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Phytochemical screening and antimicrobial study of *Rosmarinus officinalis*

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Abstract
Plants have been a source of inspiration for novel drug compounds, providing significant contributions to human health and wellbeing. Their naturally occurring chemicals are biologically active substances, known as phytochemicals, which provide defence against environmental stressors. The aim of the present study is to examine the phytochemicals present in *Rosmarinus officinalis* L. (Family-Lamiaceae) and to study its antimicrobial potential against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*. To get the plant extract, two techniques (decoction and maceration) were applied, with three different solvents (acetone, methanol and double distilled water), to check the presence/absence of various phytochemicals in the plant extract. The preliminary phytochemical screening of the extract prepared through decoction, showed good results for the presence of phenolics, flavonoids, alkaloids, saponins and tannins in comparison to maceration in double distilled water (DDW) extracts, followed by the methanol and acetone extracts. Quantitative estimation of total phenolic contents (TPC) and total flavonoid contents (TFC) were carried out by using Folin Ciocalteu method and aluminum chloride method, respectively, using UV-vis spectrophotometer (Systronics, 119). The methanolic extracts processed through maceration had exhibited maximum value of TPC (55.44 µg GAE g^{-1} extract) and TFC (2.70 µg QE g^{-1}), as compared to acetone, decoction and DDW extracts. The maximum zone of inhibition (13 mm) with significant antibacterial activity against *S. pyogenes* were shown by acetone extract, followed by methanol extracts while no effect was observed in the DDW extract. Therefore, utilising *R. officinalis* may help to combat the problems, associated with the usage of synthetic antibiotics, because these plant based natural antibiotics have great therapeutic potential without any side effects.

Keywords: Phytochemicals, *R. officinalis*, decoction, maceration, antibacterial

Introduction
Herbal remedies have stood the test of time and in that aspect, India has a huge repository of medicinal plants, since time immemorial. Plants are an essential part of our life as we rely upon them for umpteen purposes, be it for food, medicine or cosmetics. They have been the subject of wonder for man ever since the advent of human civilization. In Indian scenario, plants are of great ethnobotanical value, having been documented in numerous ancient texts (Marak and Adela, 2021) [16]. India is well-known for its traditional medical practices, which include Unani, Siddha and Ayurveda. Herbal medicine application is also mentioned in the Atharva Veda (3000–2000 BC). The 19th century saw the beginning of scientific attention to the active ingredients in herbs, which paved the way for molecular research.

A wide range of chemical compounds are synthesized by plants, with a variety of physiological functions that can be used as medicines and in industry. These naturally occurring, biologically active substances are phytochemicals, with significant physiological effects on the human body and they encompass a broad spectrum of chemical entities, such as polyphenols, flavonoids, steroids, saponins, terpenoids, etc. They can be found in different plants (leaf, stem, root, inflorescence, fruits, seeds etc.), also vegetables, grains, nuts and tea, etc. These phytochemicals provide defence system against environmental stressors, like pollution, drought, salinity, UV radiation and pathogenic invasion. These phytochemicals are also capable of controlling/preventing diseases in human beings. Plants with such phytochemicals can potentially address common issues, related to the excessive use of antibiotics, chemical medications and ultimately, developed resistance of bacteria towards these compounds (Kumar *et al*., 2023) [13]. Depending on their role in plant metabolism, phytochemicals can be classified as primary metabolites and secondary metabolites.

Primary metabolites are directly involved in the growth and development of plants whereas, the secondary metabolites provide defence against biotic and abiotic stresses.

Plants respond to microbial infections by producing antimicrobial compounds present in the form of phytochemicals (Vaou *et al*., 2021) [27]. Therefore,it is worth mentioning about the medicinal properties of plants, having a variety of phytochemicals, including flavonoids, alkaloids, sterols, terpenoids, phenolic acids, stilbenes, lignans, tannins, and saponins. Many scientific studies have demonstrated the benefits of these bioactive compounds, including their antibacterial, antioxidant, immune-stimulating, detoxifying enzyme-modulating, anti-platelet aggregating and hormone-metabolizing properties, as well as their anticancer potential (Nyamai *et al*., 2016) [18]. Herbal prescriptions have drawn a lot of attention recently as a potential substitute for perceived shortcomings in conventional pharmacotherapy on a global scale.

