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Anti-Diabetic, Anti-Oxidant and Antibacterial Activity of Leaves Extract of *Salvia Rosmarinus (Rosemary)*

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Abstract: One of the plant species that is known for its therapeutic utilize is Salvia Rosmarinus. This experimental study aimed to evaluate anti-diabetic potential, anti-oxidant and antibacterial activities of the plant leave extracts with different solvent having varying polarity. Successive extractions were carried out with petroleum ether, ethyl acetate, and methanol and gave percentage yields of 3.00%, 10.01% and 14.04% respectively. The screening test for metabolites reveals the presence of Alkaloids, Anthraquinones, Phenols, Flavonoids, Steroids, Terpenoids, Saponins, Tannins and Glycosides from different crude extracts under some experimental protocols. The anti-diabetic ability of extract of Salvia Rosmarinus was evaluated by alpha-amylase inhibitory assay which implies that all methanol, ethyl acetate and petroleum ether extracts have greater potential to anti-diabetic activities at a higher concentration. The methanol extract showed the highest anti-diabetic activity from 59.77% to 94.19%, which is very close to the activity of acarbose a standard (72.36% to 95.71%). The ethyl acetate and petroleum ether extracts showed very slow at minimum concentration but rapidly increase their activity when their concentration becomes large from 11.31% to 94.11% and from 6.33% to 76.6% respectively. The total flavonoid concentrations of the petroleum ether, ethyl acetate, and methanol extracts were, respectively, 222.70, 152.1, and 113.71 mg QE/g. And the total phenolic content was 339, 182, and 28 mg GAE/g in methanol, ethyl acetate and petroleum ether extract respectively. Methanolic extract has higher total phenolic and flavonoid content and expected to the highest scavenging activity for free radicals. Lastly, an assessment was conducted on the antibacterial properties of the crude extracts. The highest minimum zone of inhibition was recorded in methanol crude extracts against a negative bacterium E. coli with minimum zone of inhibition 31 + 0.14 mm. Growth inhibition tests against bacteria showed a good result at a higher concentration in methanol and ethyl acetate extracts.

Keywords: In Vitro Antibacterial, Anti Diabetic, Agar Disk Diffusion, Rosemary Leave, Phytochemical Screening, Alpha-Amylase Inhibitory Assay

1. Introduction

All living things on earth depend on nature for a variety of essential resources. Plants are vital to mankind, animals, other living things [1]. Since the beginning of time, humans have used plants for a wide range of reasons, including food, flavor, cosmetics, clothing dye, and medication for a number of maladies. There has been a connection between people and plants ever since humans first appeared on the planet [2].

As seen in figure1, Salvia Rosmarinus, or rosemary, is a typically upright, rounded, evergreen shrub with fragrant, needle-like, gray-green leaves and tiny, two-lipped, pale blue

to white flowers. Synonymous with Rosmarinus officinalis. The genus name Salvia comes from the Latin word salveo meaning "to save or heal", in reference to the purported medically curative properties attributed to some plants in the genus [3].

Scientists have isolated chemicals from Salvia Rosmarinus's many parts due to the plant's wide range of therapeutic applications. Some of the metabolites isolated and characterize from this herb are shown in figure 2 below. Scientific studies are still being conducted by various research teams to examine the biological activities of crude extracts or pure compounds isolated from Salvia Rosmarinus, and most of them showed promising activities. Some of the compounds isolated from various parts of the plant have already been tested for their biological activities [4, 5].

