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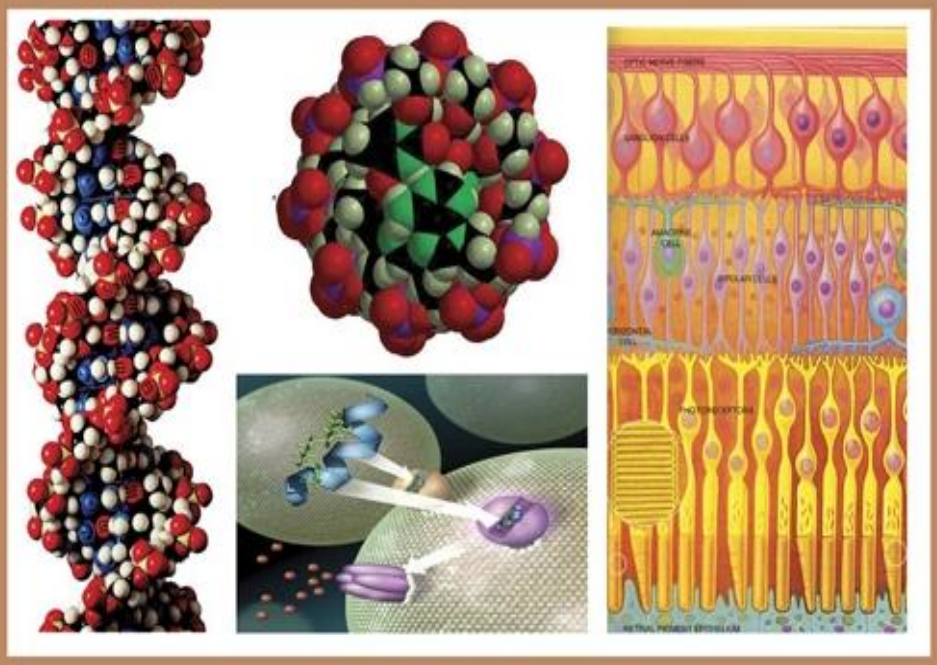

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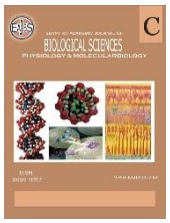

Phytochemical Screening and Evaluation of Antibacterial and Antifungal Properties of Extracts of *Matricaria pubescens* (Desf) Growing in Southwest Algeria

Ghouti Dalila [1], Ammam Abdelkader [1], Belmamoun Ahmed Reda [2], Houamria Moufida [1] and Maghnia Djamila[3].

1- Department of Biology, Faculty of Sciences, University of Saida, BP 138 ENNASR 2000 Saida, Algeria.
2- Laboratory of Process, materials and environmental engineering, Djillali Liabes University, BP 22000, Sidi-Bel-Abbes, Algeria.
3- Laboratory for Experimental Biotoxicology of Biodepollution and Phytoremediation , University of Oran Ahmed Ben Bella 1 . Algeria

***E-mail:** vetokadi@yahoo.fr

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ABSTRACT

This study was devoted to phytochemical screening and evaluation of biological properties, namely, the antimicrobial activities of hydroethanolic and infusion extracts prepared from Algerian Sahara *Matricaria pubescens (Desf)*. Phytochemical tests applied to the studied plant showed the presence of several families of chemical compounds, including tannins and flavonoids. The *in vitro* antimicrobial activity of *M. pubescens* extracts was moderate, while the gram-positive bacteria were more sensitive than the gram-negative strains (MIC values between 10 and 20 mg/mL). The contemporary presence of bioactivities suggests that the Saharan *M. pubescens (Desf)* may be a source for such new preservatives in the food and pharmaceutical industries.

INTRODUCTION

Medicinal plants are recognized for their physico-chemical properties and richness in natural drugs belonging to different molecule classes such as terpenoids and phenolic compounds like flavonoids, tannins, coumarins, and phenolic acids that contribute to effective protection against numerous pathological processes (Hosamani, 2009). There is considerable research interest in the biological properties of phenolic compounds due to their important health effects, which are caused by their preventive properties. Thus, the disastrous effects of the free radicals can, however, be stopped by the antioxidant substances, which scavenge the free radicals and detoxify the organism (Halliwell, 2008; Cui et al., 2010). Plant phenolic compounds are very common in different plant parts and are widely exploited and used in the food, cosmetic, and pharmaceutical industries (Bruneton, 1995; Merzouki *et al.*, 2000; Hosamani, 2009). They also have been found to exhibit several biological effects, including antioxidant (Hosamani, 2008), anti-inflammatory (Guo *et al.*, 2014), antifungal (Martins *et al.*, 2015), anti-cancer (Carocho and Ferreira, 2013a), antithrombotic, and vasodilatory actions (Middleton and Kandaswami, 1994; Di Carlo *et al.*, 1999).

