American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Phytochemical Components and Antimicrobial Efficacy of Rosemary Plant (Rosmarinus Officinalis) Extract

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Received: 2024, 15, Oct
Accepted: 2024, 21, Oct
Published: 2024, 26, Nov

Annotation: Rosemary is a well-known restorative plant for its aromatic characteristics. The confirmed diagnosis of R. officinali was made in the laboratory of the Faculty of Pharmacy/University of Babylon. Numerous extracts and chemical compounds were achieved as well the antimicrobial impacts of R. officinali were exanimated against 3 bacterial strains: Escherishia coli (E. coli), Pseudomonas aeruginosa (P. aeruginosa) Staphylococcus aureus (S. aureus), and one type of fungi: Candida albicans (C. albicans) applying agar well diffusion method and con. ranging from 500 to 3000 mg/mL. The outcomes of the chemically analysis of R. officinali leaves extracts demonstrated that the aqueous extract contained phenols, Flavonoids, Terpeniods, while be lacking Resins, Tanins and glycosids. The Alcohol one contained phenols, Flavonoids, Terpeniods, and Resins, while be lacking Tanins and glycosids. All exanimated isolates showed sensitivity to both extracts of R. officinali at a con. of 3000 mg/ml, where this con. of both extracts displayed increased inhibition zone diameters in S. aureus, C. albicans and E. coli respectively, as compared with P.

aeruginosa. The ultimate effectual inhibition for R. officinali aqueous extract was recorded against E. coli, while the least inhibition zone had found in P. aeruginosa. As regards R. officinali alcoholic extracts, the maximum inhibition zone had found in S. aureus, while the least impact was observed on P. aeruginosa. Conclusion: Current results imply that rosemary extracts may be used as naturally antibacterial and antifungal agent in medicinal setting.

Keywords: R. officinali, Rosemary, plant extracts, phenols, Flavonoids, antibacterial, antifungal.

Introduction

Rosmarinus officinalis (*R. officinalis*) plant is an evergreen herbaceous plant that has many names, including rosemary, dairy gravel, acacia, sea dew, poplar, and rosemary. It is a member of the Lamiaceae family; its scientific name is *Salvia rosmarinus*. Rosemary reaches a height of one to two meters and has a smell resembling camphor. Its stem is branched, its leaves are opposite, with a smooth surface. Nomi is green and its surface is under fine black hairs. The flowers are multi-colored, including dark blue, white and purple with fanudi [1]

Mediterranean basin is the home of plant, as it growing in Algeria, France, Spain, and Portugal [2]. As well it is planted in India, Central Asia, America, Southeast Africa, US, Brazil, and in a number of states of the world. It can be grown in fields, farms and home gardens [3]. All aerial parts of the plant (leaves and floral branches) can be used in extracting the plant [4].

The rosemary plant has many medicinal benefits, as it was second-handed as antispasmodics in cases of renal gripes and dysmenorrhea, alleviating respiratory diseases, and boosting hair health. Moreover, the active substances found in rosemary have the therapeutics potency to cure or prohibit bronchial asthma, hyperglycemia, and peptic ulceration. Rosemary contains: Antioxidant substances that have been used as a preservative for meat from rotting, as it was added to it to prevent it from rotting [5][6]. Rosemary is added to the herbal anti-headache mixture. Rosemary is famed for its anti-bacterial and anti-inflammatory characteristics, in addition to its antioxidant properties. Chemical compounds of Rosemary contain The active compounds are caffeic acid, phenols, diterpenes, triterpenes, flavonoids and volatile oil [7]. Also its' leaves contain volatile oil at a rate ranging from (0.44-0.73%). This oil includes many components, including champhor, barneol, 1,8-cineole) and others. The contents of it's leaves include 2-3 % of phenolics acids, like caffeic acid, chlogenic acid, labial acid, neochlorogenic acid, and rosmarinic acid [8]. This plant has anti-toxin, anti-bacterial characteristics, antioxidants, anti-diabetic as well as anti-mutagenic properties [9]. Herbs are usually rich in certain coumpound with antioxidants properties, like vitamins E and C, glutathione, enzymes and phenolics acids. A number of spice extracts had exhibited their importance to avert the autoxidation of unsaturate triacylglycerol, in particular, the naturalist extract from the Lamiaceae family ex. *R. officinali* was known in various reports for their

anti-oxidative efficacy [10].

