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Original article

# Phytochemical Characterization and Antimicrobial Potentialities of Two Medicinal plants, *Chamaemelum nobile* (L.) All and *Matricaria chamomilla* (L.)

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**Abstract**

Objective of the study was to evaluate the phytochemical characterization and antimicrobial effectiveness of two medicinal plants belonging to the Asteraceae family, growing spontaneously in the region of Boumerdes (Northeast Algeria) namely *Chamaemelum nobile* (L.) All and *Matricaria chamomilla* (L.). For this purpose, it was proposed to optimize the extraction parameters of the phenolic compounds of the aerial parts of two chosen species. The first step was intended to study the effect of different extraction solvents (water, chloroform and methanol) on the contents of different metabolites of these species. The qualitative screening of the aerial part of chamomile allowed to highlight different families of chemical compounds namely; flavonoids, total tannins, condensed tannins, gallic tannins, alkaloids, saponosides, glucosides, mucilages and total absence of anthocyanins and starch. This was confirmed by a quantitative analysis based on the determination of total phenolic compounds by spectrophotometry in the presence of the Folin-Ciocalteu reagent determined from the calibration curve of gallic acid. The results showed that the water was the best extraction solvent. At the second stage of our study, antimicrobial activity of the extracts was determined on six microbial strains such as *Staphylococcus aureus*, *Bacillus thuringiensis*, *Escherichia coli* and *Fusarium* sp., according to the disk diffusion method, and gave zones of inhibition ranging from 7 to 15 mm. Thus, the extracts had a moderately inhibitory activity and have reacted positively on at least one of the microbial strains tested with the exception of the fungal flora. However, the methanolic extract of *M. chamomilla* revealed a strong activity against to *Pseudomonas* sp. with an inhibition zone estimated at 22.5 mm.

**Keywords:** *Chamaemelum nobile* (L.) All - *Matricaria chamomilla* (L.) - Qualitative screening - Phenolic compounds - Organic and aqueous extract - Antimicrobial activity.

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## INTRODUCTION

The therapy of bacterial infections is mainly based on the use of antibiotics (Billing and Sherman, 1998). Since the discovery of penicillin by Alexander Fleming in 1929, until today antibiotics are used massively against bacterial infections. Due to this excessive use, bacterial resistance has developed against the majority of antibiotics (Gopal Rao, 1999).

For a long time, natural remedies and above all medicinal plants have been the main recourse of the medicine of our grandparents, despite the important development of the pharmaceutical industry, which has allowed modern medicine to treat a large number of diseases often threatening. About 80% of the world's population benefits from traditional herbal medicine, recognizing the empirical knowledge of our ancestors (EL Rhaffari and Zaid, 2002).

To this end, it is necessary to direct our research towards new therapeutic approaches and especially towards plants which have always been a source of inspiration for new drugs from products of secondary metabolism (Hammer et al., 1999).

*Matricaria chamomilla* (L.) and *Chamaemelum nobile* (L.) Allare often used as a medicinal plant because of their anti-inflammatory, analgesic, sedative, antimicrobial, anti-allergic, anti-hyperglycemic and antispasmodic effects. It is also used in several food, cosmetics and pharmaceutical industries (Haghi et al., 2014, Jianping et al., 2014, Ghaed et al., 2015).

Among the major originalities of plants their ability to reproduce very diversified natural substances. In fact, alongside conventional primary metabolites, carbohydrates, proteins, lipids, they frequently accumulate secondary metabolites. The latter represent an important source of molecules that can be used by humans in fields as different as pharmacology or agri-food (Macheixet al., 2005). Phenolic compounds are plant secondary metabolites. From a therapeutic point of view, these molecules form the basis of the active principles found in medicinal plants (Macheix et al., 2005).