Figure 1. *Salvia Rosmarinus herb.*

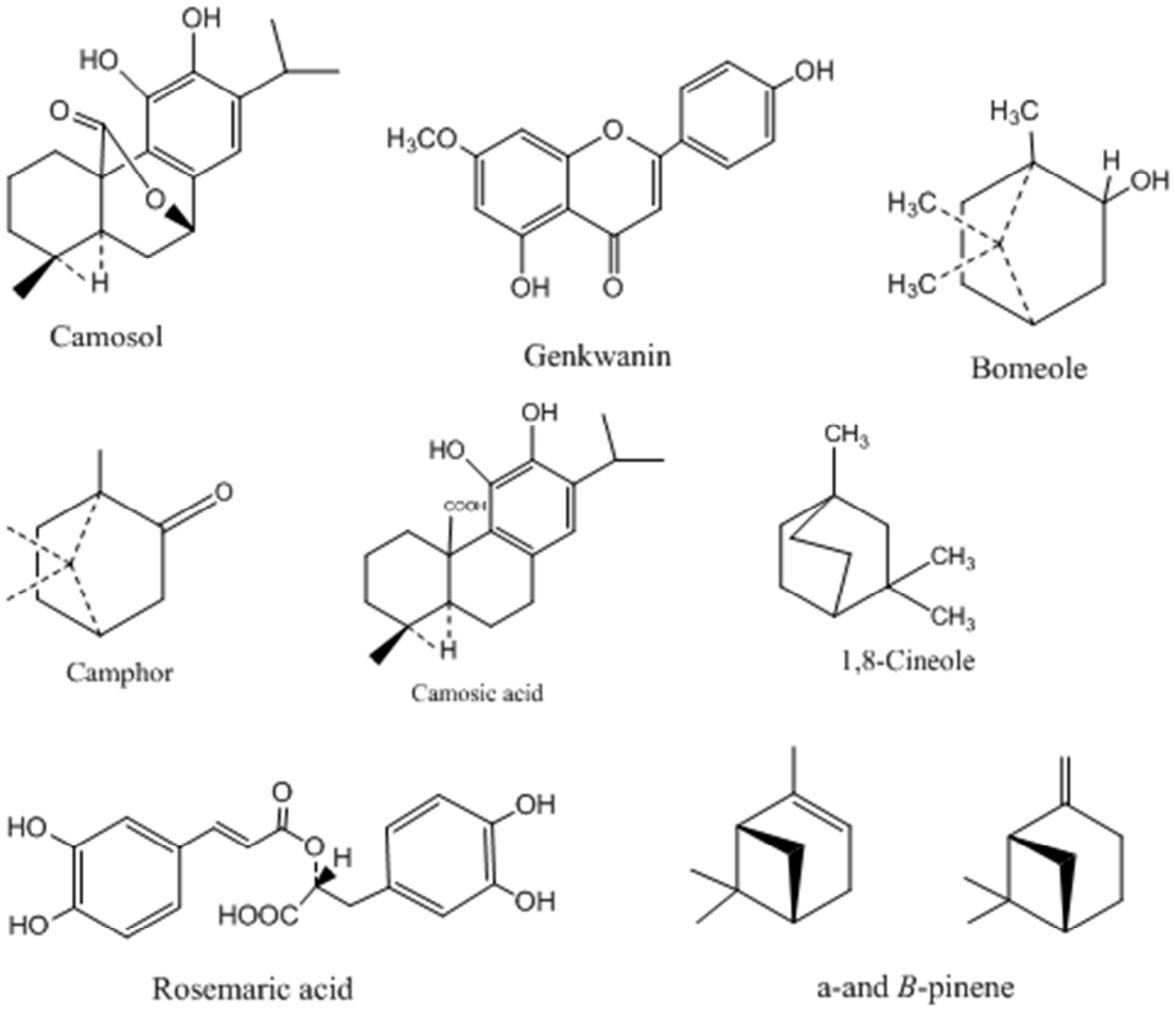


Figure 2. *Some isolated phytochemicals from different parts Salvia Rosmarinus herb.*

Rosemary has therapeutic properties and has been used in folk medicine as an oral preparation to relieve renal colic, dysmenorrhea, and muscle spasms. Rosemary has antifungal, antiviral, antibacterial, anti-inflammatory, antitumor, antithrombotic, antinociceptive, antidepressant, antiulcerogenic, and antioxidant activities [4, 6-9].

The demand for herbal treatments is currently rising in both developed and developing nations. This may be partially attributable to discontent with traditional medicine in developed nations, whereas in developing nations, it is attributable to a lack of pharmaceutical items and prohibitively high prices [10].

Diabetes mellitus is understood to be a syndrome, a group of illnesses characterized by hyperglycemia and glucose intolerance. This syndrome can result from insulin insufficiency, decreased insulin action, a combination of these factors, or both. There are two main types of diabetes; type I diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is defined by the sudden development of severe symptoms, a need for exogenous insulin to maintain life, and a propensity for ketosis. In type 1 diabetes, the body produces insufficient insulin to regulate blood glucose levels. Ketones are produced by the liver as an alternative source of energy; however, high levels of the ketones can lead to a dangerous condition called ketoacidosis. And non-insulin dependent diabetes (NIDDM, type II diabetes) Patients with NIDDM are not dependent on exogenous insulin for prevention of ketonuria and are not prone to ketosis. The pancreas produces insulin but the body does not utilize the insulin correctly [11].

