ejpmr, 2025, 12(1), 471-477

SJIF Impact Factor 7.065

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EFFECT OF AQUEOUS, ALCOHOLIC AND ACIDIC EXTRACT OF ROSEMARY LEAVES ROSMARINUS OFFICINALIS IN INHIBITING THE EFFECT OF FREE RADICALS MANUFACTURED AND INHIBITORY EFFECT IN SOME MICROORGANISMS AND DETECTION OF SOME ACTIVE COMPOUNDS

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Article Received on 28/11/2024 **Article Revised on 18/12/2024** **Article Accepted on 08/01/2025**

ABSTRACT

The current study was conducted at the College of Science, Diyala University, in the Microbiology Laboratory. Ecoli Klebsilla and Proteus, psudomanase. The study showed that the type of solvent extracted has an effect in determining the amount of antioxidant effectiveness whether the solvent is hot water, cold water, alcohol or acid. Acid but with a concentration of 5% where they had an effect inhibition of free radicals and the acidic extract added to it 5% hydrochloric acid effect of 72% in the inhibition of free radicals manufactured DPPH. The results showed that the type and concentration of the extract used in the study had an effect on the inhibition of the bacteria. Cold water extract has the highest inhibition of Psudomonas. The results of the qualitative detection of chemical compounds showed that the plant contains a large group of active compounds, including claycosides, tannins, flavonoids, turbines, resins and soaps, which are important therapeutic materials.

KEYWORD: Extracted of plant, antimicrobial, antioxidant.

INTRODUCTION

Humans are severely affected by exposure to chemicals, including Nitrate Sodium, which causes many functional disorders, including Methemoglobinia due to lack of oxygen. Methemoglobin occurs when oxidizing agents are associated with hemoclobin (Bruce, 2013). When the nitrate concentration in the body increases, it becomes toxic and causes damage to the cells of the body. Nitrates are converted by gut bacteria into nitrite, which is associated with hemoclobin to convert to methemoglobin, which separates iron associated with hemoclobin. Nitrates cause smooth muscle relaxation as well as fatal poisoning in children due to nitrate ingestion with water (Koppenol et al., 1992. (Nitrates generate free radicals that cause damage to the cells of the body. These include nitric oxide (NO), which inhibits the generation of steroid hormones in the ovary cells (1997, al et Syira). Wild plants are one of the most commonly used plants in the medical field. Basil and peppermint are blossoms of the oral family that possess thyme and multiple medicinal benefits (1991, Potter). And Steinmetz) Rosmarinus officinalis: It is an aromatic medicinal plant, an evergreen herbaceous plant of the oral family. Its flowers are small, indigo or blue in color, smelling an aberrant favorite since ancient times (Abraham and his group, 2009). As a result, the corona of giving electrons to free radicals and then becoming more so in the mountain gives protection to biological molecules such as proteins, sugars, fatty acids, amino acids and DNA as it has a high ability to remove various types of oxygen and nitrogen effective and many the roots Saber and Hawazen, 2012) Free radicals in the body consistently and permanently as long as life lasts. They can be generated from various enzymatic and non-enzymatic reactions in different tissues of the body as accidental products or chemical compounds that perform various physiological functions such as information transfer or a means of communication. There is a natural balance between the production of these roots and the production of antioxidants and antioxidants that are present in food, some of which are formed inside the body, in the case of increasing free radicals and loss of balance will cause these damage to the cells and tissues of the body. 2005, Colli Natural herbs such as rosemary and other plant species contain antioxidants as rosemary is widely used as food additive, because it has protective effects on the body and this antioxidant activity comes from the fact that it contains a large amount of phenolic compounds. Compounds Phenolic, Flavonoids and Natural Acids (Foster and Leung, 1996).

The present study aims to know the effect of rosemary plant extracts in the inhibition of free radicals manufactured DPPH and the qualitative assessment of

the active compounds by reagents in the plant because of its great therapeutic importance and knowledge of the antifungal activity of the plant extract on four types of bacteria, namely, E. coli, proteins, klebsella and sidomones because of its effects On human health in addition to its impact on food and the damage caused by them and perhaps find a contribution to find alternatives to antibiotics, which has become very widespread use, which leads to the emergence of strains of bacteria resistant to them.