The extraction of active ingredients with high added value from the plant material, especially the case of phenolic contents, which are currently attracting a lot of interest thanks to their antimicrobial and antioxidant power, is a very important step in isolation as well as in the identification of phenolic compounds. As a result, many authors have investigated the influence of different extraction conditions on the extraction yields of plant-based phenolic compounds (Jokić et al., 2010; Bonnaillie et al., 2012). The solubility of phenolic compounds depends on their chemical nature in the plant, which varies from simple to highly polymerized compounds. Vegetable materials may contain varying amounts of phenolic acids, phenylpropanoids, anthocyanins, and tannins (Morales-Soto, 2010).This structural diversity is responsible for the great variability of physicochemical properties influencing the extraction of secondary metabolites (Koffi et al., 2010).

The objective of this work is to test several phenolic extracts of two species of chamomiles *Chamaemelum nobile* (L.) All and *Matricaria chamomilla* (L.) on the microbial agents responsible for certain human and plant diseases in order to valorize the plant species in pharmaceutical and phytopharmaceutical preparations and cosmetics.

## MATERIAL and METHODS

### Biological material

The plant materials used in this study were collected in the region of Isser and Thenia located at Boumerdes area (Northeast Algiers) in the months of March-April, 2017. They consisted of the aerial parts (leaves and flowers) of two medicinal plants: *M. chamomilla* and *C. nobile* (Figure 1).


**Figure 1.** Geographical situation of the sampling areas of the two plant species (Thenia and Issers towns of northeastern Algiers)

The botanical identification of the species was carried out at the botanical laboratory of the Higher National School of Agronomy in El Harrach (Algeria). The plant material (leaves and flowers) was milled after drying at room temperature in a dark place. The crushed product obtained was stored in hermetically sealed glass vials. The ground material was used for the extraction of phenolic compounds.

**Table 1.** Microbial strains used in the evaluation of antibacterial and antifungal activity

| | Strains | Gram +/- | Origin |
|---|---|---|---|
| **Bacteria** | *Staphyloccoccus aureus (ATCC 6538)* | *Gram+* | Pasteur Institute of Algeria (IPA) |
| | *Pseudomonas aeruginosa (ATCC 9027)* | *Gram-* | |
| | *Streptococcus* sp. *(ATCC 6633)* | *Gram-* | |
| | *Escherichia coli (ATCC 4157)* | *Gram-* | |
| | *Bacillus* sp. | *Gram+* | Isolated from soil and identified in the laboratory |
| **Fungi** | *Candida albicans (ATCC 24433)* | / | Hospital of Thenia Boumerdes, Algeria |
| | *Fusarium* sp. | / | National Institute of Plants Protection (INPV), Algiers, Algeria |

**Qualitative screening**

Chemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the bioactive compounds as described by Paris and Nothis (1978), Trease and Evans (1989), and Harborne (1973). Any change of colors or the precipitate formation was used as indicative of positive response to these tests. Phytochemical parameters were determined as follows:

***Preparation of infusion***: 5 g of vegetable powder are introduced into 100 ml of boiling water and let infuse 15 to 30 minutes.

**Phenolic compounds**

***Tannins:*** 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added. A brownish green or a blue-black coloration was observed.

***Gallic tannins:*** 5 ml of the infusion was added to 2g of sodium acetate with a few drops of $FeCl_3$, and observed for a dark blue coloring.

***Condensed tannin:*** 15 ml of infusion was added to 10 ml of 40% formalin and 5 ml of concentrated HCl. A red color was observed.

***Flavonoids:*** 5 ml of HCl, traces of Mg and 1 ml of isoamyl alcohol were added to 5 ml of infused, a red-orange coloring was observed.

***Anthocyanins:*** 5 ml of infusion was mixed with a few drops of HCl, a red color gives a positive result.

**Reductive compounds**

***Mucilage:*** 1 ml of infusion was added to 5 ml of ethanol, after a rest period of 10 minutes a fluffy precipitate was observed.

**Terpene compounds**

***Saponosids***: 2 ml of infusion was mixed with a few drops of lead acetate, and observed for the formation of a white precipitate.

