ANTIBACTERIAL ACTIVITY AND DPPH• RADICAL SCAVENGING OF DIFFERENTS METABOLITES EXTRACTED FROM TWO PLANTS: ESSENTIAL OIL FROM (*Matricaria recutita* L.) AND FLAVONOIDS FROM FLOWERS AND LEAVES OF (*Hibiscus rosa-sinensis* L.)

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Abstract. The aims of this study were to evaluate the DPPH• radical scavenging and antibacterial activity of different metabolites extracted from two plants: essential oil from *Matricaria recutita* L. and flavonoids from flowers and leaves of *Hibiscus rosa-sinensis* L. The essential oil extracted by steam distillation method have presented a yield of 0.55%, with a blue color, as for the flowers extract of *H. rosa-sinensis* is the richest in flavonoids (FFR: 3.19%). Flavonoid extracts from *Hibiscus rosa-sinensis* L. flowers provided the highest antioxidant activity against free radical inhibition of DPPH. The essential oil of *Matricaria recutita* tested on pathogenic germs revealed a presence of positive effects on all the bacterial strains used. While *Hibiscus rosa-sinensis* leaf and flower extracts inhibited only the growth of certain pathogens such as *Serratia rubidae* and *Enterobacter sp*. The results obtained in this study and the comparison with other works in the same field, allowed us to unveil the importance attributed by traditional medicine to *Matricaria recutita* L. and *Hibiscus rosa-sinensis* L. as source of natural antiseptics.

Keywords: *Matricaria recutita*; essential oil; flavonoids; *Hibiscus rosa-sinensis*; antioxidant activity; antibacterial activity.

INTRODUCTION

The Compositae (Asteraceae) family contains very useful medicinal genera such as *Matricaria, Achillea, Artemisia, Tussilago, Calendula, Silybum* and *Taraxacum.* The chemical composition is very different, many compounds being identified in all species like (triterpenic saponosides, terpenoids), but some of them being specific. These compounds are mainly responsible for the therapeutic properties of extracts from Compositae family plants (anti-inflammatory, antiseptic, antihemorrhagic, antispastic, hepatoprotective properties) [23].

Chamomile is one of the most widely used and well-documented medicinal plants in the world [46], it is included in the pharmacopoeia of 26 countries [47]. In Germany, where chamomile sales exceeded 8.3 million $ in 1996 [15], more than 4,000 tons of chamomiles are produced yearly [7].

The use of chamomile as a medicinal plant dates back to ancient Greece and Rome. The name "chamomile" comes from two Greek words meaning “ground apple” for its apple-like smell. In Europe it is considered a “cure all”, and in Germany it is referred to as “alles zutraut”, meaning “capable of anything” [7]. Although there are numerous varieties of chamomile, the two most popular are Roman chamomile (*Anthemis nobilis*) and German chamomile (*Matricaria recutita*); both are from the Asteraceae family. German chamomile is considered the more potent of the two, has received more scientific evaluation, and is more widely cultivated than Roman chamomile; it is believed to possess anti-inflammatory, vulnerary, deodorant, bacteriostatic, antimicrobial, anticatarrhal, carminative, sedative, antiseptic, and spasmolytic properties [10, 40].

One hundred twenty chemical constituents have been identified in chamomile, including terpenoids, flavonoids, and coumarins [1]. The essential oil of both German and Roman chamomile is a light blue color due to the terpenoid *chamazulene*. Chamazulene is an artifact formed during heating and comprises about 5% of the essential oil. It has anti-inflammatory, antiallergic, and antispasmodic properties [3].

The malvaceae family comprises about 120 genera and 1700 to 2000 species [41]. The species of this family has been used as herbal plants in folk medicine for treatment of different diseases. All parts of these plants are antiphlogistic, astringent, demulcent, diuretic, emollient, expectorant, laxative and salve [36].

Hibiscus rosa-sinensis L. (*Malvaceae*) is a profusely flowering, perennial, woody ornamental shrub distributed widely in the tropical regions. Previous studies have indicated *H. rosa-sinensis* to possess bioactive properties and is recommended to be used as an herbal alternative to cure many diseases [42].

Furthermore, it has reported that *H. rosa-sinensis* possesses anti-complementary, anti-diarrheic and anti-phlogistic activity. According to Reddy & coll. (1997), various extracts of *Hibiscus rosa-sinensis* flowers have an anti-spermatogenic, androgenic and anti-tumor activities in albino mice [36].