MATERIALS AND METHODS

1- Plant samples

The samples were collected from the inside of the gardens where the leaves were taken and transferred to the laboratory and washed with sterile distilled water and then dried on the temperature of the laboratory after that the leaves were grinded by an electric mill until it was dissolved into powder (powder) and then kept in opaque cans and these were wrapped The cans are made by aluminum foil to avoid the oxidation process and then placed in the refrigerator until used.

2- Prohibition of plant extracts

Plant extracts were prepared with five different solvents: cold water, hot water, 96% ethanol alcohol, distilled water added to HCL acid by 1% and distilled water added to Hcl acid by 5%.

Cold distilled water 100mg of leaf powder was added and 10 ml of distilled water was added to room temperature. This amount was used for each experiment to determine phenolic content, antioxidant activity and inhibition of microorganisms, thus obtaining 10 mg per 1 ml.

2-2- boiling distilled water
100 mg of leaf powder was taken and 10 ml of boiling distilled water was added.

2.3- Ethanol Alcohol Concentrate 96% Diluted 50%
Ethyl alcohol was diluted with a concentration of 96%. 50 ml of alcohol was added with 50 ml of distilled water. The alcohol was diluted to 50%, then 10 ml of diluted alcohol was added to 100 mg of powder, where 10 mg was obtained per 1 ml.

2-4- Acid extract (distilled water added with 1% HCL acid) where 1 ml of acid was added to 99 ml of distilled water and thus the acid concentration became 1% after that 10 ml of diluted acid was taken and added to 100 mg Of the powder.

2.5- Acid extract (distilled water with HCL acid at 1% concentration)1 ml of acid was added to 99 ml of distilled water and the acid concentration became 1%. After that, 10 ml of diluted acid was taken and added to 100 mg of powder.

3- Microbiology used

4 bacteria were obtained from the central laboratory in Diyala province - Baquba. The species are E.coli, Protue, Klebsella and Psudomonas.

4- Activation of pure farms for microorganisms

The bacterial isolates were activated for all four species before the inhibition of the plant extracts were tested for 24 hours before the test was placed in 35 degrees Celsius using Nutrient Broth medium for E.coli, Protuse, Klebsella and Psudomonas.

5- Test the inhibitory effectiveness of plant extracts on bacteria

The Micro Titer Plate calibration method was used as follows

The development of the four bacterial species under study on the center of Muller Hinton Brosh for 24 hours and then pulled by a pipette Micro pipette 150بت l of bacterial suspension for each isolation of isolates and transferred to the drilling of the plate consisting of 96 holes where each isolation was made from 3 isolates Then the plant extracts were added and thus became each bacterium 15 holes and then add the center of Nutrient broth to 4 pits without adding bacteria to him and considered this is negative control and add to 4 other pits bacteria alone without adding the extract to it is a positive control and I thought in Hathina for 24 hours degree 37 Centigrade, after fortune emptied drilling The components were gently washed about 2-3 times. The cells were adhered to the walls of each hole with 200من l of methyl alcohol for 10 minutes and then dyeed with a diluted dye of Crystal Violet at a concentration of 0.5% for each digging. It took 15 minutes after the dye was washed with water. Distilled about 2-3 times and then add 95% ethyl alcohol and 200بت l per hole for 10 minutes to remove the dye attached to the cells (Tang et al., 2011) and then absorbed the absorption of all drilling by the ELISA device at a wavelength of 630 nm Tang et al., 2011) where the amount of absorbance per drill was compared Cultivated with the amount of absorbance to drill control.

As follows

$$OD = A\text{-}B / A * 100$$

Where A represents the absorption value of the control group only on the bacteria without adding the extract.
B / represents the absorption value of the plant-based mixture of bacteria with plant extracts.

