World Academy of Science, Engineering and Technology
International Journal of Agricultural and Biosystems Engineering
Vol:10, No:9, 2016

Effectiveness of the Flavonoids Isolated from *Thymus inodorus* by Different Solvents against Some Pathogenis Microorganisms

N. Behidj, K. Benyounes, T. Dahmane, A. Allem

***Abstract*—**The aim of this study was to investigate the antimicrobial activity of flavonoids isolated from the aerial part of a medicinal plant which is *Thymus inodorus*by the middle agar diffusion method on following microorganisms. We have *Staphylococcus aureus, Escherichia coli, Pseudomonas fluorescens, AspergillusNiger, Aspergillus fumigatus and Candida albicans.* During this study, flavonoids extracted by stripping with steam are performed. The yields of flavonoids is 7.242% for the aqueous extract and 28.86% for butanol extract, 29.875% for the extract of ethyl acetate and 22.9% for the extract of di - ethyl. The evaluation of the antibacterial effect shows that the diameter of the zone of inhibition varies from one microorganism to another. The operation values obtained show that the bacterial strain P fluoresces, and 3 yeasts and molds; *A. Niger*, *A. fumigatus* and *C. albicans*are the most resistant. But it is noted that, *S. aureus* is shown more sensitive to crude extracts, the stock solution and the various dilutions. Finally for the minimum inhibitory concentration is estimated only with the crude extract of *Thymus inodorus* flavonoid.Indeed, these extracts inhibit the growth of Gram + bacteria at a concentration varying between 0.5% and 1%. While for bacteria to Gram -, it is limited to a concentration of 0.5%.

***Keywords*—**Antimicrobial activity, flavonoids, strains, *Thymus inodorus.*

I. Introduction

SINCE ancient times, human uses plants as medicines and remedies. Until now, the plants are still used for human health despite the efforts of chemists trying to synthesize new molecules [1]. Since 1990, the interest of large pharmaceutical companies has been diverted from natural products to combinatorial chemistry, claiming that in a few years the number of drugs would be higher. Therefore, the number of drugs has remarkably dropped knowing that for the synthesis of a single drug 10000 molecules must be synthesized and tested [2].

N. Behidj is with the Laboratory of Soft Technologies, Valorisation, Physico-Chemistry of Biological Material and Biodiversity, Department of Biology, Faculty of Sciences, University M'HamedBougaraof Boumerdes, 35000, Algeria (e-mail: behidj_nassima@ yahoo.fr).

K. Benyounes is with the Faculty of Hydrocarbons and Chemistry, DGMP, University M'HamedBougaraof Boumerdes, 35000, Algeria (e-mail: khaled_benyounes@yahoo.fr).

T. Dahmaneis with the Laboratory of Soft Technologies, Valorisation, Physico-Chemistry of Biological Material and Biodiversity, Department of Biology, Faculty of Sciences, University M'HamedBougaraof Boumerdes, 35000, Algeria.

A.Allem is with the Department of Biology, Faculty of Sciences, University M'Hamed Bougara of Boumerdes, 35000, Algeria.

Based on these data, biologists and chemists recognize the major importance of natural products and the limitedness of the methods and the techniques of biotechnology and chemistry. This may explain the great interest in the search for compounds from natural sources.

The unlimited number of plants constitutes a huge reservoir of new medicinal compounds with great potential, thanks to their molecules which have the advantage of a wide variety of chemical structures and biological activities. Aromatic plants are characterized by their richness in active ingredients and substances such as polyphenols and flavonoids. These products possess very important properties [3].

Despite the heterogeneous nature of the huge biodiversity of the African continent in general and Algeria in particular, there has been little effort devoted to the development of therapeutic agents of plant origin. That is why we are interested in the study of *Thymus inodorus*(labiates) which is a widespread Mediterranean species [4].