Salvia rosmarinus Spenn. (Formerly known as *Rosmarinus officinalis* L.), is a native Mediterranean shrub from the Lamiaceae family that is widely used as a flavoring and spicing agent. In addition to its traditional use, it has attracted attention for its biological properties, particularly the presence of polyphenols such as carnosic acid and rosmarinic acid, as well as phenolic diterpenes in the carnosol (Musolino *et al*., 2023) [17]. Our decision to study rosemary was influenced by the potential health benefits and antioxidant contents of the plant. The vision of the present study is to examine the phytochemicals present in *R. officinalis* extracts and also its anti-microbial properties against certain Gram positive and Gram negative bacteria. Therefore, the main objectives of the present work are: (i) To check the solubility of phytochemicals in three different solvents, namely- (a) Methanol (b) Acetone (c) DDW, (ii) To check the best methods of extraction for the preparation of plant extract, using the selected solvent, such as- (a) Decoction (b) Maceration (c) Soxhlet extraction, (iii) Qualitative screening: Screening of various phytochemicals present in the plant extract by performing specific tests for- (a) Phenols (b) Flavonoids (c) Alkaloids (d) Terpenoids (e) Cardiac glycosides (f) Saponins (g) Tannins (h) Steroids, (iv) Quantitative screening: Total phenolic content (TPC) and Total flavonoid content (TFC) and (v) Determination of anti-bacterial activity.

Materials and methods

Plant samples: The leaves of rosemary (*Salvia rosmarinus*; Lamiaceae) were collected from the local nursery of Gandhi Maidan, Patna.

Pathogenic isolates: Three bacterial isolates (*Staphylococcus aureus*, *Streptococcus pyogenes* and *Escherichia coli*) were obtained from the Department of Biotechnology, Faculty of Science, Patna University, Patna, Bihar, India.

Sample Preparation

The fresh leaves of rosemary were collected and washed under running water. After washing, leaves were shade dried for four to five days, to preserve the phytochemical properties. The shade dried leaf samples were ground into fine powder. The powered samples were then stored in air tight containers for further tests.

Optimization of Extraction Conditions for Obtaining Phytochemicals

Decoction

For the purpose of decoction, 2g of dried powdered sample was added to 100 ml of double distilled water (DDW) at 100 °C for 30 min in a water bath. It was filtered through Whatman filter paper. After filtration, the extracts (filtrates) were stored for further use (Li *et al*., 2007) [14].

Maceration

For the purpose of maceration, 4g of powdered sample was added to 100 ml of DDW, methanol and acetone separately. These samples were then kept in rotary shaker (Rivotek) at 120 rpm for about 72h. After the completion of required period, the plant samples were filtered, using Whatman filter paper. All of the filtrates were then stored in air-tight bottles for further tests (Chong *et al*., 2018) [4].

Soxhlet extraction

Soxhlet extractor: The soxhlet extractor setup consists of a round bottom flask, distillation path, condenser, expansion adapter, siphon tube, cooling water inlet, cooling water outlet, heat source and thimble. The selection of the solvent for soxhlet extraction is based on the phytoconstitutent isolation process (Boopathi *et al*., 2017) [2].

In the process of plant's phytochemical extraction by soxhlet apparatus, 10g powdered samples were added to 250 ml of solvent in the thimble chamber of the soxhlet apparatus for 6h. The liquid extracts were then kept in air tight bottles for further analysis (Jorgensen and Turnidge, 2007) [11]. To carry on the soxhlet method, different solvents were mixed to extract the phytochemicals.

Methanol as solvent

In this process, 10g powdered samples were taken in the thimble channel of the soxhlet apparatus and 250 ml of methanol was taken in the round bottom flask and these were allowed to boil for 4-5 cycles at 65.4 °C for proper extraction.

Acetone as solvent

In this process, 10g powdered samples were taken in the thimble channel of the soxhlet apparatus and 250ml of acetone was taken in the round bottom flask and these were allowed to boil for 4-5 cycles at 56 °C for proper extraction.

DDW as solvent

In this process 10g powdered samples were taken in the thimble channel of the soxhlet apparatus and 250ml of DDWwas taken in the round bottom flask and these were allowed to boil for 4-5 cycles at 100 °C for proper extraction.

Preliminary Phytochemical Screening

Test for phenolic compounds: For the identification of phenolic compounds, 1ml of filtered plant extract was taken in a test tube. Further, 4 drops of 10% (w/v) aqueous ferric chloride ($FeCl_3$) solution was added into it. The formation of blue or green hue indicates the presence of phenols (Rondon *et al*., 2018) [22].