The inhibition of the digestive enzymes α-glucosidase and α-amylase, modulation of glucose uptake and the expression of glucose transporters, stimulation of insulin secretion and pancreatic β-cell proliferation, control of insulin resistance, and regulation of oxidative stress are some of the mechanisms of anti-diabetic action. The practice of using plants for management of diabetes is also documented in Ethiopia just like other ailments [12]. As demonstrated by many scholars, the bioactive compounds contained within the plants have shown beneficial effects that can delay the onset of diabetic problems and adjust the metabolic disorders.

In Ethiopia, to the best of my knowledge, no enough research works have been studied scientifically so far on the hypoglycemic activity, Antimicrobial activities, physicochemical characteristics, molecular docking and their chemical constituents of *Salvia Rosmarinus* plant species. Taking advantage of this research gap, researching on pharmacological properties and chemical constituents on the use of these selected medicinal plants was needed. Hence, the present study was investigating on vitro anti-diabetic, total antioxidant content and in vitro antibacterial activity of the crude extracts. We trying to cover the rest merit on the coming article.

The information gathered may be used to increase awareness of people on the role of the traditional uses of these plants and providing scientific evidence to support the claimed ethno medicinal uses of the selected plant species. The outcome of this study will also be expected to pave a way for the scientific communities to carry on further studies on drug designing by providing novel bioactive compounds. Besides, it could give valuable information to the public and also serves as baseline data for researchers engaged in search of selected medicinal plant species.

2. Materials and Methods

2.1. Study Area and Study Design

The Experiment was conducted at Wolaita Sodo University Department of Chemistry. Wolaita is located at 350 km. southwest of the capital city of Ethiopia Addis Abeba, between 6º40" and 7º58" N Latitude and 37º14" and 37º56" E Longitude. With a total area of 438,370 hectares, The Wolaita people are one of the indigenous people of Ethiopia who have their own culture, language, tradition, political legacy and kingdom according to the projected CSA final report of 2007.

2.2. Chemicals and Apparatus

General analytical laboratory grade solvents petroleum ether, ethyl acetate, methanol, acetone, DMSO, α-Amylase, 3,5-Dinitrosalicylic acid, sodium carbonate, sodium potassium tartrate tetrahydrate, sodium hydroxide, starch, sodium chloride, Gallic acid, Quercetin, Folin-Ciocalteu reagent, Aluminum chloride, Sodium hydroxide, Sodium nitrite, phosphate buffer, Gentamycin, Mueller Hinton agar and Nutrient broth ware used.

2.3. Extraction

The 250gram powdered plant sample was sequentially extracted with petroleum ether, ethyl acetate and methanol using maceration technique for 72 hours in each solvent. The extract was filtered and residual solvent from each extract was removed using Rotary evaporator under reduced pressure. The resulting semidried mass of each fraction was stored in shade area until used for experiments.

2.4. Phytochemical Screening

2.4.1. Test for Alkaloids (Wagner's Reagent Test)

About 0.5mL leave extracts of *Salvia Rosmarinus* were dissolved individually in dilute hydrochloric acid and filtered. Then the filtrate was treated with Wagner's reagent (iodine in potassium iodide). Formation of brown/ reddish precipitate indicates the presence of alkaloids [13].

2.4.2. Test for Terpenoids (Salkowski's Test)

To 0.5 mL of the leaves extract, 2 mL of chloroform was added and concentrated Sulphuric acid was added carefully. Formation of red brown colour at the interface indicates the presence of terpenoids [13].

2.4.3. Test for Steroids (Salkowski's Test)

To 2 mL of chloroform extract, 1 mL of concentrated H_2SO_4 acid was added carefully along the sides of the test

tubes. A red color formation in the chloroform layer confirms the presence of steroids [14].

2.4.4. Test for Flavonoids

2 mL of the extract was treated with 2 mL of dilute NH_3 solution and a few drops of concentrated H_2SO_4. A formation of yellow color indicates the presence of flavonoid [13].

2.4.5. Test for Saponins

To a little amount of each of the sample in a test tube, 2 mL of distilled water was added and vigorously shaken for 15 minutes. Formation of 1 cm foam confirms a positive result [13].