6- Chemical detection of some active substances in the leaves of the plant

Myrtus communis L.
6-1- Detection of GlycosidesOne ml of plant extract was mixed for each of the four extracts and 2 ml of Benedict reagent was added and then transferred to a boiling water bath for (5) minutes and inferred the positive examination (the presence of kalekosides) through the

appearance of red color (Harborn, 1973).

Detection of Tannins

The method in Shihata (1951 and Shami, 1982) was used to detect tannins as follows:

Take (50) ml of each of the four extracts and after filtering extracts extracted into two sections was added to the first section (1%) lead acetate lead acetate to infer the presence of tannins with the emergence of a gelatinous precipitate. While the second section added a solution (1%) ferric chloride Ferric chloride, as the appearance of blue color on the presence of tannins.

Detection of Saponins

The source was adopted in the above paragraph to detect saponins. Add (5) ml of each of the four extracts to (3) ml of mercuric chloride solution (Mercuric chloride), and the appearance of a white precipitate on the positive detection, can also be inferred by the presence of saponins tower (5) ml of plant extract strongly in a tube Test for half a minute and leave the tube in a vertical position for (15) minutes and inferred by positive examination by the appearance of dense foam.

Detection of Flavones

The method contained in (Jaffer et al., 1983) was used to detect flavonoids. The solution (B) was prepared by adding (10) ml of ethyl alcohol and concentration (50%) to the extract.

Detection of resins

The method used in (Shami, 1982) was used to detect resins. (10) ml of each of the four extracts was taken and added (20) ml distilled water acid HCl (4%) has been inferred the positive detection of the emergence of Turbidity.

Detection of Phenolic Compounds

Harbrn (1973) method was followed by adding (3) ml of plant extract for each of the four extracts to (2) ml of ferric chloride prepared by dissolving (1) g of ferric chloride in (100) ml of distilled water, if the appearance of a bluish green color Proof of positive disclosure.

7. Method of measuring free radical inhibition (DPPH)

This assay is based on the ability of plant extracts to inhibit the synthesized free radicals DPPH (Scavenging of 2,2- diphenyl-picrylhydrazyl radical), a method modified by Molan et al. (2009). With 200يت l of DPPH, which was prepared to dissolve 12المادة l of the substance in ethyl alcohol 96%. The plates were used Micro-plate for this purpose, after you were blended plant extracts with the root of the plant in the drilling The plate was cured for half an hour at room temperature and then The absorbance was measured by ELISA (plate reader) on The wavelength of 490 nm was then calculated as the antimicrobial activity as a percentage based on the following calculation equation

$$OD = A - B / A * 100$$

Where A represents the absorption value of the control group only on the bacteria without adding the extract.

B / represents the absorption value of the plant-based mixture of bacteria with plant extracts.

8- Statistical analysis

The statistical analysis of the current study was carried out according to SPSS program. An analysis of variance (ANOVA) was used to compare between more than two groups where the lowest statistically acceptable level of moral difference is 0.05 or equal to it (Elliott and Woodward, 2007). The experiment of inhibiting manufactured free radicals was 3 replicates.

RESULTS AND DISCUSSION

1- Inhibition and deactivation of free radicals manufactured DPPH

Table (1) shows the efficacy of banned plant extracts from rosemary leaves using several solvents to inhibit and inactivate the processed free radicals DPPH. The results showed that the highest inhibition rate was found in the banned extracts of distilled water added to HCL acid by 5% and then distilled water added. HCL acid by 1% and then diluted ethyl alcohol extract by 50% followed by cold water extract and then hot water extract where gave the lowest inhibition rate and the table shows the significant differences between the types of solvents used at (P-0.05), rosemary plant is a source of natural antioxidants because Second metabolic activity Where the plant contains Rosmarinic acid and Rosmarol, which is an antioxidant (Ayensa and Duke, 1985) and essential oils are used in many essential oils in the preservation of food, medicines, alternative medicine and natural remedies As shown in several studies, the antioxidant ability of plant extracts with This activity is not due to a single phenolic compound but to a wide distribution among phytochemical components, especially anthocyanins.