Test for flavonoids*:* For the identification of flavonoids, 2 ml of filtered plant extract was taken in a test tube and 4 drops of dilute sodium hydroxide 2 N (NaoH) solution was added into it. Formation of intense yellow color was observed and then the solution turned colorless on addition of few drops of HCl, this indicates the presence of flavonoids (Sankhalker *et al*., 2016) [23].

Test for alkaloids: For the identification of alkaloids, 2 ml of filtered plant extract was taken in a test tube and 3 drops of Wagner's reagent, along with 3 drops of HCl were added to

the above solution, formation of reddish brown coloration indicates the presence of alkaloids (Dahanayake *et al*., 2019) [5].

Test for terpenoids: For the identification of terpenoids, 2.5ml of filtered plant extracts were taken in a test tube and 1 ml chloroform, along with 1.5 ml of sulphuric acid (H_2SO_4) were added to the above solution, presence of reddish brown color indicates the presence of terpenoids (Pandey *et al*., 2017) [21].

Test for cardiac glycosides: For the identification of cardiac glycosides, 2 ml of filtered plant extracts were taken in a test tube and 1 ml of glacial acetic acid followed by 2 drops of 5% $Fecl_3$ and 2 ml of H_2SO_4 were added to the above solution. Appearance of brown ring at the interface confirms the presence of glycosides (Hou *et al*., 2021) [9].

Test for saponins: For the identification of saponins, 2 ml of filtered plant extracts were taken in a test tube and 2 ml of DDW was added to it and the mixture was then agitated vigorously. The formation of consistent foam for 10-15 min, confirms the presence of saponins (Vasudeva *et al*., 2015) [28].

Test for tannins: For the identification of tannins, 2 ml of filtered plant extracts were taken in a test tube and 3 drops of 5% ferric chloride ($Fecl_3$) was added to it. Formation of greenish black or dark blue coloration confirms the presence of tannins (Arinaa and Harisuna, 2019) [1].

Test for steroids: For the identification of steroids, 1 ml of filtered plant extract was taken in a test tube. Further, 2 ml of chloroform along with few drops of conc.H_2SO_4 were added to the above solution. Appearance of reddish brown color, confirms the presence of steroids (Gul *et al*., 2017) [7].

Quantitative Analysis of Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Estimation of total phenolic content (TPC)

The TPC of *R. officinalis* leaf extracts were determined spectrophotometrically, using Folin-Ciocalteau reagent. According to the methodology of Kim *et al*., (2003) [12], 1ml of extract sample (1 mg ml^{-1}) was added to 1 ml (1:10 dilution) of Folin-Ciocalteau reagent. After an interval of 5 min, 4ml of 7% sodium carbonate (Na_2CO_3) solution was added to the mixture, followed by the addition of 15.5 ml of DDW and mixed thoroughly. The mixtures were stored at 25 °C in dark for 60 min and the absorbance was measured at 650 nm, using UV-vis spectrophotometer (Systronics, 119). The TPC was determined by extrapolating, through calibration curve, prepared by 10% gallic acid solution and expressed, as µg of gallic acid equivalent per mg of dried sample (µg GAE mg^{-1}) (Fatima *et al*., 2023) [6].

Estimation of total flavonoid content (TFC)

Total flavonoids were calculated colorimetrically, following the advanced method of Chang *et al*., (2002) [3]. In brief, 1 ml of each sample solution (1 $mgml^{-1}$) was mixed with 3 ml of 96% methanol. After 5 min, 0.2 ml of 10% $AlCl_3$ was added, followed by 0.2 ml of 1M Potassium acetate (CH_3CO_2K) and thoroughly mixed with 5.6 ml of DDW. The mixture was kept at room temperature for 10-15 min with intermittent shaking. The absorbance was taken at 510 nm, using UV-vis spectrophotometer (Systronics, 119). The TFC was determined by extrapolating through calibration curve, prepared by 10% quercetin and expressed, as µg of quercetin equivalent per mg of dried sample (µg QE mg^{-1}) (Fatima *et al*., 2023) [6].

2.7 Antibacterial Activity

In this study, three bacterial isolates (*S. aureus*, *S. pyogenes and E.coli*) were obtained from the Department of Biotechnology, Faculty of Science, Patna University, Patna, Bihar, India.

Media preparation: A growth media or culture media is a solid, liquid, or semi solid substance designed to support the growth of a population of microorganisms or cells via the process of cell proliferation (Madigan *et al*., 2005) [15]. Solid Nutrient Agar (NA) media was prepared to allow the growth of bacterial colonies.

