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Research Article

ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM *SALVIA OFFICINALIS* AND *ROSMARINUS OFFICINALIS* OBTAINED BY SONICATION AND MACERATION METHODS

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ABSTRACT

Objective: Our study attempts to compare the phenolic contents and the antibacterial activity of the methanol extracts obtained by sonication and classical maceration from *Rosmarinus officinalis* and *Salvia officinalis.*

Methods: The extracts were obtained from two plants belonging to *Lamiaceae family; S. officinalis* and *R. officinalis*. These plants were collected at February 2011 in the National Institute of Medicinal and Aromatic Plants (Morocco). The total phenolic content was evaluated using Folin Ciocalteu reagent. Furthermore, the antibacterial effect of the extract was tested against eight bacterial strains using the disk-diffusion method.

Results: Our results show that sonication was more effective for the extraction of phenolic compounds than the classical maceration. Moreover, all extracts showed antibacterial activity against Gram-positive *bacteria* tested (*Bacillus subtilis* and *Staphylococcus aureus*). Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were found to be resistant.

Conclusion: The greatest antibacterial activity was exhibited by the extracts obtained using sonication method.

Keywords: Sonication, Maceration, Phenolic compounds, Antibacterial activity, Methanol extracts.

INTRODUCTION

Salvia officinalis L. (common sage) and *Rosmarinus officinalis* L. (common rosemary) two important medicinal and aromatic plants from *Lamiaceae* family. Traditionally leafs infusion of *Salvia officinalis* is used by the Moroccan population against chill, rheumatism and cough [1]. Also, leaves decoction of *Rosmarinus officinalis*are used as remedies for intestinal parasites, rheumatism and kidney. Rosemary (*Rosmarinus officinalis*) from plants that have been extensively studied for their biological activities; antioxidant[2,3], antimicrobial [4–6], anti-inflammatory [7], antidepressant[8], anticancer [9], antiulcerogenic [10],antinociceptive [11], Hepatoprotective [12], antidiabetic [13] and spasmolytic [14].

The plant secondary metabolites spans an extremely large and diverse range of chemical compounds derived from plants. Several methods are suggested for more or less selectively extracting specific classes of these compounds. For most applications, relatively simple techniques, such as percolation and maceration are used. However, some specific applications require more sophisticated and costly extraction techniques using specialized equipment such as supercritical-fluid extraction[15].

Nowadays, a lot of causes associated with resistance and toxicity of microorganisms are present several problems to the Scientifics: The nosocomial infections, the biofilms [16] and the emergence of multidrug-resistant microorganisms which become a major global healthcare problem[17].The situation is particularly complicated in hospitals, with resistant *Staphylococcus aureus*, *Streptococcus pneumoniae* and the *Enterococcus* strains [18]. Moreover, food is a rich environment for most bacteria. Food-borne illnesses caused by consumption of food contaminated with pathogenic bacteria and/or their toxins are a great problem in public health [19].

Among the solutions taken was the exploitation of natural resources, as plant natural products like flavonoids, tannins, coumarins, alkaloids, terpenoids, lectins and polypeptides, which present an important antimicrobial activity [20]. This multiplicity of metabolites can inhibit the multi-resistance of bacteria which was processed only by a single molecular entity [19]. Therefore, they could have important implications for the development and implementation of therapeutic antimicrobial strategies and have a potential to be used in the food industry as a preservative. The main goal of our work is to evaluate *in vitro* the antibacterial activity of methanol extracts from *Salvia officinalis* and *Rosmarinus officinalis* cultivated in the Garden of National Institute of Medicinal and Aromatic Plants- Taounate (Morocco). Moreover, we determine the most effective extraction method maceration or sonication, while comparing the total phenolic contents and the antibacterial activity of crude extracts obtained.

MATERIAL AND METHODS

Plants material

The aerial parts of *Salvia officinalis* and *Rosmarinus officinalis* were collected from the garden of the National Institute of Medicinal and aromatic Plants-Taounate (NIMAP) (Morocco) on 20 February 2011. Plants were identified and deposited in the herbarium of the institute.

Preparation of Extracts

Maceration

One hundred gram of dried and crushed aerial part of each plant was macerated in first time using 300 ml of hexane during 2 hours at room temperature. Extracts were filtered using Whatman paper n°1. Second maceration was performed with 300 ml of methanol during 10 hours. After filtration each mixture was evaporated under vacuum to obtain crude extracts. Two repetitions were performed.