In this study essential oil of German chamomile (*Matricaria recutita* L.) and flavonoids from flowers and leaves of *Hibiscus rosa-sinensis* L. were examined for their DPPH• radical scavenging and antibacterial activity.

MATERIALS AND METHODS

Plant material

The aerial parts of *Hibiscus rosa-sinensis* was harvested in March 2016 at the Ain zaatout region (wilaya of Biskra, Algeria). Area located between the wilaya of Biskra and Batna (35,14°N and 5,83°E).

The flowers and leaves were initially separated from the main plants body and shade dried at room temperature for twenty days, then homogenized into fine powder and stored in air tight bottles.

The flowering aerial parts of German Chamomile (Matricaria recutita L.) are used for the extraction of the essential oil.

Extraction of the essential oil

The essential oil extracted by the method of steam distillation, the yield is expressed as a percentage of the ratio between the weight of the oil and that of the drug used. [4].

Extraction of flavonoids

For extracted the Flavonoids of *H. rosa-sinensis* according to [21]; 30 g of drug are macerated in 100 ml of methanol for 72h, after filtration and evaporation of the methanol solution by rotavapor, taken up by 50 ml of boiling water the dry residue.

- First extraction of the aqueous solution obtained with ether petrol (3×30 ml);
- Second extraction of the aqueous solution obtained with ethyl acetate (3×30 ml);
- Third extraction of the aqueous solution obtained with 1-butanol (3×30 ml).

The flavonoids ethyl acetate phase and the flavonoids 1-butanol phase were mixed after evaporated for determine the yield of flavonoids of flowers (FFR) and leaves (FFL).

DPPH• radical scavenging

The determination of the DPPH• radical scavenging activity of an extract is based on the global entrapment effects of the DPPH• radical. This technique is commonly used in the determination of antioxidant assays. This method is fast simple and practical. It is similarly approved and widely accepted in the field of research, because it is independent of the polarity of the extracted samples and is effective in screening a large sample volume [2, 37].

The capacity of the essential oil and flavonoids extracts to scavenge DPPH• radical (2,2-diphenyl-1-picrylhydrazyl) was measured based on the method described by [48]. The results obtained were expressed as the percentage inhibition of DPPH based on the following formula:

$$\% \text{ inhibition of DPPH}^{\bullet} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100$$

EC_{50} value (µg/mL) is the effective concentration at which scavenged of 50% of DPPH• radical. This was obtained by interpolation and using linear regression analysis. Ascorbic acid was used as a positive control. According to [35], the value of EC_{50} is inversely related to the antioxidant capacity of a compound because it expresses the amount of antioxidant required to decrease the concentration of 50% of free radical. The lower of the EC_{50} value indicated the higher the antioxidant activity.

Antibacterial activity

Eight pathogenic bacteria (obtained from bacteriology laboratory of Hakim SAADAN hospital, Biskra, Algeria) was used for evaluating the antibacterial activities of three extracts, including three referenced (ATCC: American Type Culture Collection); *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and five strains clinical isolates from patients; *Streptococcus agalactiae*, *Serratia rubidae*, *Klebsiella pneumoniae*, *Enterobacter* sp and *Enterococcus* sp.

The strains were first reactivated by inoculation in a suitable agar medium at 37°C for 24h, and from 3 to 5 and similar isolated colonies, bacterial suspensions obtained sterile saline was prepared and its adjusted to 0.5 Mac Farland standards, at a concentration of 10^8 CFU/ml (Colony Forming Unit).

The cultivation method in Mueller-Hinton medium according Standardization antibiogram nationally [57].

Disk calibrated and sterile blotting paper are impregnated with the test solutions using a micropipette (10 µl for each disk). The stock solution (SS) is prepared from a milligram of oil or flavonoids and one milliliter of DMSO, five dilutions with DMSO was prepared from oil essensiel (1/2, 1/4, 1/8, 1/16, and 1/32) and three concentrations with flavonoids was prepared (0.5 and 0.25 mg/ml). Then these discs were placed directly on the surface of Muller Hintom agar plates, swabbed with the test organism and the plates were incubated at 37°C for 24 h.

The reading is done by the millimeter measurement of the diameter of inhibition zone around each disk [17, 24]. All results expressed are mean of three individual replicates (n= 3±SD).

The sensitivity of the bacteria to the extract can be determined as a function of the diameter of the zone of inhibition obtained [18].

RESULTS

Determining Of Extraction Yield

Steam distillation of the dried flowering aerial parts of *Matricaria recutita* gave 0.55% ± 0.045 of blue essential oil. But The flowers extract of *H. rosa-sinensis* is the richest in flavonoids (FFR: 3.19%). It was noted that there are significant differences between the flavonoid content of each exract (FFL: 1.53%).