Preparation of inoculants*:* The inoculants, obtained from the Department of Biotechnology, Faculty of Science, Patna University were revived on NA media by incubating them at 37 °C for 24-48 h. For further work, the freshly prepared Muller Hinton agar media plates were swabbed with the above mentioned inoculants of bacterial suspension ($1x10^8$ CFU ml^{-1}).

Well diffusion method*:* For the study of antibacterial activity of plant extracts, well diffusion method was used. According to Obeidtal *et al*., (2012) [19], wells were created on the previously swabbed bacterial plates using a cork borer. Now in each well, 100µl of individual extract samples were poured and then the plates were incubated at 37 °C for 24-48h. For each sample, the zone of inhibition was measured, using Himedia zone scale.

Result and discussion

Qualitative analysis

In the preliminary phytochemical screening, the potential phytochemicals present in the different plant extracts were identified.

Decoction- The extract prepared via decoction showed good results for the presence of phenols, flavonoids, alkaloids, saponins and tannins while average results were shown by the extract for the presence of cardiac glycosides and steroids. However, terpenoids were present in trace amounts (Table 1).

Maceration

The extract prepared via maceration showed good results in the DDW extract followed by the methanol and acetone extract. The methanolic extract showed good results for the presence of phenols, flavonoids, tannins and average result for the presence of alkaloids and cardiac glycosides whereas terpenoids, saponins and steroids remained absent. The acetone extract showed good results for the presence of phenols and alkaloids and average results for the presence of flavonoids, terpenoids and cardiac glycosides while saponins, tannins and steroids were absent in the extract. The DDW extracts showed good results for the presence of alkaloids, terpenoids, cardiac glycosides, saponins, tannins, steroids and average results were exhibited for the presence of phenol and flavonoids (Table 1).

Soxhlet extraction

The leaf extract prepared via soxhlet extraction showed good results in the DDW extracts followed by acetone and

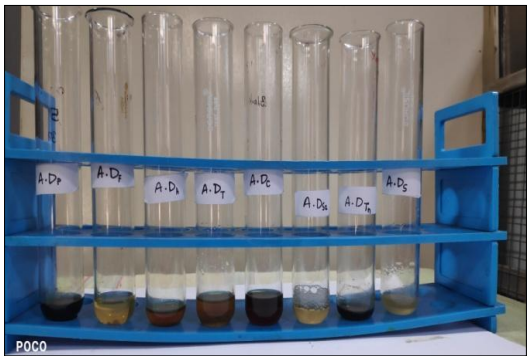
methanol extracts. The methanol extract showed good results for the presence of phenols and tannins and average results for the presence of flavonoids, alkaloids, cardiac glycosides and steroids while terpenoids and saponins remained absent in the extract. The acetone extract showed good results for the presence of phenol, cardiac glycosides and tannins and average results for the presence of flavonoids, alkaloids, steroids while terpenoids and saponins remained absent in the extract. The DDW extract showed good results for the presence of phenols, flavonoids, alkaloids, terpenoids, saponins, tannins and steroids while cardiac glycosides were absent in the extract (Plate 1, Table 1).

Table 1: Phytochemical screening of Rosemary

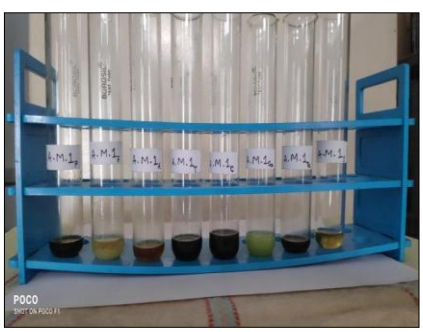
Extraction method	**Decoction**	**Maceration**			**Soxhlet**		
Solvent	**DDW**	**Methanol**	**Acetone**	**DDW**	**Methanol**	**Acetone**	**DDW**
Phytochemicals							
Phenol	+++	+++	+++	++	+++	+++	+++
Flavonoids	+++	+++	++	+	+	+	+++
Alkaloids	+++	++	+++	+++	+	+	+++
Terpenoids	+	-	++	+++	-	-	+++
Cardiac glycosides	++	+	++	+++	+	++	-
Saponins	+++	-	-	+++	-	-	+++
Tannins	+++	+++	-	+++	+++	++	+++
Steroids	++	-	-	+++	+	+	+++