Ultrasonic Extraction

Extraction was carried out in an ultrasonic bath (Elma–Transsonic TI-H-15) (Power: 100 W, Temperature: 30°C). Flasks containing 50 g of air-dried and crushed plant material and 200 ml of methanol were immersed in the ultrasonic bath. Sonication was performed with ultrasound frequency 35 KHz for 30 min (Two repetitions were performed). After filtration each mixture was evaporated under vacuum to obtain crude extracts.

Phytochemical Screening for Flavonoids

This qualitative test was carried out according Shinoda reaction (cyanidin reaction)[21]. 1 ml of the methanol extract (crude extract dissolved in 1ml of methanol) was mixed with 1 ml of hydrochloric alcohol (1:1:1 HCl-Ethanol-Distilled water) and approximately 0.5 g of Mg metal. After 3 min of reaction the change of coloration was watched. The presence of flavonoids was confirmed by the pink-orange coloration (flavones), pink or purplish red (flavonones) or red (flavonols and flavononols).

Total Phenolic Content

Folin-Ciocalteu reagent was used for the quantification of total phenolic content described by [22], with some modifications as follows: 4 mg of methanol extract were added to 4 ml of distilled water, 20 µl of this mixture was then pipetted and added into a tube containing 1.58 ml of distilled water and 100 µl of Folin-Ciocalteu reagent. After 5 min, 300 µl of $NaCO_3$ (25%) were added and the reaction mixture was allowed to stand for 2 hours at room temperature, then the optical density at 765 nm was measured. Gallic acid was used as a standard, the results are given as mg gallic acid equivalent per gram of methanol extract. Two repetitions were performed.

Bacterial Strains

The methanol extracts were tested for antibacterial activity against the following bacterial strains: *Staphylococcus aureus* ATCC 25922, *Staphylococcus aureus* CIP 543154, *Bacillus subtilis* ILP 1428B, *Bacillus subtilis* ILB142B, *Escherichia coli* 0128B12, *Escherichia coli* CIP 5412, *Pseudomonas aeruginosa* A22, and *Pseudomonas aeruginosa* ATCC 27853.

Antibacterial Assay

The antibacterial activity of the methanol extracts was examined by disk-diffusion method [23] with some modifications. Briefly, bacterial strains were cultured overnight at 37 °C on Luria-Bertani broth, then inoculum consisting of 0.5 McFarland was prepared in physiologic saline.

Bacterial inoculum (100 µl) was inoculated in Petri dishes containing a sterile Luria-Bertani Agar medium. Sterile filter paper discs (5 mm diameter) were deposited on medium and impregnated with 10 µl of extract solution (500 mg/ml of DMSO to 2%). The control was performed with discs containing 10 µl of DMSO to 2%. Each experiment was performed in duplicate

RESULTS

Yield of Methanol Extracts

The methanol extract yield which corresponds to the maceration extraction was slightly higher than that of sonication extraction for both plants (Table 1). Common rosemary gave a maceration extract with a higher yield than common sage. However, the extract of sage was obtained by sonication with a higher yield compared with rosemary.

Table 1: Yield of methanol extracts of *S. officinalis* and *R. officinalis*

Plant	Extraction method	Yield % (w/w)
S. officinalis	Maceration	13.88 ± 1.24
	Sonication	11.78 ± 0.42
R. officinalis	Maceration	15.8± 0.56
	Sonication	8.7± 0.70

Presence of flavonoids

The results of phytochemical screening for flavonoids compounds are represented in Table 2. The extracts obtained from *R. officinalis* and *S. officinalis* by sonication contain flavonoids compounds, particularly the flavones. Of same the sage extract obtained by classical maceration also contains flavones compounds. While, extract obtained by maceration from rosemary was negative for this test.

Table 2: Revelation of flavonoids of *S. officinalis* and *R. officinalis* extracts

Plant	Extraction method	Presence of flavonoides	Type of flavonoides
S. officinalis	Maceration	+	Flavones
	Sonication	+	Flavones
R. officinalis	Maceration	-	-
	Sonication	+	Flavones

Table 3: Results of total phenolic contents of *R. officinalis* and *S. officinalis*

Plant	Extraction method	Concentration of total phenolic compounds (mg gallic acid equivalent/g of extract)
S. officinalis	Maceration	116.5±9.19
	Sonication	195.1±11.31
R. officinalis	Maceration	181.4±9,89
	Sonication	193.6±8,48

Table 4 : The growth-inhibitory diameters (mm) of methanol extracts against the tested bacteria