**Nitrogen compounds**

***Alkaloid***: 5 g of powder was moistened with 50% ammonia and then macerated in 50 ml of the ether chloroform mixture (3 volumes / 1 volume) for 24 h, followed by filtration. The filtrate was exhausted with 2N hydrochloric acid (HCL) and a few drops of Dragendroff reagent were added. Formation of red precipitate indicated the presence of alkaloids.

**Search for the best extraction solvent by maceration and decoction**

***Aqueous extraction***

The aqueous extract was obtained by decoction of 20 g of ground material from the aerial part of the plant (leaves and flowers) in 500 ml of distilled water for 10 min with magnetic stirring. The solution was allowed to stand on the hot plate for 15 minutes; the mixture was first filtered on gauze and then on Whatman paper (No.3). Aliquots of the filtrate were placed in an oven at 40 °C for 24 hours to dry. The dry extract was stored in the refrigerator for subsequent analyzes (Belhattab, 2007; Majhenic et al., 2007).

***Chloroform extraction***

50 g of the plant material was macerated in 300 ml of chloroform for 24 hours at room temperature in the dark with magnetic stirring, and then filtered through sterile gauze. Once the pellet recovered, the operation was repeated a second time by 300 ml of chloroform. The filter paper was used for the final filtration. The filtrate was concentrated with a rotary evaporator at 50°C (Belhattab, 2007).

***Methanol extraction***

The conditions of the conventional solvent extraction (ECS) were chosen according to the data of the literature (Li et al., 2006; Tumbas et al., 2010; Ojeil et al., 2010; Revilla et al., 2001). The solvents giving the highest content of total phenols were methanol and ethanol (Li et al., 2006).

20 g of vegetable powder was macerated in 100 ml of methanol for 3 days at room temperature. The mixture was filtered using Whatman filter paper. The recovered filtrate was evaporated in a Rotavapor at 70 ° C for 20 min to remove the methanol. Extract was stored in small glass vials at 4°C until use.

***Extraction yield***

The extraction yield was calculated by the formula given by Falleh et al. (2008):

R (%) = 100 $M_{ext}/M_{ech}$. R (%): Yield was expressed in %, Mext was the weight of the extract after the evaporation of the solvent in mg and $M_{ech}$ was initial weight of the plant sample in mg.

***Determination of total phenolic content***

The determination of the total phenolic contents of each extract were carried out using the Folin-Ciocalteu assay according to the method of Boizot and Charpentier (2006) and Wong et al. (2006) 100 µl of extract was mixed with 500 µl of Folin-Ciocalteu (10 fold diluted) reagent and 400 µl of 7.5 % (w / v) of sodium carbonate $Na_2CO_3$. After incubation in the dark at room temperature for 10 minutes, the absorbance was recorded at 760 nm, using UV spectrophotometer (Perkin Elmer). The results were expressed as mg gallic acid equivalents (GAE)/g of dry weight (dw), with reference to the calibration curve of gallic acid. Data was reported by means of at least three replications.

**Evaluation of antibacterial and antifungal activity**

The microbial strains used are listed in Table 1. These are preserved in Mueller -Hinton medium (MH) for bacterial strains and in Sabouraud medium for fungi.

The evaluation of the antimicrobial activity of the extracts of both plants was carried out by estimating the diameter of growth inhibition of the microorganisms tested. This activity was evaluated by the antibiogram method or the agar diffusion method using sterile discs (Cavallo et al., 2006).The strains provided on preservation media were revived on nutrient agar medium for bacteria and Sabouraud for fungi. Bacteria were incubated at 37 ° C for 24 hours. Fungi, on the other hand, were incubated at 28 ° C for 72 hours to obtain young cultures to prepare the inoculum (Meena and Sethiv, 2007).The results were read by measuring the diameter of the zone of inhibition around each disk using a caliper or ruler (in mm). The zones of inhibition were expressed in diameters according to the sensitivity of the strains to the extract of the plant (Ponce et al., 2003).