DPPH• radical scavenging

In the present study, the leaves extract of hibiscus exhibited rich scavenging effects on DPPH, while the flowers extract had the lowest (Table 1). Both flowers and leaves extracts of hibiscus exhibit a percent inhibition of DPPH• radical less than ascorbic acid. Also, the essential oil extract showed activity with an EC_{50} of 416.57µg/ml.

Antibacterial activity

This antibacterial activity was quantitatively determined by the presence or absence of inhibition zone around the discs containing extract. All the data are presented in Table 1 (Results were reported as the mean values of three different experiments).

From the results obtained with oil essential of *M. recutita* (Table 2; fig. 1), it can be said that the sensitivity of bacteria; *P. aeruginosa* ATCC 27853, *K. pneumoniae* and *Enterobacter sp* is of medium type, whereas bacteria; *Enterococcus sp*, *S. rubidae*, *E. coli* ATCC 25922, *S. agalactiae* et *S. aureus* ATCC 25923 have lower sensitivity.

According to the results obtained (Table 3, Figure 2), the largest zone of inhibition obtained with the extract of flowers (FFR) on *Enterobacter sp* (13 mm) and slightly active on *S. rubidae* (9.67 mm), while the leaves extract (FFL) habe an effect on *S. rubidae* (11.33 mm) followed by *Enterobacter sp* (7 mm). On the other hand; the other strains showed resistance to our extracts.

DISCUSSION

The Essential oils of *Matricaria recutita*

We can say that our results are in agreement with the results of [43], which estimated that the yield of oils is 0.3 to 1.3% for the same species. As well, close results have been obtained by [45, 49], values of 0.4 and 0.47% were reported for *Matricaria recutita* and *Cladanthus mixtus*, respectively.

According to [1, 14] this variation is due to several factors: intrinsic factors, the organ concerned, the interaction with the environment (soil, climate, humidity... etc.) and degree of plant maturity, harvested period, geographical region and the extraction method.

It has been proved by [52] that the essential oil of *Matricaria recutita* have blue color, this is due to the presence of chamazulene (terpenoids azulenes) in its chemical composition. Chamazulene is formed during warm periods, with proportions limited between 1 and 15% of the essential oil.

Table 1. EC_{50} DPPH test of *H. rosa-sinensis* and *M. recutita* extracts

Extracts	FFR	FFL	Ess. Oil	Ascorbic acid
EC_{50} (µg/ml)	44.515 ± 0.08	36.407 ± 0.003	416.57 ± 0.06	30.99 ± 0.09

Table 2. Inhibition zone (mm) of different concentrations of essentiel oil of *M. recutita*

Strains	Essential oil of *Matricaria recutita*						
	T-	SS	D 1/2	D 1/4	D 1/8	D 1/16	D 1/32
***E. coli* ATCC 25922**	6	14.33 ± 0.57	12.66 ± 0.57	12.33 ± 0.57	12 ± 1	8.33 ± 0.57	8 ± 1
S. agalactiae	6	10 ± 1	8.66 ± 0.57	8.66 ± 0.57	8 ± 0	8 ± 0	8 ± 0
***S. aureus* ATCC 25923**	6	10.67 ± 0.57	11.67 ± 0.57	11.67 ± 0.57	8.66 ± 0.57	8.33 ± 0.57	8 ± 1
Enterobacter sp	6	15 ± 0	9.66 ± 0.57	8.66 ± 0.57	7.33 ± 0.57	7 ± 1	7 ± 1
Enterococcus sp	6	14.66 ± 1.15	12 ± 0	12 ± 0	11.33 ± 0.57	11 ± 1	11 ± 1
S. rubidae	6	14.33 ± 0.57	11.33 ± 1.15	11 ± 1	8.66 ± 0.57	8.66 ± 0.57	8 ± 0
***P. aeruginosa* ATCC 27853**	6	18.33 ± 1.15	13 ± 1	10.66 ± 1.15	9 ± 1	8.33 ± 0.57	8 ± 1
K. pneumoniae	6	15.66 ± 0.57	9.66 ± 0.57	9.66 ± 0.57	8.66 ± 0.57	7.33 ± 0.57	7.33 ±0.57