Through this study, there has been extraction by steam distillation of flavonoids from the aerial part of the Thymus inodorus, and evaluation of their antimicrobial activity by the agar diffusion media of the crude extract.

II. Materials and Methods

A. Material

In the plant, the used part is the aerial one, precisely the fruits which are rich in natural substances [1]. In this study, we have used an aerial parts of *Thymus inodorus* (Fig. 1). Harvesting is done in the third of april 2014 in the forest of Bainem (Algiers).The antimicrobial effect of the flavonoids isolated from *Thymus inodorusis* evaluated on many microorganisms.

Fig. 1 *Thymus inodorus*

It was tested on three bacterial stumps; *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Escherichia coli*.It was

studied on one kind of yeast (*Candida albicans*). Finally, two mouldes which are the *Aspergillus niger*, and the *Aspergillus fumigatus*. It's noteworthy that these agents are characterized by an important frequency of contamination and phatogynicity.

B. Methods of the Study

1. Flavonoids Extraction

Flavonoids extraction is performed according to Markham method [5] with modification inspired from Bruneton method [6]. It is based on the solubility degree of flavonoids in organic solvent. The method comprises two main steps. The first step is done with methanol to dissolve the flavonoids. The second is carried out with ethylic ether to remove free genius, ethyl acetate (extraction of monoglycosdes) and n-butanol (extraction of di and tri glycosides). The macerate is filtered and then evaporated to pressure at 35° C (RotaVapor, Stuart, England). The obtained aqueous phase is stored for 48 h at 4 ° C to accelerate the diffusion of molecules in the solvent. At this level, the filtrate is freed of waxes, fats and chlorophyll by three successive washings with petroleum ether (V/V) to have an aqueous phase in order to separate flavonoids from aglycones fractions, monoglycosides and riglucosides. This recovered phase is mixed with ethylic ether (V/V) to obtain an organic phase containing the aglycones flavonoids and methyl aglycones. The remaining aqueous phase undergoes three extractions with ethyl acetate, to recover in the organic phase some aglycones flavonoids, mono- glycosides in particular. The remaining aqueous phase is mixed with the n_butanol to recover di and triglucosides flavonoids. The final aqueous phase contains the most polar glycolysis flavonoids. The four collected fractions are concentrated by evaporation at low pressure at 35 °C.

2. Antimicrobial Activity

The antimicrobial activity of flavonoids taken from *Thymus inodorus*L. aerial parts is determined by the method of diffusion in an agar environment [7], [8]. The first step is the preparation of the microbial strains. It is followed by an antibiogram. This method has the advantage of being very flexible in the choice of thetested antibiotics, to be applied on a big number of bacterial species, and to be largely evaluated during 50 years of world usage [9].

A. Determinationof Minimum Inhibitory Concentrations

Defining is necessary to describe the antimicrobial activity of a compound of simple parameters. For the antimicrobial activity, the most common is the minimum inhibitory concentration (MIC) which may be determined by direct contact in agar or liquid medium. It corresponds to the concentration required to completely inhibit the growth of a determined number of germs after a given incubation time.

1. Method of Dilution in a Solid Medium

According to Hulin et al. [10], the MIC is defined as the lowest flavonoid concentration capable of inducing a reduction in microbial growth of 90%; therefore, allowing the survival of only 10% of the population.Several substances are used for this purpose namely ethanol, methanol, tween-20, DMSO, n-hexane, poly ethylene glycol, Tween-80 and agar by several authors [11]-[16].

2. Preparation of Dilution Series

To prepare the dilution series, first 200 ml of liquified culture medium at 95 ° C in the water bath, by adding 1 ml of Tween 80. A dilution is prepared in 2% flavonoids tested by diluting 1 ml of the flavonoid extract in 50 ml of medium. After, 1 bottle is homogenized. Then, is poured 25 ml of this content to another flask.Then adjusted to 50 ml of the medium for a dilution of 1%. This continues until obtaining 0.03%. From each prepared dilution, 20 ml was poured into each petri dish and let to be solidified. Boxes are partitioned into two parts, one part is seeded through spotage by using a micro syringe 3UI of each microbial suspension of 10^{-4} dilution.