## RESULT and DISCUSSION

**Phytochemical screening**

The phytochemical screening carried out on the two species of chamomile allowed us to identify the existing biomolecules in this plant in order to obtain an overall evaluation of the types of phenols existing in this plant. The tests were carried out either on the powder of the plant, or on their infusion. Phytochemical screening revealed the presence of the same biomolecule components for both chamomile species, such as glucosides, mucilages, flavonoids, total tannins, saponosides, condensed tannins, gallic tannins and alkaloids. However, they lacked anthocyanins and starch (Table 2).

**Table 2.** Phytochemical screening of *M.chamomilla* and *C. nobile*

| Chemicals compounds | Positive results | Plant | Observed results |
|---|---|---|---|
| **Alkaloids** | Red precipitate | M.c | ++ |
| | | C.n | ++ |
| **Total tannins** | Coloring black blue | M.c | +++ |
| | | C.n | +++ |
| **Condensed tannins** | Coloring red | M.c | +++ |
| | | C.n | +++ |
| **Gallic tannins** | Coloring dark blue | M.c | +++ |
| | | C.n | +++ |
| **Mucilage** | Fluffy precipitate | M.c | +++ |
| | | C.n | +++ |
| **Anthocyanins** | Coloring red | M.c | – |
| | | C.n | – |
| **Flavonoids** | Coloring orange red | M.c | +++ |
| | | C.n | +++ |
| **Saponosids** | White precipitate | M.c | +++ |
| | | C.n | +++ |
| **Starch** | Coloring blue purple | M.c | – |
| | | C.n | – |
| **Glucosids** | Coloring brick red | M.c | +++ |
| | | C.n | +++ |

M.c *: M. chamomilla* ; C.n : *C. nobile* ; (+++) : High positive reaction; (++) : Averagely Positive reaction ; (-) : Negative reaction.

Results summarized in Table 2 indicated that the powder of the leaves and flowers of the two species studied were very rich in glucosides, mucilages, flavonoids, total tannins, saponosides, condensed tannins, gallic tannins, and alkaloids. However, there was a complete absence of anthocyanins and starch. From these results, it was noted that two plants had the same chemical composition. It is well established that the phytochemical profile of a plant is directly related to environmental conditions such as climate, geographical location, temperature, photoperiod, vegetative stage, etc. These factors affect the synthetic pathways of the active compounds in the plant (Tsai et al., 2008).

**Yield extraction**

The aerial parts of the two plants *C. nobile* and *M. chamomilla* have been subjected to 3 types of extractions –for the observation of the phenolic compounds. Methods were based on maceration of the powder with solvents (methanol and chloroform) and in order to get closer more traditional preparations, hot water was used to obtain the aqueous extract (Aq). Table 3 presented the yields of the different extracts.

**Table 3.** Extraction yield of the different extracts obtained by maceration and decoction of *M. chamomilla* and *C. nobile*.

| Plants | Extracts obtained | | |
|---|---|---|---|
| | Methanolic extract (maceration) | Chloroformic extract (maceration) | Aqueous extract (decoction) |
| *C. nobile* | 10.40% | 8.50% | 16.60% |
| *M. chamomilla* | 9.40% | 6.40% | 19.92% |

Yields of *C. nobile* for aqueous, methanolic and chloroformic extract were 16.6 %, 10.4 % and 8.5 %, respectively. On the other hand, yields of *M.* chamomilla were 19.92 %, 9.4% and 6.4 %, respectively.

Through the analysis of the extraction yields, it appeared that the aqueous extract gave the best yield for the two species of chamomile followed by methanol while the chloroformic extract gave the lowest yield. This variation in yields is related to the extraction method itself.

In general, our results show slightly different extraction yields between these two species; which can be explained by various factors including the diameter of the powder and the temperature (Escribano-Bailon and Santos-Buelga, 2003).

On the other hand, the dry matter contents vary not only from one plant to another of the same family but also according to the conditions of development and growth, maturity, packaging, storage conditions and methods of extraction (Zang and Hamauru, 2003).