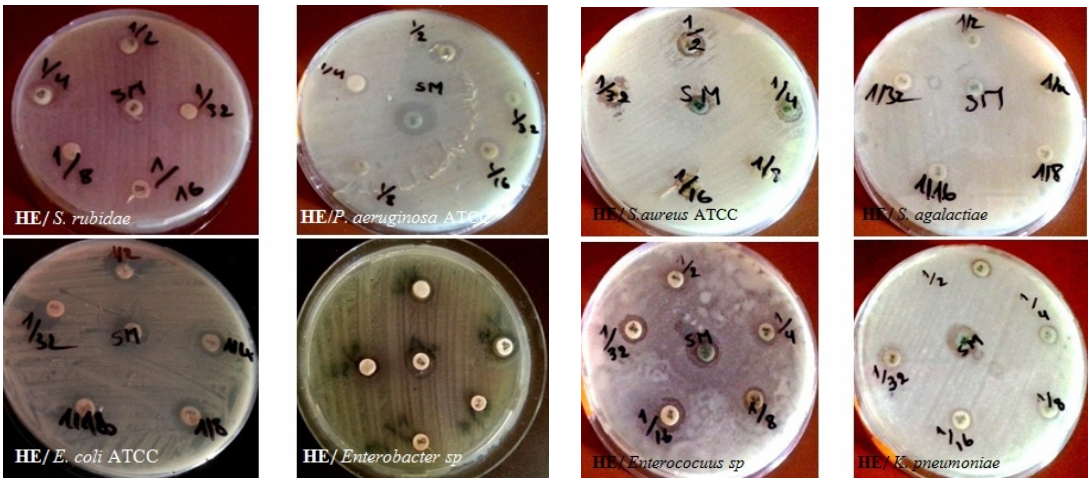

Figure 1. Antibacterial activity of essential oil of *M. recutita* against eight strains pathogenic bacteria.

Table 3. Inhibition zone (mm) of hibiscus extracts at various concentration on some pathogenic bacteria

Strains	FFR			FFL		
	1 mg/ml	0.5 mg/ml	0.25 mg/ml	1 mg/ml	0.5 mg/ml	0.25 mg/ml
***E. coli* ATCC 25922**	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
S. agalactiae	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
***S. aureus* ATCC 25923**	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
Enterobacter sp	10.67 ± 1.53	13.00 ± 02	11.00 ± 01	08.66 ± 1.15	07.33 ± 0.57	07.00 ± 01
Enterococcus sp	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
S. rubidae	09.67 ± 2.08	00 ± 00	00 ± 00	11.33 ± 0.57	10.66 ± 1.15	09.00 ± 01
***P. aeruginosa* ATCC 27853**	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00
K. pneumoniae	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00	00 ± 00

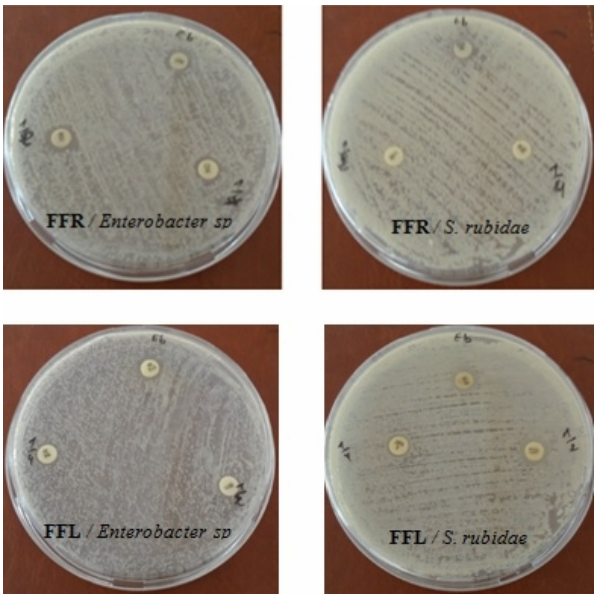

Figure 2. Antibacterial activity of the flavonoids of *H. rosa-sinensis*

According to [31], the DPPH• radical scavenging of essential oils may be related to phenolic content. According to an in vitro study on the reduction of the DPPH• radical by different chemotypes, they demonstrated that the phenolic chemotypes showed more strongly expressed and antioxidant activity than the non-phenolic chemotypes.

Generally, essential oils were shown to protect against oxidative stress by contributing to the total antioxidant defense system of the human body. Recently, a number of essential oils isolated from several medicinal and aromatic plants were shown to possess considerable antioxidant potential [19] and, consequently, protect against some cardiovascular and degenerative diseases [28].

In this study, the essential oil of German chamomile has an antibacterial activity against some bacterial strains tested. It should be noted the presence of activity of essential oils diluted against bacterial strains compared to the crude or concentrated oils [4].