+++ Abundant; ++ moderate; + trace amount; - not obtained

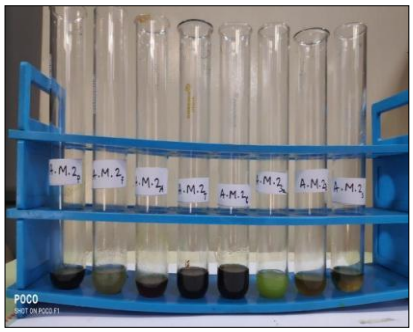
Decoction



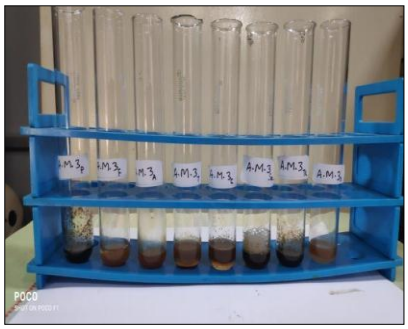
Maceration



(a) Methanol extract

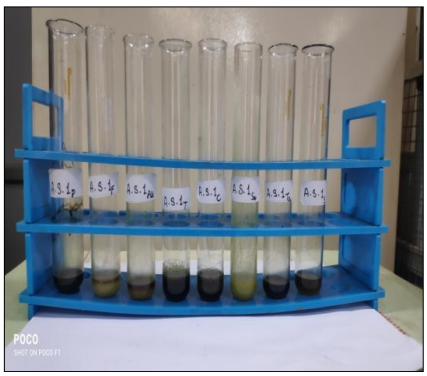


(b) Acetone extract

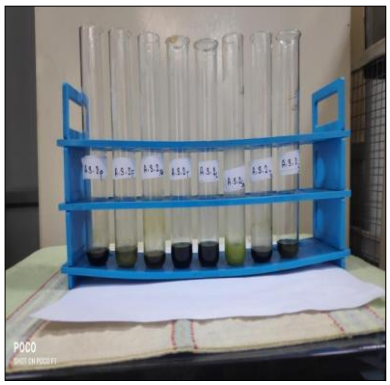


(c) DDW extract

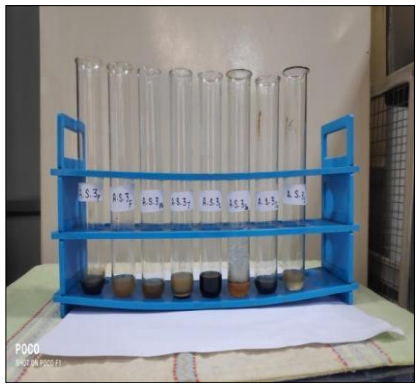
Soxhlet extraction



(a) Methanol extract



(b) Acetone extract



(c) DDW extract

Plate 1: Phytochemical screening of Rosemary

Quantitative Screening

In the quantitative screening, TPC (total phenolic content) and TFC (total flavonoid content) were calculated.

TPC (Total Phenolic Content)

Quantitative screening of total phenols was done on the basis of a Gallic acid standard curve and the linearity of calibration curve (R^2= 0.9926) was achieved, using its different concentrations from 50 to 450 µg ml^{-1} (Fig. 1). TPC of the different extracts used in the present study are represented in Table 2.

The extract prepared via decoction exhibited a TPC value of 24.25 µg GAE mg^{-1}. The extracts prepared via maceration showed the maximum value of TPC in the methanolic extract (55.44 µg GAE mg^{-1}) followed by the acetone extract (25.55 µg GAE mg^{-1}) while the minimum value of TPC was exhibited by the DDW extract (13.60 µg GAE mg^{-1}). The extracts prepared via Soxhlet extraction method showed the maximum value of TPC in the methanolic extract (68.56 µg GAE mg^{-1}) followed by the acetone extract (56.29 µg GAE mg^{-1}) and the minimum value of TPC was exhibited by the DDW extract (37.05 µg GAE mg^{-1}).