3. Reading

The MIC is defined as the absence of visible growth at the spots knowing that the presence of one or two colonies is not considered.

III. Results and Discussion

A. Extraction and Characterization of Flavonoids

1. Flavonoids Extraction

For the plant, the obtained aqueous extract has a gelatinous aspect, colored in orange-red. The yields obtained for the different extracts (di ethyl, ethyl acetate, butanol and aqueous). The flavonoids yields are showed in Table I.

TABLE I
Flavonoid Yield

Different extracts	Yield
Dethyl	22.9%
Ethyl acetate	29.88%.
Butanol	28.86%.
Aqueous	7.24%

B. Study of Antimicrobial Activity of Aqueous Extracts

The tests on the study of antimicrobial activity showed that aqueous extracts obtained from *Thymus inodorus* act differently on the tested strains. The diameters values of inhibition zone of the crude extracts and various dilutions are shown in Table II.

1. Solubility of Microbial Strains

The findings of this study show that *P. fluorescens* is sensitive to the crude extract, and resistant to various dilutions 1/2, 1/4 and 3/4. The minimum inhibitory concentration (MIC) obtained relates to the crude extract *S. aureus* is very sensitive to the crude extract. However, this strain seems to be sensitive to the parent strain and to dilutions1 / 2, 1/4 and ¾.. Since the strain is sensitive to all dilutions, the estimation of the (MIC) obtained relates to the crude extract. It is noticed that *E. coli* is sensitive to the crude extract, and resistant to different dilutions 1/2, 1/4, and 3/4. The minimum inhibitory

concentration (MIC) obtained concerns the crude extract. It should be noted that A. Niger is sensitive to dilution 3/4 and 1 / 2 of the crude extract. However, this strain has demonstrated to be crude extract-resistant and to 1/4 dilution. The minimum inhibitory concentration (MIC) requires the completion of other dilutions.

TABLE II
VALUES OF THE DIAMETERS OF ZONES OF INHIBITION OBTAINED WITH THE AQUEOUS EXTRACT OF *THYMUS INODORUS*

Strains	Number of test	Dilution	Diameter (mm)		Average (mm)
S. aureus	2	SM	13	16	14,5
		¾	12	14	13
		½	10	14	12
		¼	10	10	10
E. coli	2	SM	13	12	12,5
		¾	09	13	11
		½	10	11	10.5
		¼	09	10	09.5
P. fluorescens	2	SM	12	14	13
		¾	09	13	11
		½	10	12	11
		¼	09	12	10.5
A. niger	2	SM	09	09	09
		¾	09	11	10
		½	09	12	10.5
		¼	09	09	09.5
A. fumigatus	2	SM	09	10	09.5
		¾	11	10	10.5
		½	10	09	09
		¼	09	09	09
C. albicans	2	SM	09	09	09
		¾	09	10	09.5
		½	11	09	09.5
		¼	09	09	09

