

Antimicrobial Potentials of Flavonoids Isolated from *Tagetes erecta*

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Abstract—In this study, we are interested in a species of the family of *Asteraceae* (*Tagetes erecta*). This family is considered as a source of antimicrobial extracts with strong capacity. The extraction of the flavonoids is carried out by the method of liquid/liquid with the use of successive solvents. Afterwards, we evaluated the biological activity of the flavonoids on five pathogenic bacterial stocks such as *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and two stocks of yeasts to knowing *Candida albicans* and *Saccharomyces cerevisiae*, by employing the method of the chromatogramme starting from a solid disc. The result of the antimicrobial activity shows an action and a variable degree of sensitivity according to bacterial stocks tested. It will be noted that the flavonoids have an inhibiting effect on *E. coli*, *B. subtilis*, *K. pneumoniae* and *S. aureus*. But a resistance with respect to the extract by *P. aeruginosa*, *C. albicans* and *S. cerevisiae* is to be mentioned.

Keywords—Antimicrobial activity, flavonoids, microbial strains, *Tagetes erecta* L.

I. INTRODUCTION

DURING the last decades, a growing interest was noticed in the study of medicinal plants and their traditional use in different regions of the world [24], [1]. According to the World Health Organization (W.H.O), about 80% of population today depends on traditional medicine for their first care [1].

Therapeutics has enormously progressed to reach its current form. Some plants are used as a raw material to extract active principles which are part of the composition of many medicines. More than 30% of these products contain indeed active principles from plant origin [2].

A large number of medicinal and aromatic plants possess very important biological properties which are applied in many fields, like in medicine, pharmacy, beauty care and agriculture. However, the evaluation of pharmaceutical properties; anti-oxidizing and antimicrobial remain a very interesting and useful task, especially for the plants which

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have a rare use or less frequent or not known in medicine, in particular in folkloric medicinal traditions [3], [4]. These plants represent a new source of active components [5]. Indeed, the research about natural substances, especially about secondary metabolites is an expanding theme conducted by many researchers. They are inspired by molecular structure of this metabolite to imagine new medicines.

Herbal extracts as active ingredients of this medicine used to treat psoriasis do not undergo any antimicrobial contamination. Whereas some alkaloids, anthocyanines, flavonoids, quinines, steroids and terpenoids are used in biomedical, veterinary sciences and pharmaceutical fields [6]. The phytochemical and the antimicrobial investigations of stilbenoids and flavonoids isolated from three species of *Combretaceae* were studied [7].

The African continent is endowed of one of the richest biodiversity in the world, with a large number of plants used as herbs, as natural food and used for therapeutic objectives. More than 5000 different natural substances are identified. Most of these plants turned out to be useful in traditional medicine, in the treatment and the prevention of diseases. In spite of the heterogenic nature of the African continent, there was only some effort devoted to the development of the chemical-therapeutic and preventive agents of these plants [8]. In Egypt, the effect of the flavonoid quercetin on culture and isolation of *Frankia* from *Casuarina* root nodules was studied [9]. Always in the same country, the Antibacterial Activities of *Psidium guajava* (L.) extracts have been studied [10].

In Algeria, medicinal plants have an important place in the traditional medicine which is largely used in diverse health problems. The use of plants as a remedy is cheap and without unwanted effects [11]. In this country, the antibacterial activity of flavonoids extracted from *Crateagus oxyacantha* (L.) leaves of Bainem was studied [12]. Then, the antibacterial activity of flavonoids extracted from *Crataegus oxyacantha* (L.) flowers of Boumerdès was studied [13].

In this study, we are interested in studying the antimicrobial effect of *T. erecta*. *Tagete* which is a medicinal, decorative and annual plant. It belongs to *Asteraceae* family. It is widely employed in folkloric medicine, probably because of the presence of many aromatic actives responsible of its biological activities.

II. MATERIAL AND METHODS

A. Material

The vegetable material is composed of the Tagetes's flowers collected in the region of Boudouadou, (Boumerdes) at the beginning of May. This study was conducted using five bacterial strains which are *S. aureus*, *P. aeruginosa*, *B. subtilis*, *E. coli* and *K. pneumoniae* and two kinds of yeast; *C. albicans* and *S. cerevisiae*. It is about strains of collection from type A.T.C.C. (American Type Culture Collection).

B. Methods of the Study

The different used methods deal with the obtaining of plant extracts (flavonoids) as well as the antimicrobial power of these flavonoids.