2.4.6. Test for Phenols (Ferric Chloride Test)

10 mL of alcoholic solution of extract, 2 mL of distilled water followed by drops of 10% aqueous $FeCl_3$ solution was added. Formation of blue or green indicates the presence of phenols [15].

2.4.7. Test for Tannins (Ferric Chloride Test)

2 mL of the aqueous extract was added to 2 mL of water, 1 to 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins [14].

2.4.8. Test for Glycosides

A small amount of alcoholic extract was dissolved in 1 mL of water and the aqueous NaOH solution was added. Formation of yellow color indicates the presence of glycosides [15].

2.5. Antibacterial Activity Test

Agar diffusion method

The 5 mm diameter sterile discs (Whatman No 3 paper) was placed on the surface of the inoculated Agar in petri dishes, and 20 µL each test solutions ware applied onto the discs. After addition of test solutions on the discs, the extract will be allowed to diffuse for 5 minutes and the plates ware then be kept in an incubator at 37 °C for 24 hrs. The antibacterial activity will be evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with ruler and results will be expressed as Mean ±Std of replicate tests. Standard discs of the antibiotic disc (tetracycline, 30µg/disc) will serve as the positive antibacterial control. For negative control the same volume (20 µL) DMSO poured on paper disks will be used. Antibacterial activity will be recorded if the zones of inhibition will greater than 6 [16, 17]. The disk diffusion assay will be used as a preliminary test to select the most efficient extracts.

2.6. Quantitative Analysis for Antioxidant

2.6.1. Procedure for Determination of Total Polyphenols

Total phenolic content of *Salvia Rosmarinus* leave extracts were determined according to the Folin Ciocalteu method. 0.5mL of plant extract was diluted with 4.5mL of distilled water. Folin-Ciocalteu reagent (0.25mL) was added to 5mL of the solution, followed by 0.5mL of 7% sodium carbonate (Na_2CO_3) solution. The contents were mixed. The mixtures were left for 30 minutes in dark and the absorbencies were measured at 765 nm. The same procedure was used for gallic acid to obtain standard calibration curve [18, 19].

2.6.2. Procedure for Determination of Total Flavonoids

The total flavonoid content of *Salvia Rosmarinus* crude extracts was determined by aluminum chloride assay. 1mL of the sample extract was mixed with 2mL of distilled water in separate test tube, followed by an immediate addition of 0.30ml of 5% $NaNO_2$. 5-minute latter 0.30mL of 10% $AlCl_3$ solution was added. After 6 minute 2.00mL of 1.0 M NaOH solution was added. The absorption at 510 nm using Uv-Vis spectrophotometer was taken after 10 minutes. The same procedure was used for quercetin to obtain standard calibration curve (Ullah, O. S. et al. 2014).

2.7. Anti-Diabetic Activity

A number of bioassay protocols have been established for this purpose [20]. In this study, the following in vitro anti diabetic activity experimental protocols of the crude extracts was followed.

Alpha amylase inhibition assay

The in vitro anti diabetic activity of the crude extract of the selected medicinal herb was evaluated by α-amylase inhibition assays. The enzyme inhibition capacity was expressed as concentration IC50 values in mg/mL [21].

The α-amylase inhibition assay was performed using the 3, 5-dinitrosalicylic acid (DNSA) method. The crude of *Salvia Rosmarinus* was dissolved in buffer ((Na_2HPO4/ NaH_2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 50 to 1000 mg/mL. A volume of 200 mL of a-amylase solution (Molychem) (2 units/mL) was mixed with 200 mL of the extract and was incubated for 10 minutes at 30°C. Thereafter, 200 mL of the starch solution (1% in water w/v) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3, 5-DNSA solution) and was boiled for 10 minutes in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technologies). The blank with was prepared by replacing the plant extract with the buffer. A blank reaction was similarly positive control sample was prepared using acarbose (Bayer) and the reaction was performed similarly to the reaction with plant extract as mentioned above.

The % α-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph.

2.8. Analytical Methods

Calibration curves of standards were constructed to determine total flavonoid contents (TFC) and total phenolic content (TPC).