Aim of the study

Due to the continual global growing in antimicrobial resistance, the present study aimed to analysis organic components of Rosemary plant extracts and its effects on some types of Gr+ve, Gr-ve bacteria and one type of fungi to provide natural plants based anti-pathogenic agent

Materials and Methods

Plant materials collect: we were brought plant from one of gardins in Hilla city and cutting leaves with some branches then dried it at room temperature with continuous ventilation, this was done over a period of 3 days Dry leaves of rosmarine, grinding them well with home grinding. The plant substance sample was grinded by the electric grinder into a fine powder that had kept in a tightly closed glass container and then kept in the drug laboratory at the College of Pharmacy until it was used to make the plant extract.

Preparation of plant extract

Aqueous extract

A conical flask containing (25g) of plants powder that dried in the air was used, added 500 ml of boiling distilled water in the flask 1000 ml. in size and the mixture was boiled for 2hours, then shake a solution for 30 minute, then put in the test tube in the centrifuged at 3000rpm for 10 min. Top was taken and left the sediment that collect and dried by putting it in the oven (45^0c) until it was wholly dry. The dry extract was preserved at (4°C) in order to be used later [11][12].

Alcoholic extract

The method of alcoholic extraction was adopted according to the method adopted by in preparing plant extracts, where the weight of 25 gm of the powder for each sample of the plants under study, each separately, was added to it 500 ml of ethanol, with a concentration of 98% in the flask 1000 ml. in size, Mix well and leave for 24 hrs. at 25°C, then filter extract with a funnel using a filter paper (What man No.1). Rotary vacuum evaporator of 40°C has been employed to concentrate the formed filtrate, and left it at room temperature to completely get rid of the solvent, after which the material was skimmed off and placed in opaque sealed bottles and preserved in a refrigerator at 4°C up to use [12].

Phytochemical examination

Revelation of Phenols

The mixture was prepared of 1% aqueous iron chloride along with 1% of potassium iron cyanide, then the same amount of rosemary extract has been added, where the greenish-blue color represent an indicative for phenols content [13].

Revelation of Terpenoids

About 2ml of plant extract had taken and dissolve in 2ml of chloroform and left to evaporate for drying up. Then 2ml of concentrated sulfuric acid has been appended and heating it for approximately (2 mins). A grayish coloration represents an indicative for terpenoid content [14].

Revelation of flavonoids

About 5 ml of ethyl alcohol (95%) has been appended to 1 ml of rosemary extract in a test tube and put down in water bath for thirty mins. Then some drops of NaOH have been added, and manifestation of dark color is indicative for existence of flavonoid [13].

Revelation of resin

About 1ml of plant extracts were appended to acetic anhydride solution and 1ml of concentrated H_2SO_4. Appearance of orange to yellow, this indicates the presence of resin [15].

Revelation of tannins

A 1% aqueous solution of ferric chloride was prepared, then an equal amount of rosemary extract was added. The appearance of a bluish-green color indicates that the extract contains tannins [16]

Revelation of Glycosids

About 2mg of rosemary extracts were dissolved in 2ml methanol, then 10% Sodium hydroxide has been added drop by drop, if a yellow color has appeared and then disappeared when acidified the solution, the test was deem to be positive [17].

Antibacterial activity assay

R. officinalis water and alcoholic extracts was tested individually on brain heart infusion Agar (BHIA) medium applying well-agar diffusion technique. The study was carried out utilizing 18-20 hrs. microbial cultures of *S. aureus*, *E.coli*, *P. aeruginosa*, as well as *C. albicans* standard strains.

Agar well diffusion technique

In the present study both of water and alcoholic extracts of *R. officinalis* had tested at different con. include: 500, 1000, 2000 and 3000 mg/ml; nutrient broth has been inoculated by loop full growth of each microbial isolates, then incubating at 37°C for nearly 18 hrs. Bacterial suspensions have been dilute in normal saline egalitarian to McFarland number 0.5, to get a uniformed suspensions with cells density of 1.5*108 CFU/ml have utilized [18]. Petri dishes had prepared with BHIA, bacterial isolates have cultured on the media by streaking, after this 5 wells with 6mm diameters were formed in the medium using sterile borer. These wells have been filled with 100 ml of all cons. for both extract employing a sterile micropipette; as for center well, it had the control.