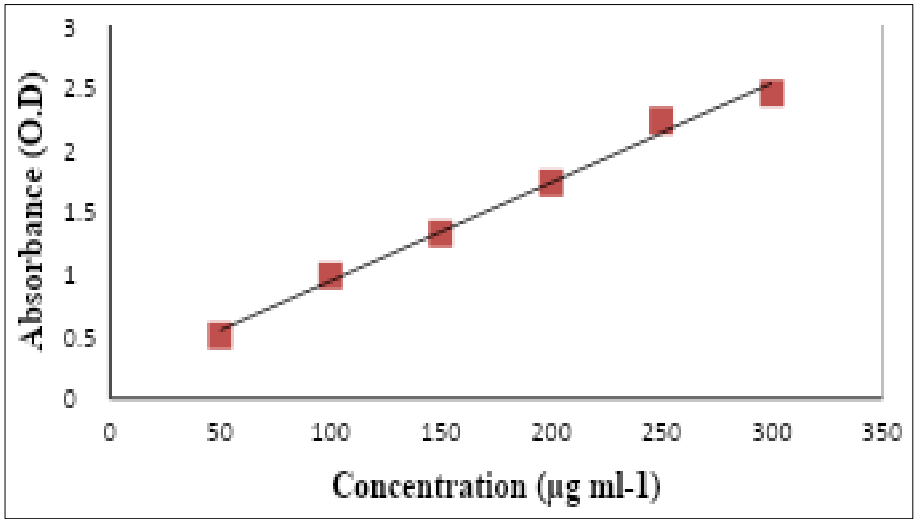


Fig 1: Gallic acid standard calibration curve

Table 2: Quantitative analysis of TPC

Extraction method	Solvent	TPC (µg GAE mg^{-1}) (Rosemary)
Decoction	DDW	24.25
Maceration	Methanol	55.44
	Acetone	25.55
	DDW	13.60
Soxhlet	Methanol	68.56
	Acetone	56.29
	DDW	37.05

TFC (Total Flavonoid Content)

The quantitative screening of TFC was done on the basis of a quercetin standard curve (R^2= 0.9988) and the linearity was achieved in calibration with a concentration from 50 to 500 µg ml^{-1} (Figure 2). TFC of the different extracts used in the present study are represented in Table 3.

The extract prepared via decoction exhibited a TFC value of 1.61 µg QE mg^{-1}. The extracts prepared via maceration showed the maximum value of TFC in the methanolic extract (2.70 µg QE mg^{-1}), followed by the DDW extract (0.64 µg QE mg^{-1}), while the minimum value of TFC was exhibited by the acetone extract (0.23 µg QE mg^{-1}). The extracts prepared via Soxhlet extraction method showed the maximum value of TFC in the methanolic extract (10.12 µg QE mg^{-1}), followed by the DDW extract (2.29 µg QE mg^{-1}) and the minimum value of TFC was exhibited by the acetone extract (1.63 µg QE mg^{-1}).

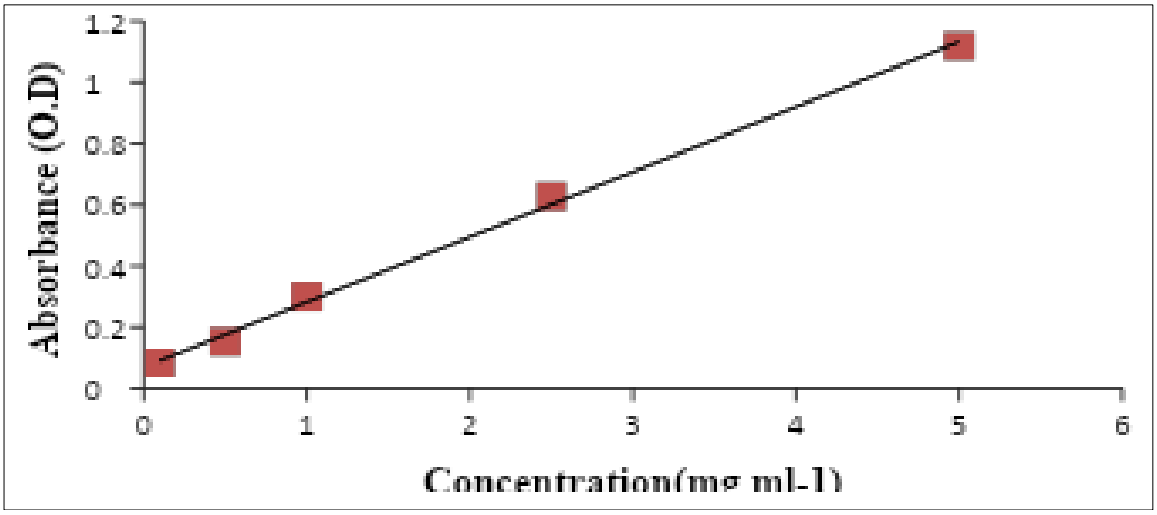


Fig 2: Quercetin standard calibration curve

Table 3: Quantitative analysis of TFC

Extraction method	Solvent	TFC (µg QE mg^{-1})
		A (Rosemary)
Decoction	DDW	1.61
Maceration	Methanol	2.70
	Acetone	0.23
	DDW	0.64
Soxhlet	Methanol	10.12
	Acetone	1.63
	DDW	2.29