Linear equations were also derived from each calibration

curves in the form of

$$Y = mX+b \quad (1)$$

Where, *m* is slope

b is y-intercept

Y and *X* is absorbance and concentration respectively

The inhibition of a-amylase was expressed as percentage of inhibition and was calculated by the following equation:

$$\text{Inhibition (\%)} = 1/4[\frac{(Ac-Acb)-(As-Asb)}{(Ac-Acb}] \times 100\%$$

Where Ac: is the absorbance of control; Acb: is the absorbance of control blank; As: is the absorbance of sample; And Asb: is the absorbance of sample blank.

The total flavonoid contents (TFC) and total phenolic content (TPC) of leaves extract of *Salvia Rosmarinus* were determined by calibration curve which was prepared by using quercetin and gallic acid as a standard. Then the total flavonoid and phenolic contents of the extracts (as shown in eq. 4) were expressed in terms of milligram of quercetin equivalent or (mg QE) or gallic acid (mg GAE) per gram of extract.

$$mg\ QEor\ GAE\ /_g = \frac{X.V}{g\ of\ sample} \quad (2)$$

Where, $\frac{mg\ QE\ or\ GAE}{g}$ are quercetin equivalents per g of dry sample.

X is concentration from standard calibration curve = $\frac{Y-b}{m}$ in the equation $Y = mx + b$

Y is Absorbance of the sample

b is the y intercept of the standard graph

m is the slope of the standard graph

V is volume of the sample.

$$\text{Percentage (\%) yield} = \frac{mass\ of\ the\ extract}{initial\ mass\ of\ the\ plant\ powder\ used\ for\ extract} \quad (3)$$

This above equation is used to calculate the percentage yield of extracts.

2.9. Statistical Data Analysis

The data will be expressed as mean ± standard error of the mean (SEM), and statistical analysis between and within group will be carried out by ANOVA (one-way analysis) followed by Tukey post hoc test (for homogenous variances and normally distributed data) and Dunnett's T3 post hock test (for data that will be normally distributed and having heteroscedastic variance).

3. Result and Discussion

The leaves powder (250g) of *Salvia Rosmarinus* was extracted successively with petroleum ether, ethyl acetate and methanol respectively. The extracts were analyzed further for; phytochemical screening, anti-diabetic activity and antibacterial activities.

3.1. Phytochemical Analysis

Qualitative preliminary phytochemical analysis was depicted in Table1 below. phytochemical analysis of the secondary metabolites showed the presence of phenols, steroids, terpenoids, flavonoids, tannins, alkaloids, and glycosides.

Table 1. *Preliminary phytochemical screening of leave extracts of Salvia Rosmarinus.*

Class of compounds	Test reagent	Confirmation color	Crude extracts		
			PE	EtOAc	MeOH
Alkaloids	Wagner's reagent	Brown/ reddish brown precipitate	+	+	+
Anthraquinone	Borntrager's reaction	Red/pink	-	+	+
Phenols	Ferric chloride test	Blue/green	-	+	+
Flavonoids	H_2SO_4	Yellow	-	+	+
Steroids	Salkowski's Test	Red	+	-	-
Terpenes	Salkowski's Test	Reddish brown interface	+	-	-
Saponins	Shaking with water	1 cm foam	-	-	-
Tannins	Ferric chloride	Dark green/ blue green	-	+	+
Glycosides	NaOH test	Yellow	-	+	+

Key: "PE" Petroleum ether, "EtOAc" Ethyl acetate, "MeOH" Methanol + = present - = absent

The qualitative analysis of phytochemical constituents showed the presence of different bioactive compounds in different solvent extracts of *Salvia Rosmarinus* by using color change as an indicator. Comparably, Methanol extract was found to have a wide range of bioactive compounds it may be of its high polarity like flavonoids, alkaloids, anthraquinone, phenols, terpinoids, Taninns, and glycosides while, the petroleum ether extracts show positive result only in some of metabolites, this may due to its non-polar characteristic nature which is different polarity from most of the metabolites.

3.2. Anti-Oxidant Quantitative Analysis

3.2.1. Total Phenolic Content

The total phenolic content in the tested plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid as standard. From the standard calibration curve, equation $y = 0.0014x + 0.3292$ with $R^2 = 0.9994$ was obtained. Where y is absorbance at 765 nm and x is total phenolic content in the different extracts of *Salvia Rosmarinus*. The total phenolic content was found to be (Table2) 339 ± 1.01, 182 ± 3.41, and

28 ± 0.66mg GAE/g in methanol, ethyl acetate and petroleum ether extract respectively. The extracts in methanol and ethyl acetate had the highest levels of phenol. However, petroleum extract has the lowest concentration of phenol. The ability of polar solvents to extract polar compounds like polyphenol in this instance is consistent with the literature.