Through Table II, it may be noted that this species is sensitive to the crude extract and 3/4 dilution. Thus, it is resistant to the stock solution and dilutions 1/2, 1/4. The minimum inhibitory concentration (MIC) requires the completion of other dilutions. Finally, *C. albicans* is resistant to the crude extract, and various dilutions.Through the obtained results, it is noted that thyme flavonoids have a strong antibacterial activity to (Gram +) bacteria and low antimicrobial effect on (Gram-) bacteria. Thus, thyme flavonoids are not active on tested yeasts and molds. According to Nakatsu et al.[17], the antimicrobial effect is explained by the presence of chemical molecules such as monoterpene alcohols, sesquiterpene which are endowed with excellent antibacterial factors. This can cause the release of cellular components that change the content and the composition of fatty acid and phospholipid bacteria. It, thus, causes disturbance of electron transport or the nutrient intake and affects the synthesis of nucleic acids. Monoterpenes may inhibit the development of enzymes responsible for the growth of these germs which are behind their antiseptic power. Nakatsu et al.[17] show that the Gram-positive bacteria are more sensitive to the action of flavonoids comparing to Gram-negative bacteria. Mebareki[18] worked on the antimicrobial effect of flavonoids on a number of species of thyme, in particular the odourless thyme of Tlemcen region, reports that the flavonoids of *Thymus inodorus*have a significant inhibitory effect on Gram + bacteria. But this inhibition is average on Gram - bacteria. Thus, the same author notes that among the flavonoids derived from the different studied species of thyme, *T. inodorus* flavonoids are the ones that have better antimicrobial activity comparing to other species of thyme.The results of this qualitative study deserve a more developed quantitative study to determine the minimum inhibitory concentration (MIC) and the minimum flavonoid concentration of T. inodorus.

C. Study of the Minimum Inhibitory

The results of the MIC are summarized in Table III. According to Table III, flavonoids of *T. inodrus* inhibit the growth ofGram $^{+}$ bacteria at a concentration varying between 0.5% and 1%. While for bacteria Gram-, it is limited to a concentration of 0.5% (Table IV).

T. inodorus flavonoids have exerted a significant inhibitory activity towards the Gram^{+} bacteria, except for *P.*fluorescens which is a bit resistant. Thus, *S. aureus* and *E. coli* are the most sensitive.

TABLE III
RESULTS OF THE MIC

Strain	Concentration(%)						
	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
E.coli	+	+	+	+	-	-	-
S. aureus	+	+	+	+	-	-	-
P. fluorescens	+	+	+	+	+	-	-

(-):Inhibition (+):Growth

TABLE IV
MINIMUM INHIBITORY CONCENTRATION (MIC) OF FLAVONOIDS OF *THYMUS INODORUSON* BACTERIA TESTED

Strains	MIC(%)
E. coli	0.5
S. aureus	0.5
P. fluorescens	1.0

They are inhibited from the minimum concentration of 0.5%. The concentration of 1% is sufficient to stop the growth of P. fluorescens.

Indeed, all the bacterial strains used were inhibited in a concentration varying between 0.5% and 1%.

The results obtained from the study of antimicrobial activity showed that *T. inodorus* flavonoids have different behaviors. Thus, it is noted that thyme flavonoids are effective on all the tested strains. This efficiency is greater towards the Gram-positive bacteria comparing to Gram-negative.

According to Nakatsuet *al.* [17], Gram-positive bacteria are more sensitive to the action of flavonoids than Gram negative bacteria. Chatenet [19] explains this phenomenon in that Gram-negative bacteria are equipped with a peptidoglycan layer wedged between the plasma membrane and an outer base formed of lipopolysaccharide and protein. This latter is not present in Gram-negative bacteria.

IV. CONCLUSION

The extraction of flavonoids recovered from the aerial part of the odourless thyme by evaporation gave an interesting

flavonoids yield which is 7.24% for the aqueous extract, 28.86% for butanol extract, 29.88% for the ethyl acetate extract and 22.9% for the extract di-ethyl.

Thus, the evaluation of the antimicrobial activity of flavonoids tested on six pathogens showed a slight inhibition on *E. coli, S. aureus, and P. fluorscens*. So a weak inhibition is observed in studied yeasts and molds.

In fact, *T. inodrus* flavonoids inhibit the growth of Gram + bacteria at a concentration ranging between 0.5% and 1%. While for Gram- bacteria, the inhibition is limited to a concentration of 0.5%.

Knowing that Algeria has a huge plant biodiversity and each plant is characterized by a fairly large reservoir of secondary metabolites with therapeutic and specific pharmacological characteristics, it is hoped to exploit its resources in the medical and pharmaceutical field.

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