Research Article

Effect of Solvent on the Antibacterial Activity of the Flavonoids in Luzerne

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Accepted on: 02-06-2015; **Finalized on:** 30-06-2015.

ABSTRACT

This work consists in identifying the influence of solvent extraction on microbiological activity of flavonoids isolated from *Medicago sativa,* that the phytochemical study shows its richness in flavonoids. Thus, three extracts obtained by three organic solvents, from the aerial parts of alfalfa harvested in the region of El Tarf (Eastern Algeria), tested on six bacterial strains and a strain of fungus. The phytochemical and microbiological analysis shows that the quantity and the quality of secondary metabolites substances in alfalfa is also variable and dependent on the solvent used. According to the results of the microbiological study and comparison with other studies in the same field, we can say that the butanol extract flavonoids isolated from the aerial part of Medicago sativa has better antibacterial and antifungal activity.

Keywords: *Medicago sativa,* flavonoids, antibacterial activity, antifungal activity, extracting solvent, butanol extract.

INTRODUCTION

Alfalfa (*Medicago sativa*) was the subject of several published works particularly its use in forage and its ability to fix atmospheric nitrogen. It is cultivated as well, especially for agricultural needs. However, its therapeutic applications remain rare or even unknown. The objective of this study is to develop *Medicago sativa* entant that medicinal plant.[1,2] Our interest has focused on the wealth of chemical compounds in this plant species giving it unique properties that allow it to be classified as plants therapeutic effects. Phenolic content of a plant depends on a number of intrinsic and extrinsic factors[3]. The study conducted on this species cover both the quantitative and qualitative aspects of a group of flavonoids extracted from the aerial part of *Medicago sativa* and their microbiological activity. The calculated performance and microbiological activity of various extracts, were used to determine the influence of the extraction solvent to the latter[4,5].

MATERIALS AND METHODS

Vegetal Material

The harvest of the aerial parts of *Medicago sativa* was performed in the month of October in the region of Ben Mehdi wilaya of El Tarf Algeria. Area located between 36° 41 N and 7° 51 E a longitude from 0 to 50 m.

The drug consists of the dried leaves and stems at room temperature in the dark for ten days.

Strains

Sixteen bacterial strains were the subject of a microbiological study including two referenced *: Escherichia coli ATCC 23, Staphylococcus aureus ATCC 12* (ATTC: American Type Culture Collection) *and fourteen strains isolated pathogen products Acinetobacter baumannii, Enterobacter cloacae19, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas, Salmonella sp, Serratia marcescens, Shigella sp, Staphylococcus aureus 240, Staphylococcus hominis 88, Staphylococcus saprophyticus66, Staphylococcus warneri 176 et Candida albicans.*

Phytochemical Screening Flavonoids and Extraction

Phytochemical Screening

Flavonoids, one of the active principles of *Medicago sativa* have been sought according to the methods described by Bruneton[6]. For this, 5 g of the powdered drug are macerated in 150 ml of 1% HCl for 24 h.

After filtering 10 ml of the mixture are basified by the addition of NH_4OH, the presence of flavonoids, expressed after 3 hours by the appearance of a light yellow color in the upper part of the tube.

Extraction

The extraction was performed by the method of Paris[7]: 40 g of drug are macerated in 800 ml 90% ethanol for one hour.

The residue is macerated again hot in 800ml of ethanol in the Sohxlet for 4 hours, after a night of rest, vacuum evaporation of the two ethanol solutions, taken up by 80 ml of boiling water the dry residue.

Extraction with Ether

After filtration, extraction of the aqueous solution obtained above four times with diethyl ether (ethoxyethane) (4x40 ml).

Extraction with Ethyl Acetate

Second extraction of the aqueous solution four times with ethyl acetate (4x40 ml).

Extraction with Butanol

Third extracting the aqueous solution five times with butanol (butan-1-ol or n-butanol) (5x40 ml). We had three excerpts: flavonoids ethereal mixture Phase: FFTPE, flavonoids ethyl acetate phase mixture: FFTPA and flavonoids butanol phase mixture: FFTPB.

The yield is the ratio of the weight of the extract and the weight of the drug treated. Three replicates were performed for each extraction.

Antibacterial Activity Test

Reactivation

The strains were first reactivated by inoculation in a suitable agar medium and from 3 to 5 and similar isolated colonies CLSI (Clinical and Laboratory Standards Institute), bacterial suspensions obtained sterile saline was prepared at a concentration of 10^6-10^8 CFU/ml (Colony-Forming Unit).

Seeding

The cultivation method on Mueller-Hinton medium according Standardization antibiogram nationally used is that of Vincent[8].

Layout of Disks

Disk calibrated and sterile blotting paper are impregnated with the test solutions using a micropipette (10 ul for each disk). The stock solution (SM) is prepared from a milligram of extract and one milliliter of DMSO.

In the first trial was conducted several dilutions of FFTPB extracted with DMSO (1/2, 1/4, 1/8, 1/16).

Reading

Reading is done by the millimeter measurement of the diameter of the inhibition zone around each disc [9-12].

We performed two microbiological tests: in the first we analyze extract flavonoids (FFTPB) twelve bacterial strains and a fungal strain; the second was tested three extracts obtained previously seven bacterial strains and the strain of *C. albicans*.

RESULTS

Phytochemical Screening

Qualitative analyzes of the samples revealed the presence of flavonoids that have been our microbiological study.