Table 2. *Total phenolic contents (TPC) for leave extract of Salvia Rosmarinus.*

Crude extracts from	Total Phenolic Content (mg GAE/g) ±STD
Petroleum ether	28 ± 0.66
Ethyl acetate	182 ± 3.41
Methanol	339 ± 1.01

3.2.2. Total Flavonoid Content

Using a colorimetric aluminum chloride assay with quercetin as a reference, the total flavonoid content of Salvia Rosmarinus leaf extracts in methanol, ethyl acetate, and petroleum ether was calculated. Different known quercetin concentrations (20, 40, 60, 80, and 100mg/L) were plotted against absorbance to create the calibration curve. The equation of the calibration curve, y = 0.0171x -0.162, R^2 = 0.9974, where y is the absorbance at 510 nm and x is the concentration of quercetin in mg/L, was used to express the total flavonoid content as mg/g of quercetin equivalent. The total flavonoid content of each plant extract was calculated using this calibration curve. The data in the Table3 below shows that the total flavonoid concentrations of the petroleum ether, ethyl acetate, and methanol extracts were, respectively, 222.70, 152.1, and 113.71 mg QE/g. The methanol extract's g/L flavonoid concentration was the highest.

Table 3. *Total Flavonoid content of different solvent extracts of Salvia Rosmarinus leaves.*

Crude extracts from	Total Flavonoid Content (mg GAE/g) ±STD
Methanol	222.70 ± 0.111
Ethyl acetate	152.1 ± 1.21
Petroleum ether	113.71 ± 2.99

3.3. Anti-Diabetic Activity

The Anti-diabetic activity of different solvent extracts from *Salvia Rosmarinus* was determined by α-amylase inhibition assay using the 3, 5-dinitrosalicylic acid (DNSA) reagent. A large decrease in the absorbance of the reaction mixture indicates significant inhibiting activity of the compound under test.

The inhibiting ability of methanol, ethyl acetate and petroleum ether extract of *Salvia Rosmarinus* was evaluated by constructing calibration curve of Acarbose standard.

The calibration curve was plotted as absorbance and % inhibition (Table4) versus different concentrations (20, 40, 60, 80 and 100ppm) of Acarbose. A straight line with a linear regression equation, y = - 0.0093x + 1.1571 and linear regression coefficient, R^2 = 0.9947 was obtained from this curve. Based on the calibration curve of Acarbose, the inhibiting ability of methanol, ethyl acetate, and petroleum ether extracts were evaluated by comparing the extracts curve with the standard linear curve and by using color change as the reagent was added and recording the absorbance of each extract at a different concentration (Table 4). Quantitively Anti-diabetic ability of methanol, ethyl acetate and petroleum ether extracts were evaluated by using color change. In this study, Maltose reduces the pale yellow colored alkaline 3, 5-Dinitro salicylic acid (DNS) to the orange- red colored. The intensity of the color is proportional to the concentration of maltose present in the sample, depending upon the concentration of reducing sugar present. The DNSA test can detect concentrations of glucose between 0.5 mM (0.09% glucose w/v) and 40 mM (0.72% glucose w/v). The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water the concentration of reducing sugar, and the intensity change in color is measured using a UV-visible spectrophotometer (Agilent Technologies). Wave length is set to 540 nm because it is the region where orange-red color absorbs.

Miller GL. [22] pointed out DNS consistently gave greater color with disaccharides than with monosaccharide. As the type of monomers and nature of bonding in these disaccharides was different it is improbable that each disaccharide was partially hydrolyzed during the assay to exactly the same extent. Interpretation of the results is somewhat complicated by the observation that the monosaccharaides fructose and galactose gave a lower color reaction than glucose. As Fructose is ketoses this difference in behavior could have been the result of different functional groups. However, the reaction of galactose, an aldose, resembled to that of fructose instead of glucose. These findings suggest that color formation with DNS and reducing sugars is not exclusively due to reduction of DNS to ANS. So, Miller GL. [22] concern it is still valid that "different sugars yield different amounts of color suggest that the chemistry of the test may actually be appreciably more complicated". For instance, the methanol extract showed the highest anti-diabetic activity from 59.77% to 94.19%, which is very close to the activity of acarbose a standard (72.36% to 95.71%). The ethyl acetate and petroleum ether extracts showed very slow at minimum concentration but rapidly increase their activity when their concentration becomes large from 11.31% to 94.11% and from 6.33% to 76.6% respectively. The high percentage inhibition for methanolic extract could be due to high polarity nature of solvent. As the concentration of phenolic compound increase degree of hydroxylation of the phenolic compound also increase which result to increase the scavenging activity (% inhibition).