C. Flavonoids Extraction Methods

All the flavonoids don't have the same properties of solubility, because some flavonoids are soluble in water and alcohol whereas others have very weak hydrosoluble properties [14]. Starting from this fact, the used principle for the extraction of flavonoids is based on the degree of solubility of flavonoids in the organic solvents.

D. Evaluation of the Flavonoids Antimicrobial Activity

Concerning the qualitative and quantitative evaluation of the antimicrobial flavonoids activity, we have conducted respectively the method of chromatogramme and the method of the determination of the minimum concentration. These methods are validated by the D. R. C. of SAIDAL.

E. Qualitative Study: the Aromatogramme

The vegetable extracts are got back by the method of diffusion in an agar medium or by the method of discs which is a well-known technique in medical bacteriology. This technique is called the antibiogramme [15].

F. Quantitative Study

The efficiency of the tested flavonoid is evaluated with the measure of two concentrations which are M.I.C. and M.B.C. The M.I.C. is the minimum concentration of antimicrobial agent which inhibits the bacterial growth, after incubation in standard conditions. The microorganisms however remain variable [16].

M.B.C. is the minimum concentration of antimicrobial agents necessary in destroying the initial inoculum after incubation in standard conditions. The microorganisms are no longer variable [17].

F.M.C. is the minimum concentration of antimicrobial agent which inhibits totally the growth of a mold after incubation in standard conditions [18].

This method consists of putting at first some drops of Tween 80 in Mueller Hinton medium (M.H.). The whole is well shaken and preserved next in the Bunsen burner. Secondly, 1ml of flavonoid of Tagetes taken, to which 50ml of the previous medium is added, and then the concentration of 2% is obtained. 25ml of this preparation is poured in the first Petri dish. The remained quantity of the preparation will be adjusted to 50ml of the medium (M.H.) to obtain a

concentration of 1% and we pour always the quantity of 25ml of this preparation in the second Petri dish. By this way, the other dilutions follow one another to obtain the concentrations 0.5%, 0.25%, 0.125%, 0.06% and 0.03%. Each dilution is poured in a Petri dish, at the same time taking care of the numbering.

Concerning the strains *C. albicans* and *S. cerevisiae*, we follow the same steps with a difference of culture medium which is Sabouraud medium.

In the septic zone of the Bunsen burner, sterile A.T. B. discs are put in Petri dishes previously prepared then soaked by a small quantity of microbial suspension corresponding in each of the used strains.

On the other side of the dish, a small quantity of microbial suspension is spotted using a Pasteur pipette. We let diffuse the discs for 30 min on the drain board, then we incubate at 37°C for 24h for the bacterial strains and at 25°C for 48h for the yeasts. Reading is done by determining the smallest concentration in which the microorganism didn't grow; it is the M.I.C. The discs corresponding to this concentration and going until the highest concentration will be put in contact in a neutral medium in order to determine the M.B.C.

III. RESULTS AND DISCUSSION

This part is about the evaluation of the antimicrobial activity.

A. Antimicrobial Activity of *T. erecta* Flavonoids

1. Qualitative Results

The values of diameters of the inhibition zone (d) of flavonoids of *Tagetes erecta* tested on many microbial strains of reference are exposed in Fig. 1:

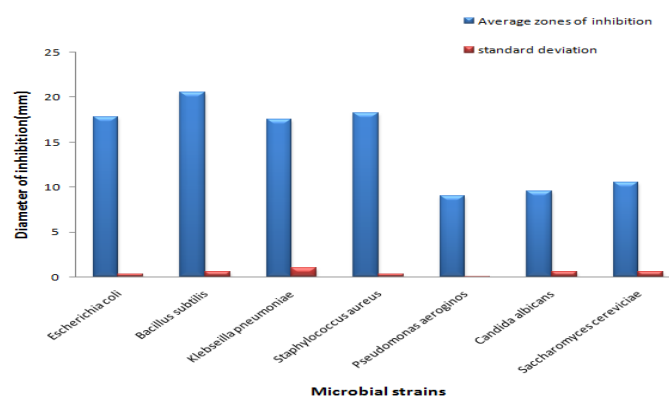


Fig. 1 Inhibition zones of the different microbial strains

It is noticed through the obtained results that *T. erecta* flavonoids have an antimicrobial action on all the tested bacteria except on *P. aeruginosa* which showed a resistance. The diameters of inhibition vary between 16.5 and 21mm. The largest diameter is obtained with *B. subtilis* (20.5mm) and the smallest is *K. pneumoniae* (17.5mm). So, the obtained results show that *T. erecta* flavonoids possess an average antimicrobial activity on G^+ and G^- bacteria.

