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# Phytochemical and antibacterial studies on the aqueous extract of *Eucalyptus gomphocephala* DC

**Authors:**
**Hayate Bouharb[1] ,**
**Khalid El Badaoui[1],**
**Ali Amechrouq[2] and**
**Jalila El Amri[1].**

**Institution:**
1. Laboratory of Environment and Health, Faculty of Science, University Moulay Ismail, BP 11201 Zitoune Meknes, Morocco.

2. Laboratory of Chemistry and Molecular Substances , Faculty of Science, University Moulay Ismail, BP 11201 Zitoune, Meknes, Morocco

**Corresponding author:**
**Hayate Bouharb**

**Email Id:**
thirubotany@gmail.com

**ABSTRACT:**

This work aims to find out the molecule responsible for the good activity against the strains of *Pseudomonas aeruginosa* reflected in a pre work done on the phytochemical study of aqueous extract of *Eucalyptus gomphocephala.* We have initially processed the quantitative colorimetric determination by a UV-Vis spectrophotometer for total polyphenols and flavonoids and a qualitative analysis by high performance liquid chromatography (HPLC) coupled with mass spectroscopy. Quantitative determinations of total polyphenols by the Folin-Ciocalteu reagent and flavonoids by $AlCl_3$ method revealed the richness of this extract in total polyphenols. Qualitative analysis by HPLC / ESI-MS revealed the presence of gallic acid. This molecule was tested by the agar diffusion method and the macrodilution method in liquid medium, which showed greater activity than the aqueous extract. The results obtained in this study suggest that the gallic acid may be used in the treatment of infections caused by *Pseudomonas aeruginosa* .
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**Keywords:**
*Eucalyptus gomphocephala*, quantitative analysis, qualitative analysis, aqueous extract, gallic acid, *Pseudomonas aeruginosa*

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## INTRODUCTION

The genus *Eucalyptus* is native to Australia where there are over 750 species (Elliot and Jones, 1983; Singh *et al.,* 1999). These kinds of species have the particularity to grow rapidly and are used to drain the swampy soil. They are also used for timber and firewood. Their essential oils are used in pharmaceutical and cosmetic industries for the manufacture of various products (Hyodo *et al.,* 1992)

The genus *Eucalyptus* is known to be a rich source of bioactive natural products, including terpenes, tannins, flavonoids and derivatives of phloroglucinol (Sing *et al.,* 1999).The eucalyptol (1.8 cineole) is the principle active essential oil of the genus *Eucalyptus* that posses different pharmacological actions (IPD, 1996).

The introduction of *Eucalyptus* in Morocco dates back to the early 19$^{th}$ century. They are spread over more than 200,000 ha, or 41% of the total area of artificial plantations, most of which is occupied by the *E. gomphocephala* and *E. camaldulensis* (80% of the planted area) (Marien, 1993).

The *Eucalyptus gomphocephala* is a plant very interesting because it grows well on limestone, upto the level of 45 meters (Boudy, 1952). The plant division has many beneficial qualities and economic uses. It is used in the national industrial wood production division and also as a honey plant. It was widely used in traditional medicine for its healing properties. In Morocco, this plant is used in the treatment of cold, bronchitis and as a fumigant against microbes.

The leaves of *E. gomphocephala* were reported to have biological activities including antioxidant and cytotoxic activities (Alsayed *et al.,* 2010), antitumor activity (Alsayed *et al*, 2012), insecticidal activity (Barbouche *et al.,* 2007; Guendouz *et al.,* 2006), antibacterial (Bouharb *et al.,* 2014) and so on.

The study of the chemistry of plants is still a burning issue despite its age. This is mainly due to the fact that the plant kingdom is a major source of immense variety of bioactive molecules (Ferrari, 2002).

The purpose of this work is the phytochemical study of aqueous extract of *Eucalyptus gomphocephala* which showed good activity against strains of *Pseudomonas aeruginosa* (Bouharb *et al.,* 2014) and to identify some compounds for highlighting the molecule responsible for the antibacterial activity.