Matricaria pubescens (Desf) preparations are frequently used in traditional medicine against mild gastrointestinal disorders, ulcers and inflammations of the mouth and throat, for irritated skin and mucosae, and the relief of the common cold, allergies, ocular affections, dysmenorrhoea, scorpion stings, dehydration, and toothache (Maiza *et al.*, 1995).

MATERIALS AND METHODS

1. Plant Material:

The samples of *Matricaria pubescens* (Del) aerial parts were collected in southwest Algeria (Bechar, 31° 01′ 00″ N, 2° 44′ 00″ Oest) during the flowering period (May 2020). The plant was identified by Okacha Hasnaoui, a professor at the University of Saida, Algeria. The samples were dried and ground to a fine powder prior to analysis.

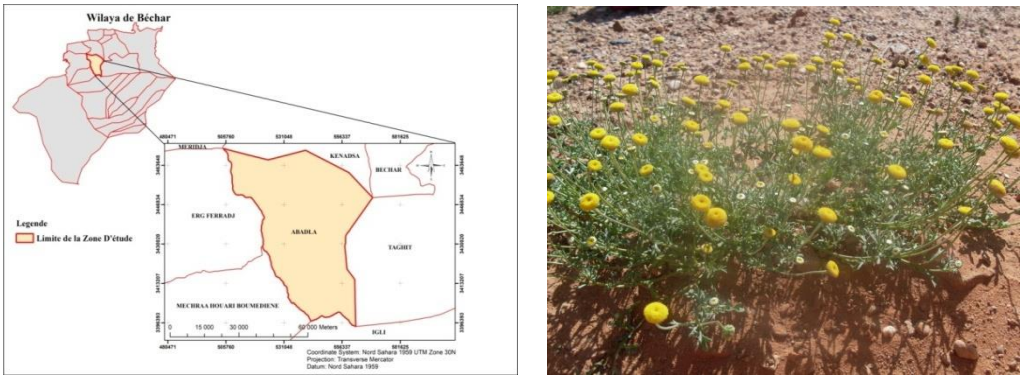

Fig.1: *Matricaria pubscens* (Del) (to the left), the harvest area of the studied plant (to the right).

2. Extraction Procedure:

Hydroethanolic and infusion extracts were prepared from *Matricaria pubescens* (Del). The hydroethanolic extraction (80% ethanol, 30 mL) was performed by maceration (150 rpm), with 1 g of each sample at 25°C for 1 h and then filtered; the residue was re-extracted, using the same methodology. Afterwards, the extracts were evaporated to remove the ethanol under reduced pressure. For aqueous extracts, 2 g of plant material was infused with boiling distilled water for 15 minutes and then filtered. Both extracts were previously frozen before lyophilization in order to obtain a dry extract.

The lyophilized hydroethanolic and infusion extracts were dissolved in ethanol/water (80:20, v/v) and water, respectively, to obtain a stock solution of 10 mg/mL for the antioxidant activity assays; 20 mg/mL in culture medium for the antimicrobial assays; and, finally, 8 mg/mL in water for the anti-inflammatory and cytotoxicity tests. In the bioactivity evaluation assays, the stock solutions were further diluted and tested.

3. Phytochimical Screening:

To get an idea about the main families that can be found in plant material, we have done a phytochemical screening. This one is either based on the formation of insoluble or colored complexes. The observed coloration is usually due to the formation of conjugation or instauration in a molecule. In such tests of characterization, we cause this induction using a suitable reagent. We have characterized the different chemical groups (tannin, flavonoids, sterols and steroids, alkaloids, and saponins) by referring to the techniques described by Paris *et al.* (1969; Trease and Evans, 1996) with some modifications.

4. Antimicrobial Activity Assays:

The antimicrobial activity of the samples was tested against a range of strains from different microorganisms: four gram-positive bacteria (MRSA-methicillin-resistant *Staphylococcus aureus*, MSSA-methicillin-susceptible *Staphylococcus aureus*, *Listeria monocytogenes,* and *Enterococcus faecalis*); five gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Morganella morganii, Pseudomonas aeruginosa*) and one yeast (*Candida albicans*). According to Dias et al., the minimal inhibitory concentrations (MIC) were determined by the microdilution method using the p-iodonitrotetrazolium chloride (INT) colorimetric assay**.** The minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC) were calculated by adding 10 µL of the MIC value to the fresh culture medium to see if the bacteria were able to grow. After 24 hours of incubation at 37 oC, MBC and MFC were registered. The antibiotic susceptibility profile of microorganisms was previously described.