**Spectral characterization of total phenolic content**

The total phenolic contents were shown in Table 4. Aqueous extracts recorded the highest phenolic contents; 23.96 mg GAE / g dw for *M. chamomilla* and 21.70 mg GAE/g dw for *C. nobile,* followed by methanolic extract which gave similar levels for the two species estimated at 11.85 and 13.11 mg GAE / g dw, respectively.

**Table 4**. Total phenolic content of *M. chamomilla* and *C. nobile* expressed in mg GAE /g dw.

| Plants | Extraction solvents | Total phenolic content (mg GAE/g dw) |
|---|---|---|
| ***C. nobile*** | Water | 21.70 ± 0.74 |
| | Chloroform | 7.75 ± 0.04 |
| | Methanol | 11.85 ± 0.09 |
| ***M. chamomilla*** | Water | 23.96 ± 4.73 |
| | Chloroform | 9.68 ± 0.63 |
| | Methanol | 13.11 ± 0.33 |

*Values represent the average of 3 replications (±SD)*

The work conducted by Koffi et al. (2010); Mohammedi and Atik (2011) confirmed our results by indicating that extraction by maceration in methanol allowed a better extraction of total polyphenols compared to chloroform.

The solubility of phenolic compounds depends mainly on the number of hydroxyl groups, molecular weight and the length of the basic backbone carbonic chain (Mohammedi and Atik, 2011). It appears from our work that to obtain fractions rich in phenolics, it is preferable to use mixtures of the appropriate organic solvent with water.

**Determination of antimicrobial activity**

Antimicrobial activities of 3phenolic extracts were determined on six microbial strains.The results relating to the diameters of the zones of inhibition of various phenolic extracts were presented in Tables 5, 6 and 7.The results of antimicrobial activity showed differences depending on the type of extract tested and the germ used.

**Table 5.** Results of the antimicrobial activity of aqueous extract of *C. nobile* and *M. chamomilla*. Data were presented as a mean diameter of inhibition zones ± standard deviation (SD).

| **Strains** | **Mean Diameter of Inhibitory Zones (mm ± SD)** | | |
|---|---|---|---|
| | *C. nobile* | *M. chamomilla* | Control |
| ***Staphylococcus aureus*** | 10 ± 1 (+) | 10 ± 1 (+) | 6.0 ± 0 (-) |
| ***Bacillus* sp.** | 11.33 ± 0.83 (+) | 11.66 ± 0.83 (+) | 6.0 ± 0 (-) |
| ***Pseudomonas aeruginosa*** | 12.66 ± 0.38 (+) | 09 ± 0.76 (+) | 6.0 ± 0 (-) |
| ***Escherichia coli*** | 12.66 ± 1.16 (+) | 10.66 ± 0.66 (+) | 6.0 ± 0 (-) |
| ***Fusarium* sp.** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Candida albicans*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |

Control : Water; (-): Resistant ; (+): Sensitive

**Table 6.** Results of the antimicrobial activity of methanolic extract of *C. Nobile* and *M.chamomilla*. Data were presented as a mean diameter of inhibition zones ± standard deviation (SD).

| **Strains** | **Mean Diameter of Inhibitory Zones (mm ± SD)** | | |
|---|---|---|---|
| | *C. nobile* | *M. chamomilla* | Control |
| ***Staphylococcus aureus*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Bacillus* sp.** | 10 ± 1.5 (+) | 9.66 ± 1.33 (+) | 6.0 ± 0 (-) |
| ***Pseudomonas aeruginosa*** | 8.66 ± 0.83 (+) | 22.5 ± 1.66 (+) | 6.0 ± 0 (-) |
| ***Escherichia coli*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Fusarium* sp.** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Candida albicans*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |

Control : Methanol; (-): Resistant ; (+): Sensitive

**Table 7.** Results of the antimicrobial activity of chloroformic extract of *C. nobile* and *M. chamomilla*. Data were presented as a mean diameter of inhibition zones ± standard deviation (SD).