Table 4. *Percent (%) of inhibition of Acarbose, petroleum ether extract (PE), ethyl acetate (EA), and methanol extract (ME).*

Concentration in mg/L	%Inhibition			
	Acarbose	PE	EA	ME
20	72.36	6.33	11.31	59.77
40	83.34	18.56	37.45	77.65
60	89.74	26.26	52.22	85.42
80	93.02	51.00	72.58	89.56
100	95.71	76.6	91.44	94.19

The % inhibition of each extract similarly increases with

concentration, as seen in Table 4, and figure 3 suggesting that all methanol, ethyl acetate, and petroleum ether extracts have a larger potential to exert anti-diabetic effects at a higher concentration.

3.4. Antibacterial Susceptibility Test

The antibacterial activity of petroleum ether, ethyl acetate, and methanol leaves extract of Salvia Rosmarinus was determined using the Agar disk diffusion method in the biology department's microbiology laboratory at Wolaita Sodo University. In this Antibacterial susceptibility test, three bacteria strains were used: two-gram positive bacteria (S. aurous and S. pyrogens) and one-gram negative bacteria (E. coli). A series of concentrations (31.25, 62.5, 125, and 250mg/ml) of each extract were made using the serial dilution method and diffused into incubated plates containing bacteria, and inhibitory zone (Tables 5 & 6) values were recorded. As shown in table 6 and figure 5, Methanol extract had shown a significant antibacterial test result whereas there was nebulous inhibition zone recorded in extracts in all three bacteria. petroleum ether extracts had lesser and even no potential antibacterial activity against the selected bacterial species as depicted in figure 7. Most of petroleum extract of this herb show nothing in gram negative bacterial strain. The antibacterial activity of mean value of each extract was statistically significant ($P \leq 0.05$).

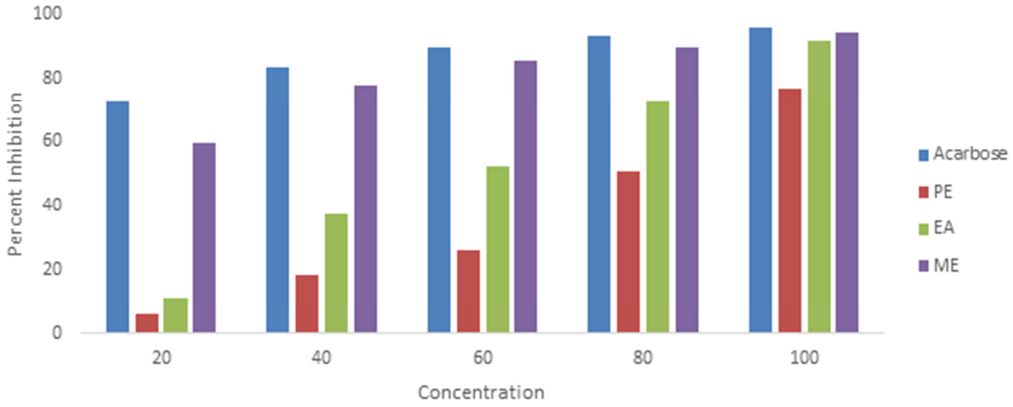
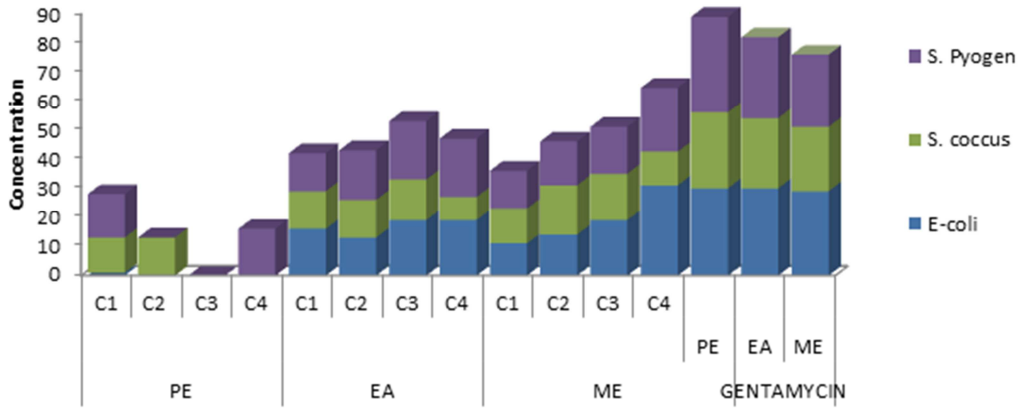