## MATERIALS AND METHODS

### Plant material

The plant was harvested in summer during the month of July. The harvest was carried out at mid-day, the collection was casually done by selecting adult tree leaves on a same tree. These are dried in shade and ground finely to make the plant material suitable for elucidating the bioactive compound. The identification of the plant has been made to the National Forestry School of Engineering (Sale).

### Preparation of the aqueous extract

100g of powder was extracted by heat reflux for two hours in water and then filtration and evaporation was carried out in a rotary evaporator at 60°C; the residue obtained is kept still until its use.

### Phytochemical study

### Quantitative analysis

### Content of total polyphenols

Polyphenols are estimated by the Folin-Ciocalteu (Wong *et al.,* 2006). 1 ml aqueous extract dissolved in distilled water was added to 1 ml of Folin-Ciocalteu reagent and diluted 10 times. After 4 min, 800 µl of sodium carbonate solution (75g / l) was added in a volumetric flask of 25ml. The absorbance was measured at 765 nm after 2h of incubation. The concentration of polyphenols were detected from the calibration ranges established with gallic acid (0-200µg / ml) and are expressed in milligrams of gallic acid equivalent per gram of dry matter MS (EAG mg / g) .

### Determination of Flavonoids

The method of aluminum trichloride (Bahorun

*et al.,* 1996) was used to quantify the flavonoids of various extracts. Quercetin was used as a standard (0.1 g / l prepared in methanol). 1 ml of extract was added to 0.1 ml of the solution of $AlCl_3$ (10% in methanol). 20 ml distilled water was added to it and mixed with methanol in a volumetric flask of 50ml. After 10 min, the absorbance was read at 430 nm. The concentration of flavonoids were detected from the calibration range established with quercetin (0-35 µg / ml) and are expressed in milligrams of quercetin equivalents per gram of dry matter (EQ mg / g).

**Qualitative Analysis**

Work on this section were performed on a HPLC chromatographic system coupled to mass spectrum in the laboratory of UATRS (CNRST, Rabat). The HPLC used was LC Surveyor thermo-electron brand, equipped with low-pressure quaternary pump with integrated degasser in isocratic mode. The separated molecules were then detected by two systems: a Photo Diode Array spectrophotometer (PDA) and Surveyor (spectral range from 190 to 800 nm) followed by a mass spectrometer (LCQ Advantage MAX) comprising an ion trap with ESI ionization. The ionization of samples was made using negative polarity. The mass range was set at: 50-2000. To analyze the aqueous extract of *Eucalyptus,* HPLC-RP-C18 was used. The stationary phase was a column (125 x 4.6mm) whereas, the mobile phase was methanol / water (60/40), and the mobile phase flow rate was 0.5 ml / min. The temperature was adjusted to 40° C (Kuntie *et al.,* 2007).

**Table 1: Identification of chemical profile of the aqueous extract**

| N° | Temps de rétention | Aire (%) |
|---|---|---|
| 1 | 2.44 | 18.79 |
| 2 | 3.36 | 81.21 |
| | % Total | 100 |

**Antibacterial tests**

*In vitro* inhibition of bacterial growth of *Pseudomonas aeruginosa* by the aqueous extract of *E. gomphocephala* was investigated by solid medium diffusion method and macrodilution method was done in liquid medium (Bouharb *et al.,* 2014). In this study, we have tested the activity of pure molecule of gallic acid (10mg / ml) against the bacterial strain.

## RESULTS AND DISCUSSION

**Quantitative study**

For there are many important biological activities in polyphenols and flavonoids, we have chosen them among different phytochemicals for quantification.

Two straight calibration **(Fig. 1 and 2)** were plotted for this purpose which are made with the standard solutions at different concentrations. The amounts of polyphenols and related flavonoids have been reported in milligrams equivalent of the standard used per gram of dry matter (ms) (mg EE / ms g) and