Then, these plates had incubated 24 hrs. in 37ºC. 5mg of Amikacin has used as positive control to detect antibacterial activity, whilst 25 mg Neostatin has deem as positive control to detect *C. albicans* activity. Distill water was deem as a negative control. The antimicrobial efficacy has evaluated by measurement the inhibition zones diameters around the wells, it expressed by mm. The evaluation has replicated 3 times and taken the mean of diameters [18].

Results and discussion

Chemically examination of *R. officinali*

The outcomes of the chemically analysis of *R. officinali* leaves extracts demonstrated that water extract content include: phenols, Flavonoid, Terpeniods, while be lacking Resins, Tanins and Glycosids. The Ethanolic one contained phenols, Flavonoids, Terpeniods, and Resins, while be lacking Tanins and Glycosids, as displayed in Table (1).

Table: (1) Chemical content for *R. officinali* Extracts:

Alcoholic extract	Result	Aqueous extract	The Result
phenols	+	phenols	+
Flavonoids	+	Flavonoids	+
Terpeniods	+	Terpeniods	+
Resins	+	Resin	-
Tanins	-	Tanins	-
Glycosids	-	Glycosids	-

The interest in using herbal plants as an antimicrobial and antioxidant has widely increased for their advantageous impacts. These pursuits are particularly important as they contribute to herbal plants (including rosemary) potential roles in boosting health and forbidding diseases.

Present finding agrees with the study of [19][20], which confirmed the phenolic content of rosemary. The phenolic profile of this plant is characterized by prime components that include carnosic acid, carnosol, rosmarinic acid and hesperidin [21]. The outcomes of our investigation are also consistent

with a previous investigation that demonstrated that the most crucial components of this plant are phenolics, flavonoids, and diterpenoids [22]. The presence of resins in the alcoholic extract as opposed to the aqueous extract may be belong to inflated solubility of complex biological substances in organic solvents versus to water base [23]

Antimicrobial activity

The antimicrobial impacts of *R. officinali* extracts were evaluated by recording zones of inhibition, as shown in Table (2). All exanimated isolates showed sensitivity to both extracts of *R. officinali* at a con. of 3000 mg/ml, where this con. of both extracts displayed increased inhibition zone diameters in *S. aureus*, *C. albicans* and *E. coli* respectively, as compared with *P. aeruginosa*. The ultimate effectual inhibition for *R. officinali* aqueous extract was recorded against *E. coli*, while the least inhibition zone had found in *P. aeruginosa*. As regards *R. officinali* alcoholic extracts, the maximum inhibition zone had found in *S. aureus*, while the least impact was observed on *P. aeruginosa*. In general alcoholic extracts of *R. officinali* was more effective than the aqueous extract against both bacterial and fungi growth.

The prime steps to control any pathogen involve recognition of pathogen as well as pick out the effective antimicrobial element with the optimal concentration. In addition to R. officinalis extracts significance as antibacterial agents [24], it had also a wide extent of useful health influences, it being as antidepressants [25], antihypertensives [26], hypocholesterolemic [27], hepatoprotective and anti-obesity [28]. *R. officinalis* extracts had deliberated as potential therapeutics factors versus numerous diseases [29]. A number of investigations revealed that the extracts of this plant displays hepato-protective, anti-diabetic, antifungal, as well anti-ulcerogenic impacts [30]. Furthermore, R. officinalis is proven to contain antioxidants and antimicrobial efficacies [31][32]

Table (2): Antimicrobials activity of *R. officinali* Alcohol and Aqueous extracts:

Microorganisms	Inhibition zone diameter (mm)								Neostatin 100ml	Amicasin 5mg
	Alcohol extract (mg/ml)				Aqueous extract (mg/ml)					
	500	1000	2000	3000	500	1000	2000	3000		
p. aeruginosa -	0	0	0	12	0	0	0	9	-	24
S. aureus +	4	5	13	17	0	4	7	11	-	-
E.coli -	0	9	11	13	0	5	9	12	-	12
C.albicanis	0	0	6	15	0	0	5	11	11	-