Flavonoids and phenolic acids appear to be responsible for

Antioxidant ability (Tuberoso et al., 2010 and Angioni et al., 2011) The principle of the work of this method depends on the process of electronic exchange, which is evident from the melting of free radicals manufactured in alcohol consists of a purple solution is constant in the temperature of the room but is reduced in the presence of antioxidant molecules and thus form a colorless solution and this technique is an effective and easy way to detect Antioxidants using a spectrophotometer (Huang et al., 2005)

The results of this study showed that the solubility of rosemary plant extract depends on the type of solvent used in the extraction process. Previous studies have shown a direct correlation between phenolic content and inhibition of free radicals (Sani et al., 2012; Molan et al., 2009).

Table (1) Antioxidant activity (ability to inhibit the action of free radical rooted DPPH) for extracts prepared from the leaves of the plant by five types of solvents.

Plant part (leaves)	المذيباتSolvents
33.75	Cold distilled water
29.16	Boiled distilled water
37.5	%50diluted ethanol alcohol with distilled water
70.83	Distilled water with 1% HCL acid
72.00	Distilled water with 5% HCL acid

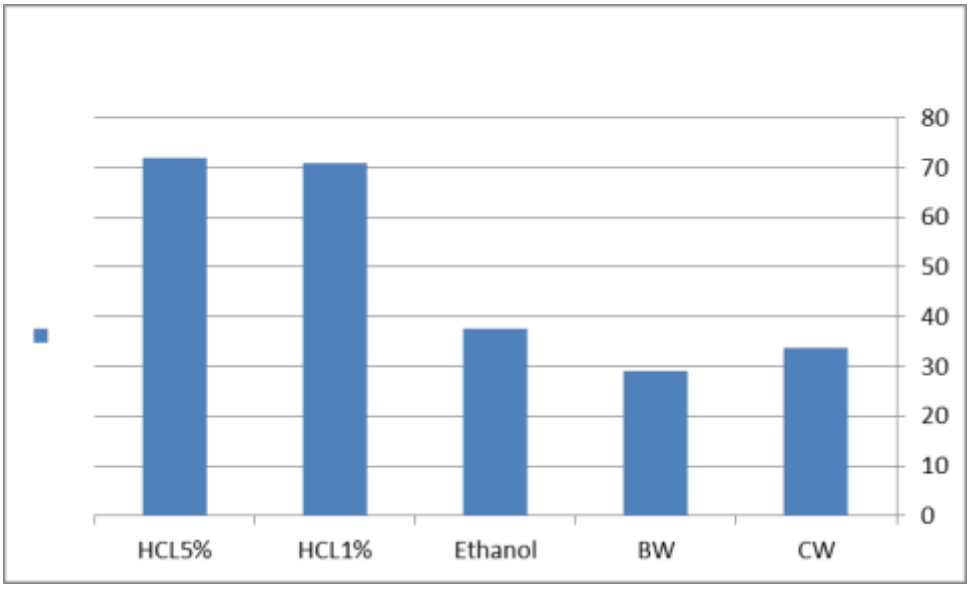

Figure 1: Comparison of Prohibited Extracts of Ace Leaf Leaves in Capacity to Inhibit Free Plant Root (DPPH).

2. Qualitative detection of some chemical compounds

In this study, specific chemical compounds found in plant leaves were detected using chemical reagents. The results shown in Table (2) showed that plant leaves contain some important active compounds that have important therapeutic value for many diseases. Alkaloids are soluble in organic solvents such as ether and alcohol while their salts dissolve in water. Therefore, alkaloids appear in aqueous and alcoholic extracts. High in inactivation of bacteria (Qutb, 1981) The reason for the inhibition of alkaloids is that they are stores for the elements that are important for the plant during its growth and some of them are growth regulators (San Miguel, 2003). They are considered to be effective compounds in medicinal plants and have medicinal and physiological significance (Shamaa, 1989). Soaps appeared in aqueous extract because they are foam when mixed with water (Taiz and Zeiger, 2006). Tannins in the water About Alklseren and lack of solubility in ether (Mahmoud, 2008) that this plant contain aggregates of these compounds emphasizes the use of effective medical Couches because of pharmacokinetics value and the multiplicity of its uses.