| **Strains** | **Mean Diameter of Inhibitory Zones (mm ± SD)** | | |
|---|---|---|---|
| | *C. nobile* | *M. chamomilla* | Control |
| ***Staphylococcus aureus*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Bacillus* sp.** | 8.66 ± 0.33 (+) | 9.33 ± 0.66 (+) | 6.0 ± 0 (-) |
| ***Pseudomonas aeruginosa*** | 10.0 ± 1.5 (+) | 10.33 ± 0.83 (+) | 6.0 ± 0 (-) |
| ***Escherichia coli*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Fusarium* sp.** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |
| ***Candida albicans*** | 6.0 ± 0 (-) | 6.0 ± 0 (-) | 6.0 ± 0 (-) |

Control: Chloroform; (-): Resistant ; (+): Sensitive

Methanolic extract of *C. nobile* and *M. chamomilla* displayed a moderate inhibitory activity on *Bacillus* with a mean inhibition diameter of 10 mm and 9.66, respectively. *M. chamomilla* had a high inhibitory activity with a high value (22.5 mm) against *Pseudomonas* sp., whereas for *C. nobile*, average sensitivity was observed with a diameter of 8.66 mm. For the rest of the microbial strains (*S. aureus*, *E. coli*, *Fusarium* sp., and *C. albicans*), they were found to be resistant to both plant species.

The study of the antimicrobial activity of the chloroform extract showed a moderate inhibitory effect against *Bacillus* and *Pseudomonas* with diameters ranging from 8.66 mm to 9.33 mm for *C. nobile* and *M. chamomilla*, respectively against *Bacillus* and diameters of 10 and 10.33 mm, respectively for *C. nobile* and *M. chamomilla* against *Pseudomonas.* However, other microbial strains (*S. aureus, E. coli, Fusarium* and *C. albicans*) were found to be resistant. As regards the controls (water, chloroform and methanol), they had no effect on the microbial strains tested. The average activity of the methanolic and chloroformic extracts can also be due to the extraction method used. Indeed, Hayouni et al. (2007) reported that the extraction method and the nature of the solvent could influence the antimicrobial activity of the phenolic compounds. On the other hand, the charge of the disc also influenced the antimicrobial activity.

Moreover, observations on the antibacterial activity of the essential oil and polyphenols of *Citrus aurantium* showed that *S. aureus* was the most sensitive strain, followed by *E. coli*, the polar extracts proved devoid of any antifungal activity against *Saccharomyces cerevisiae*, and *Aspergillus* sp. (Oulebsir-Mohand kaci et al., 2016)

If we refer to the studies of Moussaid et al. (2012), the activity of the active ingredients would be related to the drying and grinding conditions of the plant. Because grinding was also at the origin of the heat generation responsible for the loss of volatile molecules, as well as the decomposition and oxidation of thermolabile molecules (Jones and Kinghorn, 2005).

**Conclusions**

The phytochemical tests carried out showed the richness of these two species, *C. nobile* and *M. chamomilla* in total tannins, gallic tannins, condensed tannins, saponosides, alkaloids, flavonoids, mucilages and glucosides, and the total absence of starch and anthocyanins. On a quantitative level, the total phenolic content calculated by the Folin-Ciocalteu method revealed the presence of average phenolic concentrations in the two plants studied.

The extraction of phenolic compounds is a crucial step for the valorization of these active ingredients; it depends on the method and the appropriate solvent that preserve their biological properties. From this study, it appeared that aqueous decoction and maceration with methanol were the best phenolic extraction techniques.

The study of the antimicrobial activity of the chloroformic extract showed a moderate inhibitory effect against *Bacillus* sp. and *P. aeruginosa.* The inactivity of the methanolic and chloroformic extracts might be due to the extraction method used. The results described in this paper demonstrated the abundance of phenolic compounds in both plants showing the importance of their uses in local medicine as antimicrobial agents. Future studies should focus on the effect of other bioactive molecules associated

with these plants such as alkaloids and the evaluation of the antimicrobial activity on a wide range of pathogens.

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