Determining Efficiency

The butanol fraction is the richest in flavonoids (FFPB: 1.073%). It was noted that there are significant differences between the flavonoid content of each extraction phase (Table 1).

Study of the Antimicrobial Effect of Extracts

The extracts were tested on a range of bacterial strains and two fungal strain. According to Table 2, remarkable inhibition zones that obtained from the extract on FFTPB K. oxytoca (18.7mm) followed by S. aureus ATCC (16.5mm) and slightly active in K. pneumoniae (11.5mm). The fungal strain C. albicans is sensitive to FFTPB (14.2mm). Most other strains are resistant to our extracts.

Table 1: Content (Wealth) of flavonoids in drugs.

Extracts	Flavonoids yield 40 g DM	%
FFTPE	099 ± 0,078	0.247
FFTPA	0.328 ± 0.267	0.821
FFTPB	0.429 ± 0.370	1.073
TFFT	0.856 ± 0.169	2.14 ± 0,423

***Notes**: TFFT: Total of (FTP + + FTPB FTPA).*

Each value is the average of 3 repetitions.

They exist some solutions that are more active than the stock solution, but in most of the strains, they are inactive. After selection of the strains according to the most important inhibitions areas, a second test was performed on the different extracts studied (we only used the stock solution because of the inhibition zones negligible dilutions for most strains). Our choice was limited to three bacterial strains (We added some Staphylococcus species because of the diameters of the zones of inhibition remarkable about this strain). In the second test, the FFPB extract is most active for all strains with the exception of *S. aureus* ATCC, the largest zone of inhibition is that of *S. warneri* (42.3mm), Among the studied strains, *S. aureus* ATCC is the resistant strain (Table 3). Regarding fungal strain FFPB the extract has the highest zone of inhibition (16.2mm), *S. aureus* ATCC and *K. pneumoniae* are resistant to all samples.

DISCUSSION

May explain the differences between the levels of flavonoids in various phases by the polarity flavonoids vis-à-vis their extraction solvent [13-15]. The use of the drill mixing/sheets causes an increase in flavonoid rate[16] de on can say that our results are in agreement with the results of Bertin[17] et Zanin[18]. First test microbiological activity it was found that the majority of the tested strains are resistant to our extracts. They exist several dilutions more active than the mother solution, can be linked to the dissemination of DMSO in the culture medium. In the second trial and comparison with the study Athamena[19] and Treki[20]., it can be said that in general we flavonoiques extracts have antibacterial activity and very significant antifungal[21,22].

This activity varies among strains tested and according to extraction solvents[16,23]. The largest zone of inhibition is achieved by the flavonoid extract the butanol fraction of the mixture; S. aureus ATCC is the most resistant strain our extracts despite being known as a sensitive strain[24],

the resistance of the strain can be attributed to the ability of the antibacterial agent to uniformly diffuse into the agar[25-27]. It can also be linked to the agar diffusion method d'extraction[28,29].

It can be shown that each flavonoid extract acts illegally bacteria, according to their polarity in the extraction solvent[30-32].

These results indicate the study Triki[20].

This may be related to the high content of glycosylated molecules and the high content of flavonoid compound[33,34].

Cowan[35] and Bolou[36] have also shown that the phyto-molecules are distributed among the solvents according to their polarity and solubility, this confirms the results obtained by Hammoudi[37].

CONCLUSION

Through this work the importance of microbiological activity of flavonoids isolated species was determined *Medicago sativa*. The phytochemical study of this plant demonstrates its richness in flavonoids. Our results have found that there are differences between the chemical composition of secondary metabolites.

According to the results of the microbiological study we can say that the butanol extract flavonoids isolated from the aerial part of alfalfa and Medicago sativa has better antibacterial and antifungal activity so it is best to use as an antibiotic for severe infections such as sepsis and endocarditis or as an anti-fungal infections caused by *C. albicans.*

Acknowledgement: We thank the laboratory staff medical botany, plant biology laboratory and microbiology laboratory team, of the Medical Faculty of Annaba, Algeria, for the information provided and available to us the equipment and products needed to carry out this work.

Table 2: Diameter of inhibition of the first test areas.

Strains	FFTPB				
	(SS)	½	1/4	1/8	1/16
E. coli ATCC 23	9.5	6	6	6	6
S. aureus ATCC 12	16.5	12.2	8.5	11.5	11.1
A. baumannii	9.6	11.5	9.5	6	6
E. cloacae 19	10.9	8.3	7.9	6	6
E. coli	7	8.1	7.2	10.2	6
K. oxytoca	18.7	6	6	6	6
K. pneumoniae	11.5	6	6	6	6
P. mirabilis	7.2	6	6	6	6
Pseudomonas 53	7.9	8.9	8.1	7.5	6
Salmonella	9.5	9.6	7.5	7.2	6.2
S. marcescens	7.7	9.6	9.1	6	6
Shigella	10.2	10.5	9.6	6	6
Candida albican	14.2	9.1	10.1	8.2	7.7

Table 3: Diameter of the second test flavonoid inhibition zones

Strains	Extracts		
	FFTPE	FFTPA	FFTPB
S. aureus ATCC 12	6	6	6
K. oxytoca	10.5	23.9	33.1
K. pneumoniae	11.5	27.7	35.8
S. aureus	33.6	32.5	35.9
S. hominis	26.7	28.2	32.5
S. saprophyticus	18.3	22.5	30.7
S. warneri	21.5	34.7	42.3
C. albicans	10.7	15.5	16.2

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Source of Support: Nil, **Conflict of Interest:** None.