundii, Staphylococcus aureus, Klebsiella pneumoniae).* Extract was effective in inhibiting the growth of the microorganisms except for *Staphylococcus aureus* and *Citrobacter freundii.*

Keywords: *Eucalyptus globulus* trunk bark, methanolic extract, antioxidant activity, antibacterial activity and phenolic content.

I. Introduction

Eucalyptus globulus is indigenous of Tasmania and the south west of Western Australia. Because of its fast growth and good quality *Eucalyptus globulus* is being established in plantation in Mediterranean country. It has been introduced in Algeria in 1854 by Ramel. Its leaves have been used as tradional remedies for the treatment of various diseases**.** *Eucalyptus globulus* has been well known, for the volatile terpenoid constituents of the essential oil. Concerning the phenolic compounds of *Eucalyptus globulus*, they have been quantified, though only in leaves and wood. Up to now, little has been done on the activities of trunck bark of this plant. Give the important evidence of the effect of natural compounds occurring polyphénols on disease prevention; we present a study on the determination of the phenolic content, antioxidant and antibacterial activities of the methanolic extract of *Eucalyptus globulus* trunck bark.

2. Materials and Methods

2.1. Plant Material

Trunck bark of *Eucalyptus globulus*, Myrtaceae family, was obtained from his natural habitats. The bark was cleaned, dried in the steam room and reduced to thin powder.

2. 2. Preparation of the extract

Polyphenols from the dried and powdered sample were extracted with methanol (99 %). The process of extraction continued for a week at room temperature in dark place, using magnetic blender. The extract was filtered through Whatman N°4 filter paper and concentrated to dryness under reduced pressure in rotary evaporation to yield dried methanol extract.

2.3. Determination of antioxidant assays

2. 3. 1- 1, 1- diphenyl – 2 –picryl –hydrazyl (DPPH) free radical –scavenging activity

The free radical-scavenging activity of EGTBE was measured as reported by **Lee et al, (2003)**. The percent of the DPPH decolouration of the samples was calculated as:

Antiradical activity = 100 (1- absorbance of sample / absorbance of control).

2. 3. 2. Determination of hydrogen peroxide H_2O_2 scavenging activity

H_2O_2 scavenging activity of the extract was determined according to the method of **Yamasaki et al, (1977)** with some modifications.

2.4. Determination of antibacterial activity

Antibacterial activity of the EGTBE was examined by means of disk-diffusion methods with six bacterial species, *Proteus vulgaris, Proteus mirabilis, Escherichia coli, Citrobacter freundi, Staphylococcus aureus and Klebsiella pneumoniae.* These microorganisms strains were clinical isolate.

2. 4. Spectrophotometric determination of phenolic compounds

The total phenols content was determined spectrophotometrically at 740 nm using the procedure of Owen and Johns, (1999). Flavonoïds content of EGTBE was determined according to the method of Bahorun et al, (1996) and tannin concentration of EGTBE was determined spectrophotometrically at 510 nm using the protein precipitation method of Hagerman and Butler, (1978) with some modifications.

3. Results and Discussion

3.1. Extraction yield

The yield of extract obtained from EGTBE using methanol was found to be 50 % (W/W).

3.2. Antioxidant activity

3. 2. 1. Scavenging activity on 1, 1 Diphenyl -2-picryl –hydrazyl radical

The extract showed a concentration – dependent anti-radical activity by inhibiting DPPH radical with 65.17 % 50 µg / ml, the results are shown in Fig. 1. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. Following to this result we can pronounce that the EGTBE possesses hydrogen donating capabilities and acts as antioxidant.

3. 2. 2. Determination of hydrogen peroxide Scavenging activity

Hydrogen peroxide can be performed *in vivo* by many oxidizing enzymes such as superoxide dismutase. The result obtained indicates that EGTBE had strong hydrogen peroxide scavenging activity Fig. 2 by diminution of DO after 20 min.