Table 2: Results of Chemical Detection of Active Chemical Compounds in Rosemary Extracts.

Detection result	Detection method	Totals for active compounds
Red precipitate	Benedict's detector	Glycosides
Gelatinous precipitate The appearance of blue	Lead Acetate 1% Ferric Chloride 1%	Tannins
The appearance of dense foam for a long time The appearance of a white precipitate	Shake the aqueous extract Mercury chloride	Saponin
Appearance of yellow color	Mix equal amounts of plant extract with ethyl alcohol	(Flavones)
The appearance of Akura	Boil the alcohol extract and add acidified water with HCL acid	(Resins)
Appearance of bluish green color	Ferric chloride	Phenolic Compounds

3- Examination of bacterial inhibition test by extracts of the plant

The effect of the plant extract of the Ace plant on bacterial isolates under study was carried out using the Micro Titer Plate method and the reading on ELISA device. The figures show a clear variation in the effect of each extract and its concentration on each type of bacteria where the results of the present study showed a difference There was a significant probability of 0.05 between the solvent type used in the plant extract under study and the ability of the Ace plant to inhibit the bacteria due to its active compounds and acids such as phenolic acids, flavonoids, essential oils and tannins. The plant contains an anthocyanin dye which is characterized by its antibacterial activity. The plant contains a group of compounds that are abundant in the leaves, including hexanol, tricyclene, α-thujena, α-Pinene, Sabinene, abin-Pinene, Myrcene, P-cymene and limonene (Wannes et al., 2009).

Note that the type of extract and its concentration had an effect on the inhibition rate, the aqueous extract of hot water was superior to the inhibition of E. coli bacteria as shown in Figure (1) while the cold water extract was superior to the protuse bacteria than the other extracts as in Figure (2). The treatment was the highest inhibition rate for Klebsella bacteria as shown in Figure 3, while the cold water extract was superior to other extracts over Psudomonas.

The effect of acidic extract is due to its ability to process the bacterial cell wall and destroy the enzyme penicillinase (Tamimi, 2013), while the ability of the alcoholic extract to inhibit is due to the solubility of the active substances well in organic solvents (Zangana, 2004) as well as the high alcohol content of the extract. Soluble efficacy that has the potential to inhibit bacterial growth through its ability to penetrate the cell wall or its effect on important vital parts of the bacterial cell such as cytoplasm, ribosomes, or DNA (Al-Dulaimi, 2006). This is by generating hydrogen bonds formed with proteins that lead to the destruction of protein structure in the bacterial cell that leads to inhibition of bacterial growth (Mustafa. 1995) as well as because the negative bacteria of the Cram stain do not contain the peptidoglycan layer. (Majhenic et al., 2007) The effect of cold and hot aqueous extract of rosemary has shown a high inhibition rate because it contains a number of hydroxyl groups that act as a hydrogen donor which makes it very important and powerful.

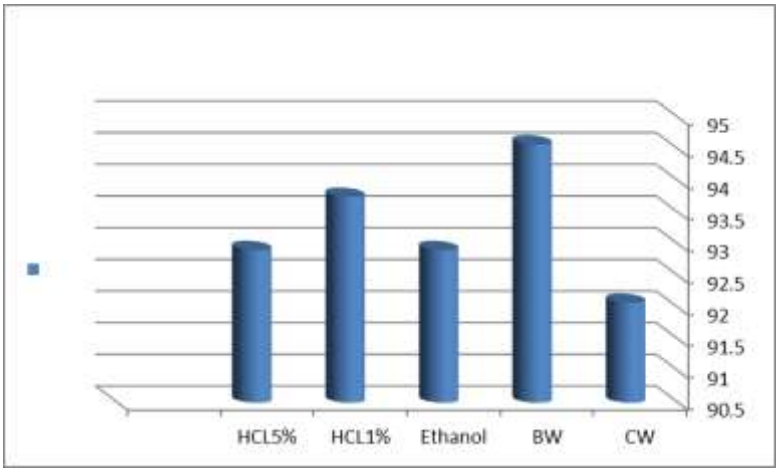

Figure 1: Effect of Plant Extract of Ace Plant on E.coli Bacteria.

