



AENSI Journals

Global journal of medicinal plant research

ISSN:2074-0883

Journal home page: <http://www.aensiweb.com/GJMPR/>

## First determination of phenolic content and antioxidant activity of *Daphne gnidium* L. flower extracts

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### ARTICLE INFO

#### Article history:

Received 8 December 2014

Received in revised form

22 December 2014

Accepted 25 December 2014

Available online 1 January 2015

#### Keywords:

*Daphne gnidium*, flowers, total phenols, total flavonoids, antioxidant activity

### ABSTRACT

**Background:** *Daphne gnidium* is mediteranean medicinal plant which is used in traditional medicine **Objective:** in the present work we determine in the first time phenolic compound content and antioxidant activity of flowers extracts of *Daphne gnidium*. **Methodology:** total phenolic was detemined by folin cicaltieu method. Total flavonoids was measured by Trichlorure Aliminium method. and antioxidant activity was evaluated by the scavenge of 2,2-diphenyl-1-picrylhydrazyl. **Conclusion:** our results highlight that *Daphne gnidium* flowers have higher quantity in polyphenols which contribute to the antioxidant activity of this organs.

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**To Cite This Article:** Mustapha Mahmoud Dif, Fouzia Benali Toumi, Mohamed Benyahia, Nabil Mekhfi, Faiza Moumen, Mohamed Rahmani, Hanane Rahmani, Wafâa Tehami. First determination of phenolic content and antioxidant activity of *Daphne gnidium* L. flower extracts. **Adv. in Nat. Appl. Sci.**, 3(2): 1-4, 2015

## INTRODUCTION

Medicinal plants play a considerable role, not only as traditional medicines used across many cultures, but also as trade commodities which meet the demand of often distant markets. Recent trends in medicinal plants studies show that there has been an appreciable increase in research activity in the area of bioactivity of natural products. For example, between 1981 and 2002, 5% of the 1,031 new chemical entities approved as drugs by the U. S. Food and Drug Administration were natural products, and another 23% were natural product-derived molecules (Clardy and Walsh 2004).

Phenolic compounds get into the human body as part of the diet. They decrease thrombocyte aggregation (Weisburger *et al.*, 1999; Pace-Asciak *et al.*, 1995) and affect apoptosis and cell proliferation through modulation of signal transduction pathways (Singh *et al.*, 2007). Their antioxidant, anticarcinogen (Rusak *et al.*, 2005) and cardioprotective effects are also proven (Yi *et al.*, 2005). These properties are remarkable facts in light of the WHO mortality statistics: nowadays cardiovascular diseases are the leading cause of death in the developed countries, causing one third of the total mortality. Plant phenolics, however, are not only present in fruits and plants but they are also traceable in derived fruit products like white and red wines (Mokni *et al.*, 2007; Castillo-Sánchez, 2007; Stervbo, 2007), determining their taste, colour and bitterness. These compounds in wines thought to account in large part for the so-called French paradox [16-17] (Goldfinger *et al.*, 2003; Iijima *et al.*, 2002).

*Daphne gnidium* L. (*Thymeleaceae*), a toxic and medicinal plant (Bruneton (1987, 2005)) that commonly grows wild in Tessala Mounts (North-West Algeria) and the whole Algerian Tell, is confined mainly to the West Mediterranean, being relieved by *Daphne linearifolia* Hart in the Eastern part of the Basin (Quézel & Santa, 1963). This evergreen shrub with leathery leaves can grow to a height of 2 m (Ziyyat *et al.*, 1997). In folk medicine the infusion of the leaves of "lazzaz" is used as hypoglycemic and to treat skin diseases (Bellakhdar, 1997; Bruneton, 1987). This plant is also used in traditional fabric dyeing (Charnot, 1945). The methanolic extract of *Daphne gnidium* L.

The present work is the determination in the first time in the word of the Total phenols, Total flavonoids and antioxidant activity of *Daphne gnidium* L. flowers extracts

## MATERIAL AND METHODS

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**Biological material:**

The inflorescence of *Daphne gnidium* is terminal panicle; the panicle rower-long 5 to 10 cm, compact, bringing together many flowers; it is entirely white, tomentose (covered with a hairy hair tight by a cottony stalk and is small, hermaphrodite and is fragrant The chalice is tubulous form 4 spreading lobes - having the appearance of number 8. The ovary is unilocular

**Collection and extraction:**

Flowers of *Daphne gnidium* were collected in August, 2014 from Tessala region then were dried in the shield then 20 g of dry matter was macerated in 200 ml of methanol (80 %) for 24 h then filtrated with watt ma paper 1 mm

**Phenolic compound assay:**

The proportioning of total polyphenols by Folin-Ciocalteu was described by Singleton and Rossi (1965). The reagent consisted of a mixture of acid phosphotungstic ( $H_3PW_{12}O_{40}$ ) and phosphomolybdic acid ( $H_3PMO_{12}O_{40}$ ). It was reduced, during the oxidation of phenols, in a mixture of blue molybdenum and tungsten oxides. The maximum absorption of color varied between 725 and 750 nm and it was proportional to the quantity of polyphenols present in the vegetable extracts.