3.3 Antibacterial Activity

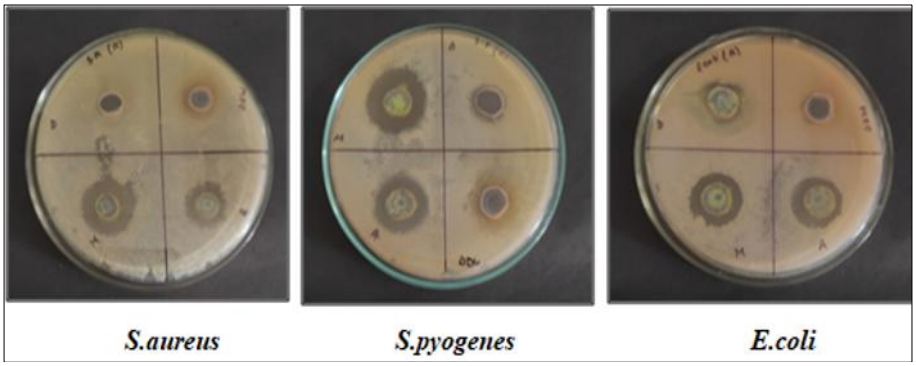
For the antimicrobial study, agar well diffusion method was used to check the antibacterial activity of the selected plant extract against three test organisms, namely, *S. aureus*, *S. pyogenes* and *E. coli*.

The extract prepared via decoction showed a significant zone of inhibition (5 mm) against *E.coli*, followed by 4 mm against *S.pyogenes*, while no zone of inhibition was observed against *S.aureus*. The extract prepared via maceration showed good results in the methanolic extract with a zone of inhibition of 10 mm against *S.aureus*, followed by 11 mm against *S.pyogenes* and 8 mm against *E.coli*. The diameter of zone of inhibition was 7 mm against *S.aureus*, 8mm against *S.pyogenes* and *E.coli* in the acetone extractwhereas no antibacterial activity was observed in the DDW extract. The extract prepared via Soxhlet extraction exhibited the maximum activity in the acetone extract with a significant zone of inhibition of 13 mm against *S.pyogenes*, 12 mm against *S.aureus* and 9 mm against *E.coli* (Plate 2; Table 4).

Table 4: Antibacterial activity of Rosemary

Extraction method	Solvent	Test organisms		
		S.aureus	***S. pyogenes***	***E.coli***
Decoction	DDW	-	4 mm	5 mm
Maceration	Methanol	10 mm	11 mm	8 mm
	Acetone	7mm	8 mm	8 mm
	DDW	-	-	-
Soxhlet	Methanol	10 mm	10 mm	6 mm
	Acetone	12 mm	13 mm	9 mm
	DDW	-	-	-

Rosemary
(Decoction+ Maceration)



(Soxhlet Extraction)

