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Bioactive components of butanolic extract of *Hypericum tomentosum* L. (Clusiaceae)

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ABSTRACT

From the butanolic extract of Hypericum tomentosum L., eight flavonoids and one phenolic acid have been isolated and structurally elucidated. The antioxidant activity of the butanolic extract was investigated by the use of the DPPH scavenging method and phenol quantification.

Keywords: *Hypericum tomentosum* L.; Clusiaceae; Antioxidant; DPPH; polyphenols.

INTRODUCTION

The genus *Hypericum* (Clusiaceae), including about 400 species, is widely distributed in the Mediterranean area. Antifungal [1], antibiotic [2], anti-viral [3], anticancer [4], antioxidant [5,6] and antidepressant [7,8] activities have been reported for this genus. In continuation of our works on therapeutic plants of Constantine province [7-39], we report here the composition and the antioxidants activity of the butanolic extract of the endemic species *H. tomentosum* [40], collected at Constantine (Eastern Algerian).

MATERIALS AND METHODS

Plant Material

Aerial parts of *Hypericum tomentosum* were collected on May 2011 at Bekira- Constantine (Eastern Algerian). The voucher specimen was identified by Professor Gérard De Bélair (University Badji-Mokhtar, Annaba) and was deposited at the Laboratory of Therapeutic Substances (LOST) under the reference LOST/Ht/05/11.

Extraction and isolation

Air-dried and powdered aerial parts (1kg) of *Hypericum tomentosum* were extracted with 70% MeOH. The residue was dissolved in water and extracted with petroleum ether, dichloromethane, ethyl acetate and n-BuOH, successively.

The butanolic extract of *Hypericum tomentosum* (BEHT) was column chromatographed on silica gel with a gradient of cyclohexane-ethyl acetate with increasing polarity then with a gradient of ethyl acetate-MeOH with increasing polarity. Further successive separations using a silica gel column eluted with a gradient of ethyl acetate-methanol with increasing polarity followed by preparative TLC silica gel 60GF plates, eluted with ethyl acetate-MeOH-AcOH (8:1:1) and 3mm Whatman paper Chromatography, using BAW (4:1:5) upper phase led to nine compounds (**1**-**9**).

Antioxidant activity

DPPH scavenging method

The radical scavenging activity of the BEHT was measured by the slightly modified method of Hatano (1988) [19, 41]. One milliliter of a 0.2 mM DPPH methanol solution was added to 4 mL of various concentrations of the extract in methanol. The mixture was shaken vigorously and left to stand at room temperature. After 30 min, the absorbance of the solution was measured at 517 nm and the antioxidant activity calculated using the following equation:

Scavenging capacity % = [(Ab of sample – Ab of blank) × 100/ Ab of sample].

Methanol (1 mL) plus plant extract solution (4 mL) were used as a blank, while DPPH solution plus methanol was used as a negative control. The positive control was DPPH solution plus 1 mM rutin. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the plot of inhibition percentage against extract concentration.

Total phenolic quantification

Total phenol concentration was determined according to the Folin-Ciocalteu method [42], using pyrogallol as a standard. The absorbance was measured at 760 nm on a Uvikon 930 UV/VIS spectrophotometer (Kontron instruments) and the results were expressed as pyrogallol equivalents in g per 100 g dry material.

RESULTS AND DISCUSSION

Isolated and identified compounds

From the butanolic extract, 8 known flavonoids and one known phenolic acid were isolated and characterized by the spectroscopic methods (UV, ^{1}HNMR, ^{13}CNMR, HMBC, HSQC) as dehydrokaempferol (**1**), luteolin **(2)**, quercetin **(3),** apigenin-7-O-β-*D*-glucoside (**4),** rutin (**5**), quercetin 7-O-α-*L*-rhamnosyl-3-O-β-*D*-glucoside (**6**), quercitrin (**7**), hyperoside (**8**) and chlorogenic acid (**9**), respectively.

Antioxidant activity

DPPH scavenging method and Phenol quantification

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. From Table 1 and Figure 1, illustrating the results of Free Radical Scavenging Activity and Phenolic amounts of the BEHP, a significant phenolic content (>10.77 g/100 g of dry extract) and a strong radical scavenging activity were found for this extract (IC_{50} = 14.5 µg/ml), compared with Queretin (IC_{50} = 12 µg /ml) and Rutin (IC_{50} = 3.01 µg/ml) standards .

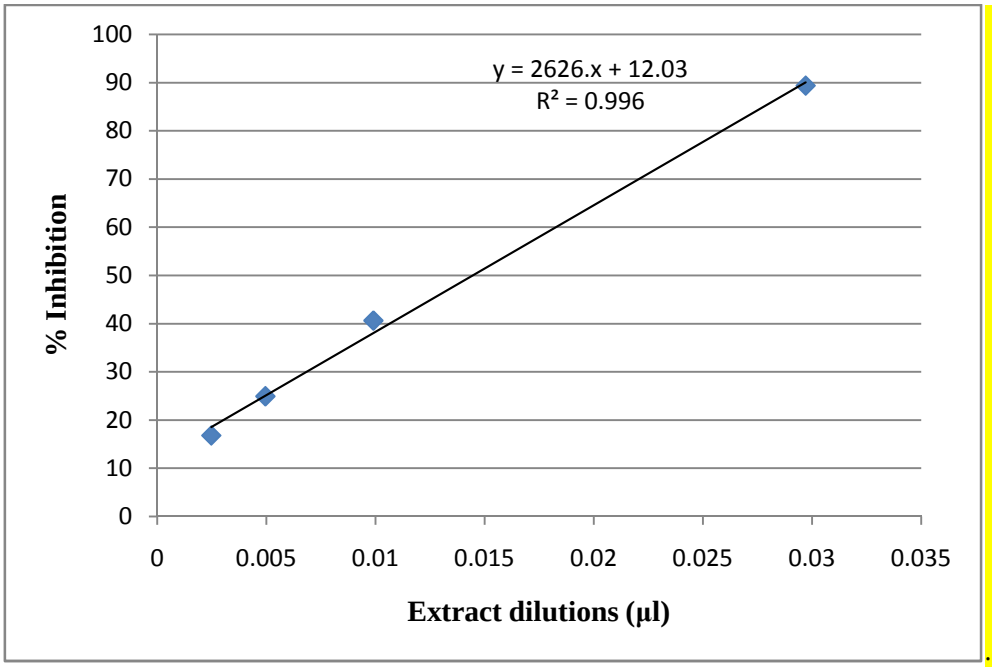

Figure (1): The BEHT scavenger effect (DPPH radical Reduction).

Table 1: Total phenolic quantification and antioxidant activity of the BEHT.

	Phenolic compounds (g/100 g equiv. Pyrogallol)	IC_{50} DPPH (µg/ml)
BEHT	10.77 ± 0.69	14.5
Quercetin		12.0
Rutin		3.0

CONCLUSION

Eight flavonoids namely, luteolin **(2)**, quercetin **(3),** apigenin-7-O-β-*D*-glucoside (**4),** rutin (**5**), quercetin 7-O-α-*L*-rhamnosyl-3-O-β-*D*-glucoside (**6**), quercitrin (**7**), hyperoside (**8**) and one phenolic acid, chlorogenic acid (**9**) have been isolated from the butanolic extract of *Hypericum tomentosum* L. This extract showed a strong radical scavenging activity (IC_{50} = 14.5 µg/ml) compared with the standards Quercetin (IC_{50} = 12 µg /ml) and Rutin (IC_{50} = 3.01 µg/ml). This may be explained by its quercetin, rutin and quercitrin content.

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