Quantification of flavonoids was performed following *Zhishen et al* method, (1999) with aluminum trichlorid and sodium hydroxide. Aluminium trichloride as a yellow complex with flavonoids and sodium hydroxide formed a pink complex absorbs in a visible 510 nm.(benhamou *et al*, 2006)

**DPPH scavenging assay:**

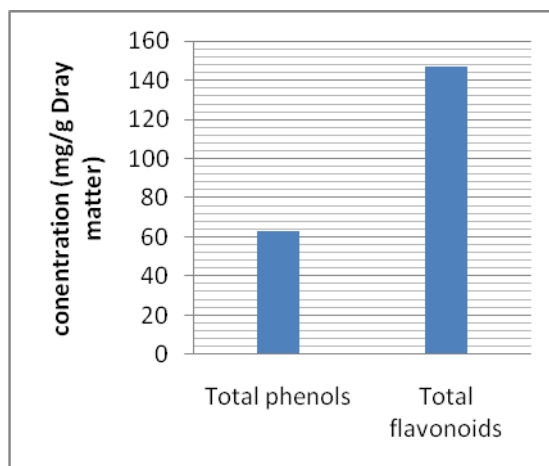
The hydrogen atom's donation ability of chemical compounds in the leaves and stems was measured on the basis to scavenge the 2,2-diphenyl-1-picrylhydrazil free radical. Fifty microliter of various concentrations of the extracts in methanol were added to 1,950 ml of a 0.025 g/l methanol solution DPPH. After about a 30 min in the incubation period at room temperature, the absorbance was read against a blank at 515 nm.

DPPH free radical scavenging activity in percentage (%) was calculated using the following formula: DPPH scavenging activity (%) =  $(A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100$

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), A sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC50) was calculated from the graph plotted of inhibition percentage against extract concentrations. The ascorbic acid methanol solution was used as positive control. (benhamou *et al*, 2006)

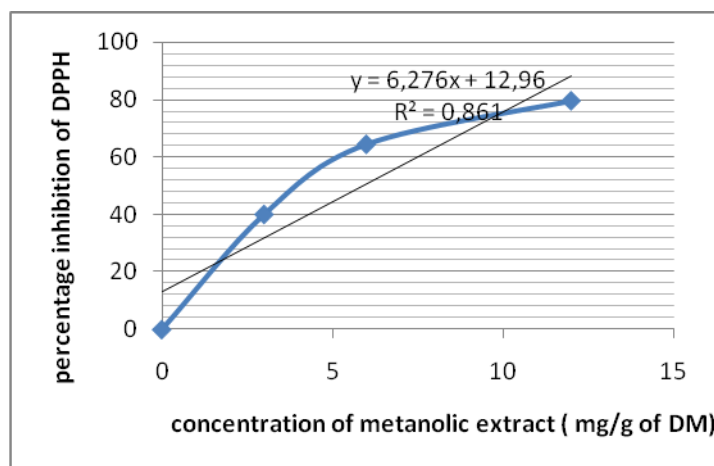
## RESULTS AND DISCUSSION

our results highlight that the methanolic extract of flower have a high content of flavonoids and total phenols (figure 1) we know that polyphenols are secondary metabolites present in all vascular plants, and constitute a large family of ubiquitous and varied substances, from simple molecules to complex structures. These natural all have in common the presence of one or several benzenic cycles bearing one or several hydroxy functions and deriving from the metabolism of shikimic acid and/or polyacetate (Bruneton,2009 ; Macheix *et al*, 2005, Sarni-Manchado et Cheynier, 2005)



**Fig. 1:** phenolic content concentration in *Daphne gnidium* flowers extract

this study is the first which determine the phenolic content of *Daphne gnidium* in other side The methanolic extract of *Daphne gnidium* L. leaves showed a presence of four coumarins (daphnetin, daphnin, acetylumbelliferon, and daphnoretin), nine flavonoids (apigenin, luteolin, quercetin, orientin, isoorientin, luteolin 7-O-glucoside, apigenin 7-O-glucoside, genkwanin, and 5-O-β-D-primeverosylgenkwanine, and α-tocopherol) which contribute to its antioxidant activity (Diana et al., 2003). Chaabane et al. (2012) have highlighted the quantification of phenolic compounds of leaf methanolic extract (total phenols = 157.47 gallic acid equivalent, total flavonoids = 114.57 quercetin equivalents, tannins = 116 tannic acid equivalents).



**Fig. 2:** antioxidant activity in *Daphne gnidium* flowers extract

The antioxidant activity (figure) of *Daphne gnidium* flowers have moderate EC<sub>50</sub> (5.90 mg/gDM).

#### Conclusion:

We conclude that *Daphne gnidium* flowers have higher quantity in polyphenols which contribute to the antioxidant activity of this organ.

We think to work deeply in this flower of this Mediterranean plant to characterize the phenolic compound and other metabolites and to evaluate the biological activities of their extracts.

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