In general, the essential oil have antibacterial activity which varies from one strain to another, it can be said that this activity can be large, low or null depending on the concentration of the sample and according to the degree of sensitivity of the strains tested [44, 51].

Our results are in disagreement with the bibliographic data, because normally Gram (+) bacteria should show greater sensitivity to Gram (-) [5, 29].

Chalchat *et al.* [13] have shown that the antimicrobial activity of essential oils is highly dependent on their chemical composition, in particular on their major constituents. Essential oils have a very wide spectrum of action due mainly to their high affinity to membrane lipids due to their hydrophobic nature [16].

Very few studies have been carried out on the mode of action of essential oils against micro-organisms. In general, essential oils prevent the multiplication, sporulation and synthesis of toxins from bacteria. The essential oils seem to possess several modes of action on the different micro-organisms. According to [29] these modes of action are:

* Interference with the lipid bilayer of the cell membrane, causing an increase in permeability and loss of cellular constituents.

* Alteration of the various enzymatic systems including those involved in the production of cellular energy and the synthesis of structural components.

* Destruction or inactivation of genetic material.

Inouye *et al.* [30], attribute the action of essential oils to the selective insertion of the constituents of the latter on the lipids of the cytoplasmic membrane to disrupt its function. This insertion leads to the loss of electrolytes and the reduction of the level of sugars and amino acids.

The flavonoids of *H. rosa-sinensis*

Presence of high level flavonoids has been reported in flowers and their extracts [60], thus supporting the results and observations done in this study.

The results of the DPPH$^{\bullet}$ radical scavenging test showed the richness of the flower extracts in molecules responsible for DPPH trapping compared to the leaf extract. Greater radical scavenging activity could be attributed to the presence of phenols, tannins or flavonols in the flower extract.

According to Turkmen *et al.* [56], this activity could be related to its richness in flavonoids which appear to be effective hydrogen donors to the DPPH$^{\bullet}$ radical, due to their ideal structural chemistry.

Additionally, studies have reported a positive correlation to occur between antioxidant compounds and antioxidant activities in plant parts (e.g. flowers, fruits, leaves, seeds) and their extracts [58]. Earlier, it has been reported that different solvent extraction systems can contribute significantly to differences in the free radical scavenging of the extracts [54, 59].

Flavonoids are recognized as potentially antioxidant substances with the ability to trap radical species and reactive forms of oxygen [8, 38]. The mechanism of the reaction between the antioxidant and the DPPH depends on the structural conformation of the antioxidant and the number of OH groups of the flavonoid structures can influence the different antioxidant mechanisms [27, 34]. Some compounds react very quickly with DPPH by reducing a number of DPPH equal to that of the hydroxyl groups of the antioxidant [11].

In this study, the majority of the tested strains are resistant to ***H. rosa-sinensis*** extracts. They exist several dilutions more active than the concentrated solution, can be linked to the dissemination of DMSO in the culture medium [4].

Reports available have shown crude plant extracts to exhibit higher antibacterial activities against Gram (+) bacteria than Gram (-) bacteria [32]. This has been attributed to structural variations observed in the bacterial cell envelope (including those of cytoplasmic membrane and cell wall components) between Gram (+) and Gram (-) bacteria [53]. However, in the present study, flower extracts of Hibiscus exhibited higher antibacterial activities against Gram (-) more than Gram (+) pathogens.

S. agalactiae et *Enterococcus sp* are strains resistant to our extracts despite being known as sensitive strains Gram (+), this confirms the results obtained by [9]. The resistance of the strain can be attributed to the weak ability of the antibacterial agent to spread evenly in the agar [22, 26]. It can also be related to the distribution method of the extract into the agar [20, 39].

The inefficiency of flavonoid extracts is probably due to the presence of differences in their chemical compositions. It can be shown that each extract acts in a completely different way on the same bacterial strain, depending on their location in the plant and their polarity in the extraction solvent [6, 33], these results are in concordant with Treki and Dehimat studies [55].

According to [12], the antimicrobial activity is related to the polarity of the bioactive substances. The less polar compounds, such as flavonoids, having no hydroxyl OH group on their Bring, are more active against microbial agents than those carrying the hydroxyl group.

Polyphenols, flavonoids and tannins present in a sample might be responsible for the antibacterial activity. These compounds are generally produced by plants as defenses against microbial infections [50]. This is attributed to the formation of complexes between tannins and microorganism enzymes as well as the transport proteins of the cell envelope. This complex renders the proteins inactive which will eventually cause the inhibition of microbial growth. [25].

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