According to the obtained results, it is noticed that both tested yeasts; *C. albicans* and *S. cerevisiae* are resistant to the effect of *T. erecta* flavonoids.

2. Quantitative Study

a) Bacteria

The results of the Minimum Inhibitor Concentration (M.I.C.) and those of the Minimum Bactericide Concentration (M.B.C.) of *T. erecta* flavonoids towards tested bacterial strains are represented in Table I.

TABLE I

M. I. C. AND M. B. C. IN %FOR THE DIFFERENT TESTED BACTERIAL STRAINS		
Bacterial strains	C. M. I.	C. M. B.
<i>B. subtilis</i> (ATCC 9372)	>2%	>2%
<i>E. coli</i> (ATCC 4157)	>2%	>2%
<i>S. aureus</i> (ATCC 6538)	>2%	>2%
<i>P. aeruginosa</i> (ATCC 9027)	>2%	>2%
<i>K. pneumoniae</i> (ATCC 4352)	>2%	>2%

According to Table I, it is noticed that M.I.C. and M.B.C. vary according to tested strains. All the bacteria have been grown in different concentrations of flavonoids starting from 0.03 to 2%. Of this fact, M.I.C. of all the bacteria is superior to 2%. The bactericide minimal concentration of all the bacteria is superior to 2%.

b) Yeasts

The results of the Minimum Inhibitor Concentration (M.I.C.) and the Fungal Minimal Concentration (F.M.C.) of *T. erecta* flavonoids towards tested yeasts are represented in Table II.

TABLE II

M. I. C. AND M. B. C. IN %FOR THE DIFFERENT TESTED YEASTS		
Yeasts	C. M. I.	C. M. F.
<i>C. albicans</i> (ATCC 24433)	>2%	>2%
<i>S. cerevisiae</i> (ATCC 2601)	>2%	>2%

Both yeasts have been grown in all concentrations from 0.03 till 2%. So, M.I.C. of yeasts is superior to 2%. So, F.M.C. of yeasts is superior to 2%.

The obtained results during this study turned out to be in accordance with those of [19]. These latter notice that flavonoids and other component with phenolic nature or free hydroxyl groups are classified as very active antibiotics [20]. Polyphenols, like flavonoids are substances with an important antimicrobial property [21], [22]. A more recent study [23] has showed the antimicrobial power of *T. erecta* glycoside flavonoids against strains of negative and positive gram bacteria [23].

During this work, the results of the chromatogram remain, however close to those of the authors cited before according to the chosen plant; it is about *T. erecta* and *Tagetes patula*.

The tested bacteria turned out to be sensitive to *T. erecta* flavonoids which possess an average antimicrobial activity on bacteria G⁺ and G⁻ except on *P. aeruginosa*. This later has relatively shown a certain resistance to the effect of *T. erecta*

flavonoids. So, the flavonoids extract from *Crateagus oxyacantha* have a very important antimicrobial effect on the Gram + bacteria and Gram- ones as well [12].

These results do not correspond to the works of [24]. However, some authors, explains this resistance by the capacity that possesses this bacterium in order to be protected against a wide range of antibiotics and antimicrobial agents [23]. This capacity is due to the formation of a bio-film or a polysaccharidic barrier (hydrophilic permeability barrier). This barrier is composed of strata linked from the inside to the external membrane permitting this bacterium at the same time to find itself in its environment and to be protected against antimicrobial agents [25]. Alvarez et al. (2006) note that when flavonoid combinations were employed, a strong effect was found against *E. coli* than against *S. aureus*. This fact is due to existence of porins in the outer membrane of G⁻ bacteria. The compound that acts as enhancer acts by blocking the charges of amino acids in the porins and thus facilitates the passage of other compound by diffusion into the bacterial cell [26].

The obtained results on five bacterial strains and two yeasts during this qualitative and quantitative study of the antimicrobial activity of *T. erecta* flavonoids show an action and a degree of variable sensitivity. The highest value of inhibition diameter resisted in bacteria is 20.5mm which corresponds to *B. subtilis*, and the lowest value is the one of *K. pneumoniae* with an inhibition diameter of 17.5mm. On the other hand, *P. aeruginosa* is resistant to flavonoids of *T. erecta*. The inhibition is maximum for *Bacillus subtilis* (29.5mm) and *K. pneumoniae* (28mm) and minimum for *P. aeruginosa* (18mm). Flavonoids have a weak fungal activity against the two yeasts. *C. albicans* and *S.cerevisiae* have shown a resistance to flavonoids extracted from *T. erecta* flowers [22].