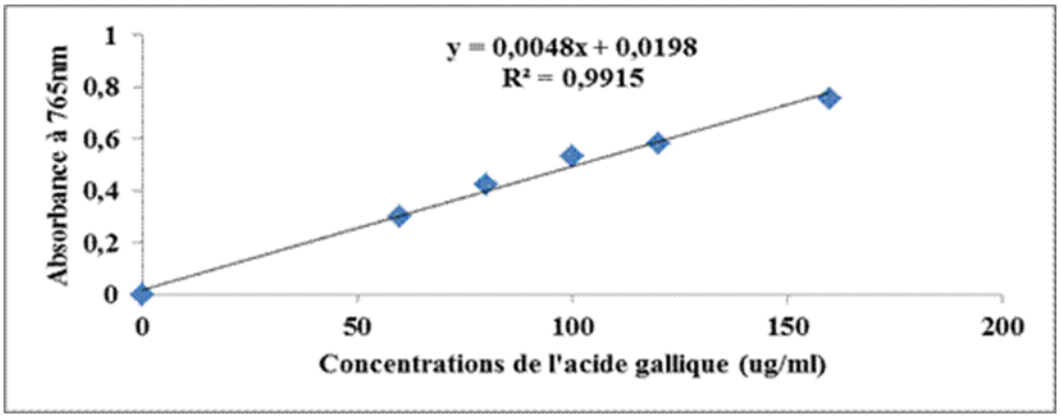


**Figure 1 : Calibration curve of gallic acid for the determination of total polyphenols**

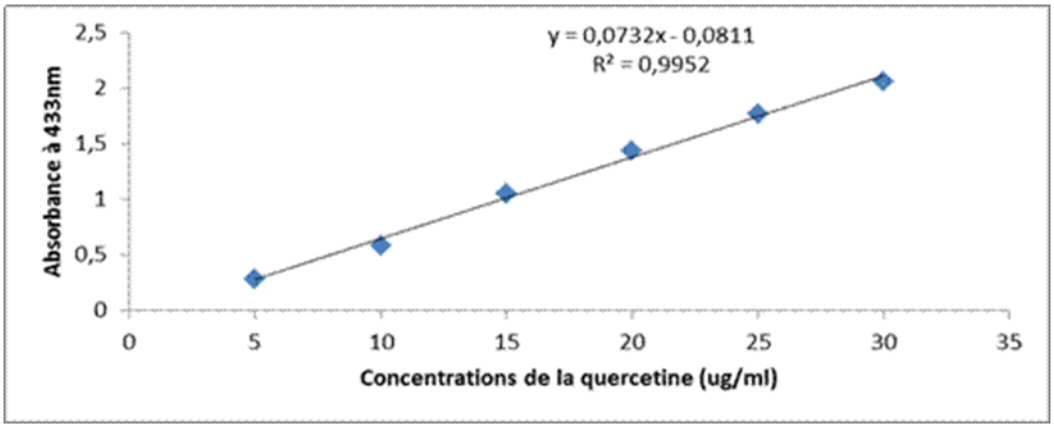


**Figure 2: Calibration curve of quercetin for determination of flavonoids**

determined by the type of equation: y = ax + b. The assay of total polyphenols was made according to the method of Folin-Ciocalteu reagent. Gallic acid was used as a standard. The flavonoids assay was performed according to the method of aluminum trichloride and quercetin was used as a standard.

**Calibration curve for the determination of total polyphenols**

This curve is established using gallic acid as reference. The formula for the linear regression of the curve y = 0.0048 x + 0.0198 is used with a correlation coefficient $R^2$ = 0.991 (**Figure 1**).

**Calibration curve for the determination of flavonoids**

The reference compound used in the preparation of this curve is quercetin. The formula for the linear regression of the curve is y = 0.0732*-0.0811 with a coefficient of determination ($R^2$ = 0.995) **(Figure 2).**

The contents of total polyphenols and flavonoids in the aqueous extract of leaves of *Eucalyptus gomphocephala* (mg / g of DM) are:

Polyphenol content: 10.64 ± 1.94 mg / g DM

Flavonoid content: 0.49 ± 0.004 mg / g DM

The only work done on the levels of phenolic compounds in the species studied is that of Alsayed *et al.* (2012). A comparison between the two studies is difficult because it is important to emphasize that the use of original plant is distinctly different from geographical and climate zone as well as different extraction and assay methods which reduce the reliability of a comparison between the two studies. Recent studies have shown that the level of phenolic compounds change in a considerable way from one