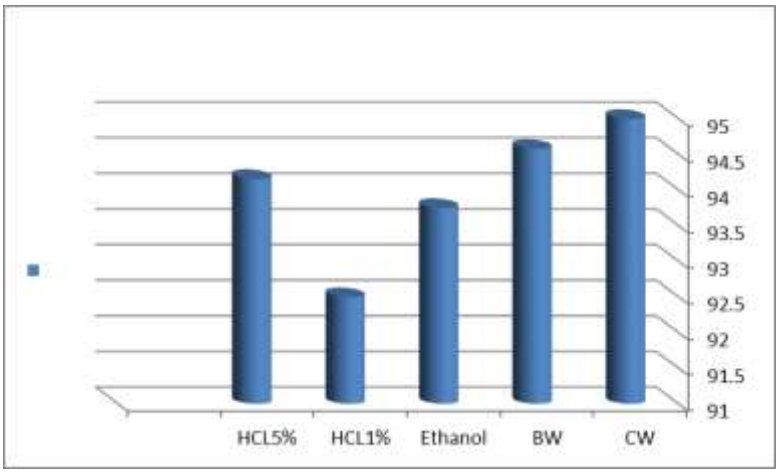

Figure 2: Effect of plant extract of Ace plant on Proteus bacteria.

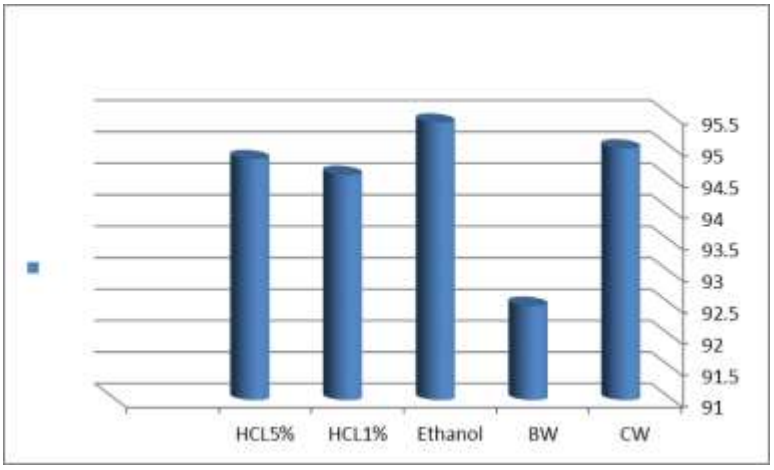

Figure 3: Effect of plant extract of the plant on the Klebsella bacteria.

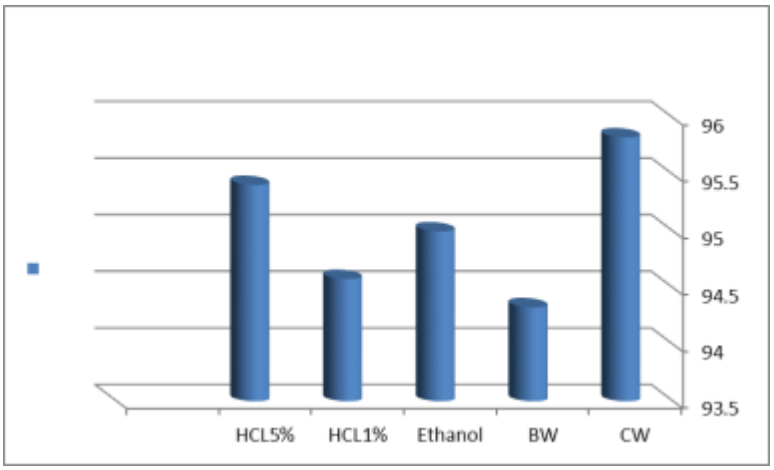

Figure 4: Effect of plant extract of Wallace plant on Pseudomonas.

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