The analysis of the different results shows clearly the sensitivity of the bacteria G⁺ comparing to bacteria G⁻. The resistance of this later is not amazing. In fact, these bacteria possess an intrinsic resistance to biocides agents which are in relation to the nature of their external membranes composed of lipopolysaccharides. In the presence of agents permeabilizing of the external membrane, inactive substances against these bacteria become active.

This qualitative study of the antimicrobial activity of this tested product turned out interesting, since that we have obtained satisfying results. According to these results, we can say that *T. erecta* flavonoids possess an average antimicrobial activity in which some strains seem to be distinguished by the highest sensitivity comparing to the others.

The bacteria and the yeasts have shown a resistance to the doses of flavonic extracts administrated to their culture medium in spite of Tween 80 which facilitates the distribution of the extract in the culture medium and which permits a considerable increase of flavonoids antimicrobial activity.

The inhibitor minimal concentration (M.I.C.) of all the microbial strains is superior to 2% whatever may be the used pathogen agent, because flavonoids don't have an inhibitor effect with weak concentrations. So, in order to have an

inhibition on bacteria or yeasts under the effect of flavonoids, we must increase the concentration (superior to 2%).

From the results of (M.I.C.), we deduce that (M.B.C.) of all the strains are superior to 2%. Flavonoids don't behave like the other oils which have inhibitor minimal concentrations superior to 2% because flavonoids are secondary metabolites. However, we cannot determine the values of M.B.C and F.M.C.

Minimum Bactericide Concentration (M.B.C.) and Fungicidal Minimum Concentration (F.M.C.) are sometimes equal to M.I.C. But, generally M.B.C. and F.M.C. are more important and higher than M.I.C. Till now, no study has been conducted on the determination of M.I.C. and M.B.C. from *T. erecta* polyphenolic extracts. The capacity of a vegetable species to resist to insects attacks and to microorganisms is often with the content of phenolic components [27].

IV. CONCLUSION

In conclusion, results of the present study demonstrate that flavonoids isolated from *Tagetes* flowers present a great potential for the biopharmaceutical applications due to their biological properties.