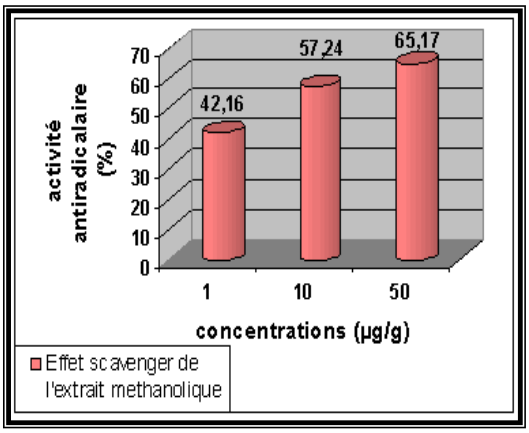

Fig.1. Free radical scavenging activity (DPPH) of different concentration (1, 10 and 50 µg/ml of EGTBE. In 3. 2. 1. Scavenging activity on 1, 1 Diphenyl -2 –picryl –hydrazyl radical

3. 4. Determination of antibacterial activity

Extract was effective in inhibiting the growth of microorganisms except for *Citrobacter freundii* and *Staphylococcus aureus,* the result is shown in Table 1. The idea that these activities were due to the presence of phenolic compounds led to investigate the

Table 1: Antibacterial of methanolic extract of EGTB. In 3. 4. Determination of antibacterial activity.

Microorganismes	Diameter of EGTBE zone (mm)
Proteus mirabilis	10,56
Proteus vulgaris	8,46
Klebsiella pneumoniae	7,54
Escherichia coli	5,18
Citrobacter freundii	4,94
Staphylococcus aureus	3,46

phenolic composition of the extract studied. The proportion of total phenolics was 256 mg CE (catechin equivalents) /g of dry weight (dw), concerning tannins and flavonoïds, the concentration obtained were 173,

67 mg ATE (tannic acid equivalents) /g of dw; 7, 5 mg (quercetin equivalents) Q E / g of dw, respectively.

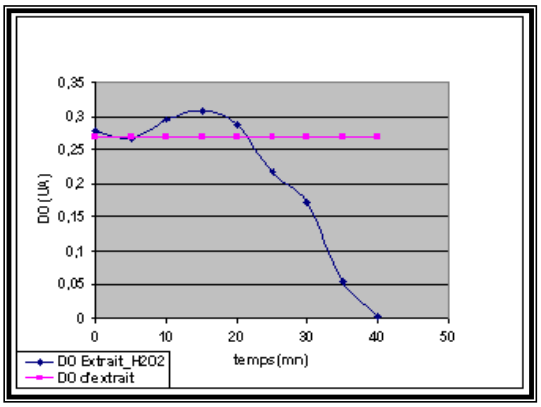

Fig. 2. Hydrogen peroxide scavenging activity of EGTBE. In 3. 2. 2. Determination of hydrogen peroxide scavenging activity

Conclusion

Overall, it could be concluded that EGTBE has potent antioxidant and antibacterial activities. The results of this study indicate that it can be used as easily accessible source of natural antioxidant and antibacterial. The preliminary chemical examination of methanolic extract of this plant has shown the presence of number of polyphénols.

currently, unclear. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidative and antibacterial components of EGTBE.

References

[1] Bahorun, T.; Gressier, B.; Trotin, F.; Brunet, C. et al. (1996). Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations. *Arzneim–Forsch Drug Research* 46: 1086 –1108.

[2] Hagerman, A.E. and Butler, L.G. (1978). Protein precipitation method for the quantitative determinationof tannin. Journal agriculture and Food Chemistry 26 (4): 809-812.

[3] Owen, P. L. and Johns, T. (1999). Xanthine oxidase inhibitory activity of northeastern north American plant remedies used for gout. Journal of Ethnopharmacology 64: 149-160.

[4] Yamasaki, H.; Sakihama, Y. and lkehara. N. (1997). Flavonoid-Peroxidase Reaction as a Detoxification Mechanism of Plant Cells against H202. Plant Physiol 11 5: 1405-1 41 2.

[5] Lee, K. W.; Lee, H. J.; Surh, Y. J. and Lee, C. Y. (2003). Vitamin C and chemoprevention: reappraisal. American Journal of Clinical Nutrition **78**: 1074-1078.