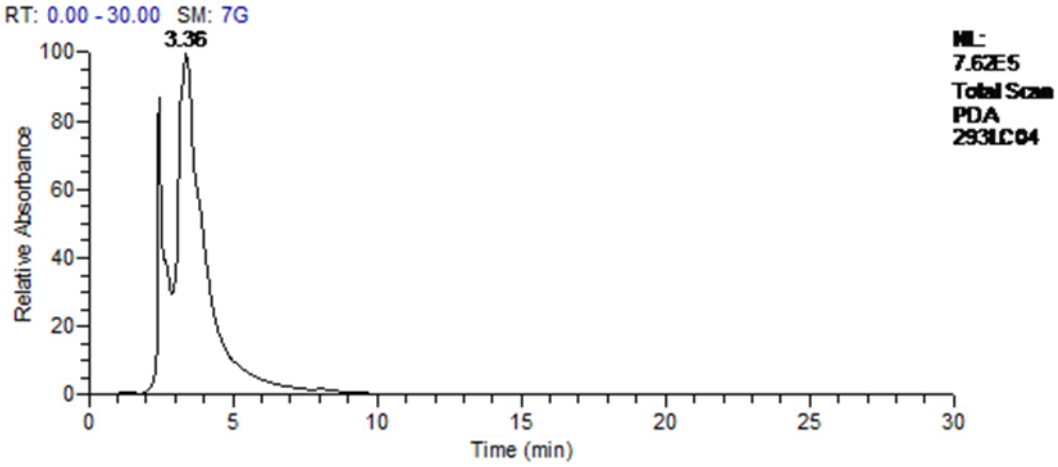


**Figure 3: Chromatogram of aqueous extract of *Eucalyptus gomphocephala***

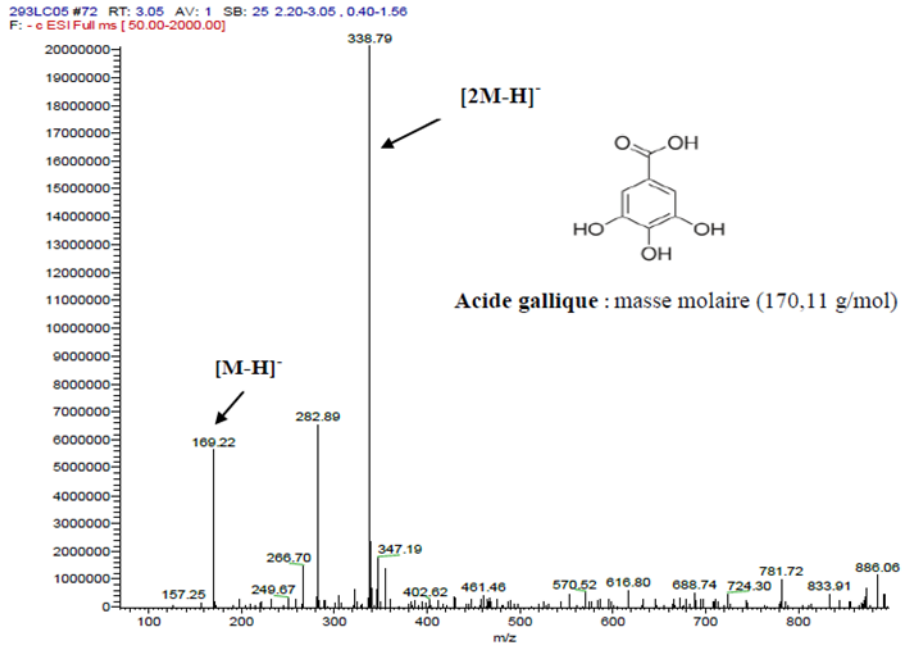


**Figure 4: Mass spectrum of gallic acid in negative mode**

species to another and within the same species (Ksouri *et al.,* 2012), because of external factors (temperature, climate, etc.,) (Ksouri *et al.,* 2008), genetic (variety and the origin of species) (Ebrahimzadeh *et al.,* 2008), physiological (the degree of maturation of the plant, nature of organs) (Maisuthisakul *et al.,* 2007) and the period of storage (Aganga and mosase, 2001).