Bacterial strains	*S. officinalis*		*R. officinalis*	
	Maceration	Sonication	Maceration	Sonication
S. aureus ATCC 25922	10 ± 0.7	13 ± 1,4	11,25 ± 0.35	13,5 ± 0,7
S. aureus CIP 543154	12 ± 0.35	15 ± 0.7	11 ± 0.35	13 ± 0.35
B. subtilis ILP1428B	12 ± 0.7	15 ± 0.35	11 ± 0.35	12 ± 0.7
B. subtilis ILB142B	8 ± 0.7	11 ± 0.7	10 ± 0.7	12 ± 0.35
E. coli 0128B12	-	-	-	-
E. coli CIP 5412	-	-	-	-
P. aeruginosa A22	-	-	-	-
P. aeruginosa ATCC 27853	-	-	-	-

Total phenolic content

As shown in Table 3. The total phenolic contents of the methanol extracts of rosemary and sage varied from 116± 9.19 and 195.1 ± 11.31 (mg gallic acid eq/g of extract). Methanol was used in this research because these phenolic compounds and most other reported bioactive compounds are generally more soluble in polar solvents[24]. Therefore, the methanol extracts obtained from both plants by sonication have the highest phenolic content than those obtained by classical maceration.

Antibacterial Activity

The results of the antibacterial activity were presented in Table 4. The negative control used (DMSO 2%) did not exert any inhibition on the strains tested. In the present study, rosemary and sage exhibit remarkable antibacterial activity against the Gram positive strains (*Bacillus subtilis and Staphylococcus aureus*). However, gram negative strains tested (*Escherichia coli and Pseudomonas aeruginosa)* were resistant to all extracts. But when we compare the activity of methanol extracts obtained from each plant we see clearly that the extract obtained by sonication has a greater antibacterial activity than that obtained by classical macerati

DISCUSSION

In our study, the maceration shows a stronger yield than sonication extraction. However, many studies have shown that the extraction by ultrasound has several economic advantages; shorter extraction time, reduction in solvent usage, the possibility of extraction of many samples at once in an ultrasonic bath and the extraction is carried out at room temperature, which makes it suitable for the extraction of thermally labile compounds. Therefore, the use of ultrasonic means for extraction purposes in high-cost raw materials is an economical alternative to traditional extraction processes, this being a demand by industry for a sustainable development [25–27].

Extracts obtained from both plants with sonication show the presence of flavonoids compounds. The previously published data indicate that *Salvia officinalis* contains: luteolin 7-O-beta-D-glucoside, luteolin 7-O-beta-D-glucuronide, luteolin 3'-O-beta-D-glucuronide, 6-hydroxyluteolin 7-O-beta-D-glucoside and 6-hydroxyluteolin 7-O-glucuronide [28]. While the rosemary contains: Luteolin 3'-O-beta-D-glucuronide, Luteolin 3'-O-(4"-O-acetyl) -beta-D-glucuronide, Luteolin 3'-O-(3"-O-acetyl) -beta-D-glucuronide [29]. Several works indicate that Luteolin has been identified in many edible plants, and is known by its antimicrobial, anti-inflammatory, antioxidant, cancer chemopreventive and chemotherapeutic and other biological activities [30]. Methanol extracts obtained from both plants by sonication have the highest phenolic content than those obtained by classical maceration. This allowed us to conclude that sonication is an effective method for the extraction of phenolic compounds from plant material. Our results are in agreement with other studies which found that ultrasound assisted extraction has a great potential as a method for the extraction of antioxidant compounds [25,27,31].

Studies that have focused on the determination of phenolic compounds from rosemary and sage showed that carnosic acid, carnosol and rosmarinic acid are the phenolic compounds responsible for antioxidant activity of these two plants [32–34]. For sage more phenolic compounds have been isolated such as; salvianolic acid, sagecoumarin, sagerinic acid, apigenin 6,8-di-C-glucoside and other flavonoids [28]. The phenolic compounds that have been found in rosemary are; augustic acid, benthamic acid, pterogynoside, kaempferide, primiverin, 7- Ethoxyrosmarol, epiisorosmanol ethyl ether, luteolin7- glucuronide, hispidulin, diosmetin, cirsimaritin and ladanein [35]. The antibacterial activity of the methanol extracts from sage and rosemary obtained by sonication was found important. This activity of both plants against bacterial strains can be attributed to the presence of phenolic compounds, simple phenols, phenolic acid, flavonoids and other phytochemical compounds which may be present in these extracts[20]. In conclusion, methanol extracts of *S. officinalis* and *R. officinalis* have great potential as antimicrobial compounds against *Staphylococcus aureus* and *Bacillus subtilis*. Thus, they can be used to fight resistant microbes in the pharmaceutical, food and veterinary. Moreover, our work shows likewise the effectiveness of sonication as a method of extraction of bioactive compounds from plant material.

Conflict of Interest statement

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript

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