Previous studies came to the same results, Sienkiewicz et al. revealed that *R. officinalis* extract has an inhibition of *E. coli* growth [33]; de Oliveira et al., as well as Manilal et al. assured the activity of rosemary toward *S. aureus* [29][34]. The findings of the current work are consistent with a recent investigation [35][36], which also indicated that higher con. of this plant extract could suppress the same bacterial strains: *E. coli*, *S. aureus*, *P. Aeruginosa*. Present study also demonstrated that alcoholic extract of rosemary was more effectual than the aqueous one in inhibiting both Gr+ve and Gr-ve bacteria along with *C.albicanis*, and from this finding could conclude that type of utilized solvent along with extraction technique were fundamental consideration to determine active components composition and the effectiveness of both extracts; and this statement was in the same line with the previous investigations [37] [36], which stated that alcoholic extract showed greatest efficacy against Gr+ve as well Gr-ve bacteria. Extracts efficacy may shift after separation and purification, where the phenolic substance in the first class differing. Besides, the extract's escalated efficacy may exert an influence on cell membrane permeability and action of the bacterial cells [36].

Furthermore, the current investigation showed the Gr+ve bacteria like *S. aureus* exhibited more sensitivity than Gr-ve bacteria (*E. coli* and *P. aeruginosa)*, and this result is consistent with those who reported that Gram-positive bacteria are great affected than other bacterial isolates, they suggested that differences in antimicrobial activities may be as a result of the modifications in the cell wall configuration of bacteria [38].

In contrast, the outcomes of current investigation disagree with previous studies that proved that rosemary extract is ineffective against *P. aeruginosa* [39][40]. These variations may be belonging to seasonal variations, plant condition, as well the extraction technique that influence the content of plant of active substance and thus refracted on the performance of extracts against microbes.

The effectiveness shown by the aqueous and alcoholic extracts against Gr+ve and Gr-ve bacteria may be due to flavonoid content of this plant, which is known to have an effective effect against these bacteria [35].

The potency of alcoholic extract to suppress the bacterial growth is belong to high solubility's of active substance in organics solvent [41], in addition to the high alcoholic contented of the extract. Soluble efficiency that had ability for inhibiting bacteria via its aptness to penetrate the cell walls or its impact on considerable pivotal parts of bacterial cells like the cytoplasm, ribosome, and nucleic acids by forming hydrogen bonds with proteins, so leading to destroy of protein structures of bacterial cells and suppress the bacterial growth; As for the destruction of Gr-ve bacteria, it is because their lack of peptidoglycan layer [42]. The effect water extract of *R. officinali* has revealed a high inhibition rate because it has many (OH) groups that serves as a H donor [43].

Nieto et al. [20] stated that the antimicrobial impacts of *R. officinali* extracts might be due to its containment of caffeic acid derivate (a phenolic compound constituent), which react with metal ions and form chelates, which consequently react with peroxides and inhibit them.

With respect to *C. albicans*, our finding is in partial consent with Sepehri *et al*. [44], who indicated that alcoholic extract of Rosemary displayed higher efficacy toward *C. albicans*, while the water extract displayed no major efficacy toward *C. albicans*. Antifungal impact of rosemary had evaluated against *Candida spp*. including *C. albicans*, where it was found to be as effectual as nystatin [45].

Previous investigations have demonstrated that the rosemary had the ability to inhibit the clinical strain of *C. albicans*, by influencing germ tubes production [46][47]. Furthermore, the antibiofilm impact of this plant versus *C. albicans* has been proven; the product of *R. officinalis* was integrated with cellulose acetate nanofibers as soon as it was a good carrier with lack of biological activity. This merging lead to a uniform structure, with a high con. of essential oils being more efficacious toward *C. albicans* [48].

Conclusion

According to current investigation, it can be concluded that *R. officinalis* extracts have inhibitory impacts on various isolates of bacteria (*E. coli, P. aeruginosa, S. aureus*) along with one fungus species (*C. albicans*). It can be introduced into the pharmaceutic industry to produce novel synthetic medications for treating infectious diseases. Current results imply that rosemary extracts may be used as naturally antibacterial and antifungal agent in medicinal setting.

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