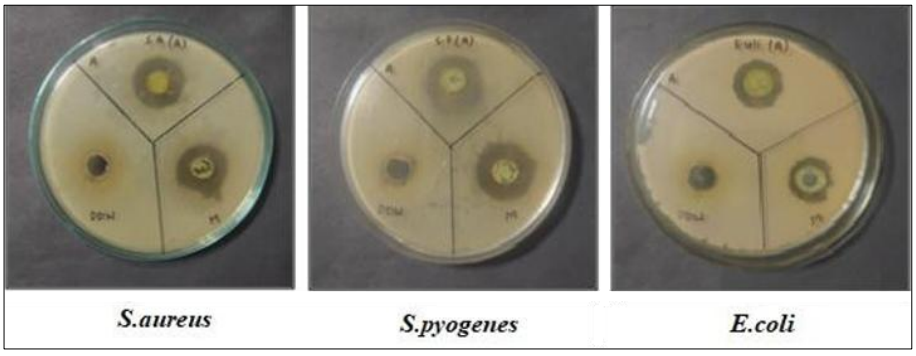


Plate 2: Antibacterial activity of Rosemary

4. Discussion

The phytochemical screening of rosemary is a vital process that allows researchers to identify and quantify the various bioactive compounds present in the plant. Rosemary (*R. officinalis*) has been recognized for its diverse pharmacological properties, including antioxidant, anti-inflammatory, antimicrobial and anticancer activities, many of which are attributed to its rich phytochemical composition (Okwu, 2001; Ibrahim *et al*., 2022) [20, 10]. Phytochemical screening typically involves the extraction of bioactive compounds from rosemary using suitable solvents, followed by the quantification of these compounds using different techniques. The study conducted phytochemical screening of *R. officinalis* and found that various compounds like alkaloids, flavonoids, tannins, phenols, steroids, glycosides, terpenoids, as well as saponins were significantly present in different extracts obtained through decoction, maceration and soxhlet extraction methods. As per the reporting of Trease and Evans, (2005) [26] also alkaloids were particularly highlighted for their cytotoxicity and applications in allopathic systems. In another work, steroids were noted for their importance in pharmacy, especially in drug production (Okwu, 2001) [20]. Glycosides were recognized in our study, which are known for their role in lowering blood pressure and treating heart-related conditions (Trease and Evans, 2005) [26]. Terpenoids were associated with cough, asthma, and hay fever treatment, while saponins were acknowledged for their traditional use as detergents, pesticides, and their health benefits (Shi *et al*.,

2004) [24]. Tannins and phenols were emphasized for their antioxidant properties (Han *et al*., 2007) [8].

Furthermore, the study explored the variation in TPC and TFC, among different rosemary extracts, obtained using various solvents and extraction techniques. Soxhlet extraction with methanol yielded the highest TPC and TFC values. In our study, soxhlet performed best in terms of TPC for (68.56 mg $GAEg^{-1}$ DW) and TFC (10.12 mg QEg^{-1} DW), especially when combined with methanol, as solvent. Methanol was followed by acetone and DDW extracts in terms of efficiency for TPC and TFC (Table 2 and 3).The antibacterial activity of *R. officinalis* extracts was evaluated, showing significant activity against various bacterial strains, with acetone and methanol extracts, exhibiting the highest activity. However, the DDW extract did not show any activity. The assessment of antibacterial activity was done by observing the presence or absence of inhibitions zones and zone diameters. The significant zone of inhibition was obtained (13 mm), against *S. pyogenes* under soxhlet extraction combined with acetone, followed by 12 mm, against *S. aureus* and 9 mm, against *E.coli*. Taking all the results of extracts into consideration, it appears that rosemary presented the highest record of TPC and TFC in methanolic extracts and antibacterial activity against *S. pyogenes* in acetone extracts under soxhlet extraction methods (Table 4).

Several studies have demonstrated that the antibacterial effects of rosemary extracts against pathogens like *S. aureus*, *S. pyogenes* and *E. coli* can be attributed to compounds like rosmarinic acid, carnosol, carnosic acid (Sienkiewicz *et al*., 2013) [25]. The antibacterial activities varied among microbial strains and extracts, with acetone extracts showing the strongest activity. This variability was attributed to differences in the solubility of bioactive compounds.

Overall, the study revealed the diverse phytochemical composition and antibacterial potential of *R. officinalis* extracts, influenced by extraction methods and solvent choice, which could have implications for its use in pharmaceutical and food industries.

5. Conclusion

On the basis of the results and discussion only, it became possible to evaluate the optimization of solvents and extraction techniques, in order to produce the best outcomes for various tests, including qualitative, quantitative and antimicrobial studies.

Therefore, it can be inferred that for qualitative screening, decoction method is the best method that can be employed to check the presence of various phytochemicals in Rosemary (*R. officinalis* L.). In case of preparation of plant extracts via maceration and soxhlet extraction, DDW appeared, as the best solvent, as it showed the presence of most of the phytochemicals (phenols, flavonoids, alkaloids, terpenoids, cardiac glycosides, saponins, tannins and steroids). In case of quantitative screening, methanol extract prepared by soxhlet extraction method showed the highest value of TPC, i.e. 68.56 µg GAE mg^{-1}. The highest value of TFC was exhibited by methanol extract prepared by soxhlet extraction method, i.e.10.12 µg QE mg^{-1}. For antimicrobial study, acetone extract prepared via soxhlet extraction method exhibited the maximum zone of inhibition, i.e. 13 mm against *S.pyogenes*.

The link between consumption of phytochemicals and health benefits has gained popularity due to recent scientific studies. Also, the use of *R.officinalis* may prove to be helpful to control different side effects, associated with the growing resistance of bacteria to antibiotics and the increased use of chemical medications. Therefore, it can be concluded that this plants has the potential to produce new products and fine chemicals that may be employed in the development of novel drugs. Thus, it can be used in the preparation of functional foods and nutraceuticals, to treat or prevent a wide range of diseases, due to its structural stability and purity.

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