REFERENCES

- [1] C.Muthu, N.Mir,S.Ignacimuthu, "Medicinal plants used by traditional healers in kanchee puran district of tamil Nado, India". *Journal of ethnobiology and ethnomedecine*. 2006; 2, 43 doi: 10.1186/1746-4269-2-43.
- [2] O. Catier, D. Roux, "Botanique, pharmacogonose, phytothérapie". 3rd Wolters Kluwer, 2007. p. 13.
- [3] D. Lamnaouer, "Composition chimique et activité biologique de quelques plantes médicinales du PNT". Programme de l'UICN en Afrique du nord, 2001. Phase III, p. 1-9.
- [4] D. Lamnaouer, "Réflexions sur l'enseignement de la botanique et des plantes médicinales". Colloque « Voyages en Botanique » Maroc; 2010. p.4.
- [5] D.S.Teixeira, "Mining the essential oil of the Anthemideae". *African Journal of Biotechnology*.v.3, n.12, p. 706-720, 2004.
- [6] L. Pacheco, M. M. Costantine, G. D. V. Hankins, "Physiologic changes during pregnancy," in *Clinical Pharmacology During Pregnancy* ed. Mattison D. R., editor. (San Diego: Academic Press;) 5-14, 2013.
- [7] D.R. Katerere, G. Graziani, K.M. Thembo, N.Z. Nyazema, A. Ritieni, "Antioxidant activity of some African medicinal and dietary leafy African vegetables". *Afr. J. Biotechnol*.11:4103- 4108, 2012.
- [8] I.Farombi, "African indigenous plants with chemotherapeutic potentials and biotechnological approach to the production of bioactive prophylactic agent". *African Journal of Biotechnology*. v. 2, n. 12, p. 662-671, 2003.
- [9] W.F.Sayed, C.T.Wheeler, "Effect of the flavonoid quercetin on culture and isolation of *Frankia* from *Casuarina* root nodules". *Folia Microbiol*. v.44, n.1, p. 59-62, 1999.
- [10] R.N Sanches, D.A.Garcia Corte, M.S.Schiavini, "An evaluation of antibacterial activities of *Psidium guajava* (L.)". *Brazilian Archives of Biology and Technology*. v.48, n.3, p. 429-436, 2005.
- [11] A. Hallimi, "Les plantes médicinales en Algérie". Ed. Berti, Algérie, 2004. p. 42.
- [12] N.Behidj-Benyounes, T.Dahmane, K. Hassak, F.Slimani, "The antibacterial activity of flavonoïds extracted from *Crateagus oxyacantha* (L.) leaves of bainem (Algeria)". *13th International Multidisciplinary Scientific GeoConference SGEM Conference proceedings, June 16-22, 2013 Albena (Bulgarie)*. 2013a. p. 288- 295.
- [13] N. Behidj-Benyounes, T. Dahmane, K. Hassak, F.Slimani, "The antibacterial activity of flavonoïds extracted from *Crateagus oxyacantha* (L.) flowers of Boumerdès (Algeria)". *13th International Multidisciplinary Scientific GeoConference SGEM Conference proceedings. June 16-22, 2013 Albena (Bulgarie)*. 2013b p. 296- 303.
- [14] J. Bruneton, "*Plantes toxiques végétaux dangereux pour l'Homme et les animaux*". 2nd. Tec and Doc, Paris, France, 2001. p. 542 - 564.
- [15] J.L. Fauchere, J.L. Avril, "*Bactériologie générale et médicale*". ed. Ellipses, Paris, France, 2002. p. 365.
- [16] M.S. Klaric, I.Kosalec, K.J.Mastelic, E.Pieckova, K.Pepeljna, "Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against mould from damp dwellings". *Letters in Applied Microbiology*. v.44, p. 36-42, 2006.
- [17] M.A.Pfaller, D.Diekema, M.Mendez, C.Kibbler, P.Erzsebet, S.C.Chang, D.L.Gibbs, V.A.Newell, "The global antifungal surveillance group, *Candida guilliermondii*, an opportunistic fungal pathogen with decreased susceptibility to fluconazole: Geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program". *Journal Clinical Microbiology*. v.44, p. 3551-3556, 2004.
- [18] C.Su, X.Zhang, J.P.Dudey, "Genotyping of *Toxoplasma gondii* PCR-RFLP markers: a high resolution simple method for identification of parasites". *International Journal of Parasitology*. v.36, p. 441-848, 2006.
- [19] T.Essawi, M.Srour, "Screening of some Palestinian plants for antibacterial activity". *Journal of Ethnopharm*.v. 70, p. 343-349. 2000.
- [20] M.C.Marjorie, "Plants products as antimicrobial agents". *Clinical Microbiology Reviews*. v.9, p. 564-582, 1999.
- [21] B.Shan, Y.Z.Cai, J.D.Brooks, H.Corke, "The in vitro antibacterial activity of dietary spice and medicinal herb extracts". *Journal International of Food Microbiology*. v.117, p. 112-119, 2007.
- [22] T. Askun, G. Tumen, F. Satil, M.Ates, "In vitro activity of methanol extracts of plants used as spices against *Mycobacterium tuberculosis* and other bacteria". *Food Chemistry*. v. 104, n. 116, p. 289-294, 2009.
- [23] D.Horikrishna, A.V.N.Approo, M.C.Probhakar, "Pharmacological investigation of flavonoid glucoside". *Indian Journal of Pharmacology*.v.36, p. 244-250, 2004.
- [24] S.Rhama, S.Madhavan, "Antibacterial activity of the flavonoid, patulitrin isolated from the flowers of *Tagetes erecta* L.". *International Journal of Pharm Tech Research*. v.3, n.3, p. 1407-1409, 2010.
- [25] M.C. Pibiri, "Assainissement microbiologique de l'air et du système de ventilation au moyen d'huile essentielle". Thèse de Doctorat, Ecole Polytechnique Fédérale de Lausanne, 2006. p. 177.
- [26] M.A.Alvarez, N.B.Debattista, N.B.Pappano, "Antimicrobial activity and synergism of some substituted flavonoids". *Folia Microbiol*. v. 53, n.1, p. 23-28, 2008.
- [27] S.Rees, J.B.Harborne, "The role of sesquiterpene lactones and phenolics in the chemical defense of the chicory plant". *Phytochemistry*.v.24, p. 2225-2231, 1985.