RESULTS AND DISCUSSION

1. Phytochimical Composition*:*

As shown in Table 1, the phyto-chemical analysis demonstrated the presence of common phytoconstituents like sterols and steroids, tannins, saponins, flavonoids, triterpenic heterisides, and the absence of alkaloids, coumarins, and anthocyanins. Our results are consistent with those obtained by Makloufi *et al.* (2012).

Table1: Phytochemical analysis of *Matricaria pubscens* (Del).

Compounds	***Matricaria pubscens* (Del) (Ariel parts)**
Starch	+
Saponins	++
Tanin cathéchique	-
Tannin gallique	+
Flavonoid	++
reducing Compounds	--
Alkaloïd	-
coumarins	-
Anthocyanin	-
Sterols and steroids	+
Steroid heterosides	+
Triterpenic heteroside	+

(-) Abscens, (+) presence, (++) strongly present.

Matricaria pubscens (Del) shows richness in phenolic compounds namely, tannins, flavonoids and saponins, these molecules have analgesic, anti-inflammatory and anti-edematous properties (Roux *et al.,* 2007). This diversity of compounds could justify their use in traditional treatments for venous insufficiency, functional signs of hemorrhoidal crisis and disorders of capillary fragility.

2. Antimicrobial Activity:

The antimicrobial activity results of *Matricaria pubescens* (Del) extracts (hydroethanolic and infusion) tested against ten pathogenic strains and expressed as the minimal inhibitory concentrations (MIC), minimal bactericidal concentrations (MBC), and minimal fungicidal concentrations (MFC) are presented in Table 2. Overall, all extracts exhibited antibacterial activity against gram-positive bacteria strains, with minimal inhibitory concentrations (MICs) ranging between 10 and 20 mg/mL. However, no inhibitory effect was observed against *Escherichia* coli, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis,* and *Morganella*

morganii; also, the yeast (candidate) showed resistance at this concentration (20 mg/mL). *Matricaria pubscens* showed the highest inhibitory effects against Methicillin-resistant *staphylococcus aureus* (*MRSA*) Strain with MICs (10 and 20 mg/mL) for hydroethanolic and infusion extracts, respectively, which is in accordance with Makhlouf *et al.* (2012). *Matricaria pubescens* extracts from Ourgla (Algeria) exhibited an inhibiting capacity against the tested strains except for *Escherichia coli* ATCC 25922 and *Acinetobacter baumannii* 867. The minimum inhibitory concentration (MIC) with a value of 0.78 mg/mL was obtained against *A. baumannii* 610 using aqueous ethanol (50%; Metrouh Amir *et al.*, 2015). The presence of secondary metabolites in *Matricaria pubescens* (Del) reveals its activity against pathogenic bacteria (Muanda *et al.*, 2010). The antibacterial effect of phenolic compounds might be related to their interaction with enzymes, their adsorptiveness to cell membranes, their substrates, and metal ion deprivation (Scalbert, 1991). Certain plant extracts at lower concentrations have been found inefficient against some test organisms; this can be related to the presence of lower antimicrobial agent contents (Nisa *et al.*, 2013).

Table 2. Antibacterial and antifungal activity of *Matricaria Pubescens* extracts (MIC, MBCand MFC values, mg/mL).

Antibacterial activity																				
		Gram-negative bacteria								Gram-positive bacteria										Yeast	
		Escherichia coli		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*		*Proteus mirabilis*		*Morganella morganii*		*Enterococcus faecalis*		*Listeria monocytogenes*		*MRSA*		*MSSA*		*Candidat albicans*	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
M. pubescecns	HydroETOHextract	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	**20**	>20	**20**	>20	**20**	>20	**20**	>20	>20	>20
	Infusion extract	>20	>20	>20	>20	>20	>20	>20	>20	>20	>20	**20**	>20	>20	>20	**10**	>20	**20**	>20	>20	>20

MRSA- Methicillin (resistant) *Staphylococcus aureus*; MSSA methicillin (susceptible) *S.aureus*; MIC-minimal inhibitory concentration; MBC- minimal bactericidal concentration.

Conclusion

Overall, the differences found in the bioactive properties of the hydroethanolic and infusion extracts of *Matricaria pubescens* (Del) can be related to the different profiles and quantities of phenolic compounds present in each sample. The studied plant proved promising in terms of antioxidant, anti-inflammatory, cytotoxic, and antibacterial activities, which could support the traditional use of *Matricaria pubescens* (Del). Further studies are required in order to establish the mechanism of action, supporting the use of this plant in pharmaceutical or food fields.

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