**Qualitative Study**

The aqueous extract of *E. gomphocephala* was analyzed by HPLC / ESI-MS to obtain information

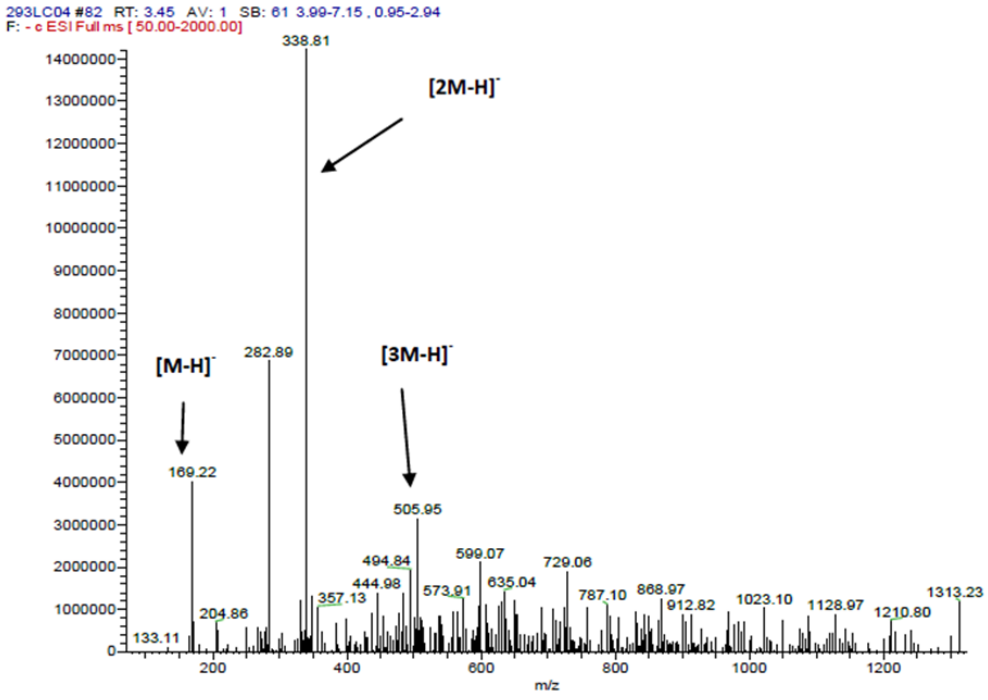


**Figure 5: Mass Spectrum of gallic acid in the aqueous extract in negative mode**

on the chemical nature of its constituents. Both standards were used: gallic acid and quercetin. The results of this study showed the absence of quercetin and the presence of large quantities of gallic acid (81.21%) (Table 1).

**Antibacterial tests**

The results of antibacterial tests (Bouharb *et al.*, 2014), from the aqueous extract of *E. gomphocephala* showed good activity against all strains of *P. aeruginosa* with inhibition zones of 12 to 18 mm and the minimum inhibitory concentration (MIC) between 6.25 and 12.5 mg / ml.

The remarkable presence of gallic acid in the aqueous extract prompted us to test this molecule against *P. aeruginosa*. The results showed higher activity than the aqueous extract with MICs of 2.5 mg / ml. Gallic acid was demonstrated effectively by several studies as antibacterial and especially against *P. aeruginosa*. Ikuro *et al.* (2000) showed that the gallic acid and its esters were evaluated as inhibitors of the enzyme p-Hydroxybenzoate recombinant Hydroxylase (PHBH) a flavin-dependent monooxygenase Nicotinamide Adenine Dinucloeotide Phosphate (NADPH) from *P. aeruginosa* . Premkumar *et al.* (2010, 2011) also has demonstrated antibacterial effect of the combination of polyphenolic compounds and gallic acid with antibiotics.

## CONCLUSION

In this study, gallic acid was shown to be a promising molecule against *P. aeruginosa.* It is recommended to do further research with different solvents and extraction methods on the *Eucalyptus gomphocephala* for identifying more active molecules.

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