Figure 3. *Percent (%) Inhibition comparison chart.*

Table 5. *A zone of inhibition of different extracts of Salvia Rosmarinus leaves against gram positive bacteria.*

Bacterial strain	Concentration in (mg/L)	Mean zone of inhibition (mm)			
		ME	EA	PE	Tetracycline
Staphylococcus aureus	31.25	12 ±0.28	13 ±1.41	12 ± 0.42	24.0±0.2
	62.5	17 ±0.99	13 ± 0.42	13 ± 0.00	
	125	16 ±0.42	14 ± 0.71	0	
	250	11.5 ±1.63	8 ± 1.13	0	
Streptococcus Pyogenes	31.25	13 ±0.99	13 ± 1.27	15 ± 0.71	28.7 ±0.40
	62.5	15 ±1.84	17 ± 0.42	0	
	125	16 ±0.14	20 ± 0.71	0	
	250	22 ±0.28	20 ± 1.27	16 ± 0.70	



Key; C1=31.25, C2 = 62.5, C3 = 125, C4 = 250mg/LPE = petroleum ether extract, EA = ethyl acetate, ME = methanol

Figure 4. *Comparison of inhibition zone among different solvent crude extracts of Salvia Rosmarinus leaves and with standard antioxidants (Tetracycline (30µg)) against different bacteria chart.*

Table 6. *Zones of inhibition of different extracts of Salvia Rosmarinus leaves against gram negative bacteria.*

Bacterial strain	Concentration in (mg/L)	zone of inhibition (mm)			
		Methanol	Ethyl acetate	Petroleum ether	Tetracycline
Escherichia coli	31.25	11 ±0.14	16 ±0.42	0	29.7± 0.058
	65	14 ±0.71	13 ±0.84	0	
	125	19 ±0.42	19 ±0.28	0	
	250	31 ±0.14	19 ±1.13	0	

All Extracts Had Lowest Antibacterial Potential As Compared with the Antibiotics Used as Standard

(Tetracycline). In this study a good antibacterial result was recorded at higher concentration. Methanol extract showed a maximum and minimum zone antibacterial result against negative bacteria *E. coli* 31 + 0.140. Ethyl acetate extract shows a maximum and minimum zone of inhibition against *S. pyrogens, and S. aureus* 20 + 0.71 and 8 +1.13nm respectively as shown in figure 6, however, most of the petroleum ether test shows null zone of inhibition. The highest minimum zone of inhibition was recorded in methanol against a gram-negative bacterium *E. coli* with minimum zone of inhibition 31 + 0.14nm. Similar results showing that the alcoholic extract having the best antimicrobial activity against gram negative bacterial is also reported [23]. Methanol extracts of this plant also showed highest inhibition zones compared to the positive control (Tetracycline) against *E. coli* bacterium. This might indicate the ingredients of the plant extract are more potent than the antibiotics. Most of the petroleum extract have no zone of inhibition. In general, the methanol and ethyl acetate extracts were shown a good result at a higher concentration than petroleum ether extract as depicted in Figure 4.

The methanolic extract result agrees with previous reports by [24]. Gram-negative bacteria have an outer phospholipidic membrane that makes the cell wall impermeable to lipophilic solutes, whereas the porines contain a selective barrier to hydrophilic solutes. Several research findings supported this justification, extracts from some medicinal plants were found to be more effective against Gram positive bacteria than Gram-negatives. This difference may be due to difference in experimental protocols and source of bacterial strains. From the phytochemical and TPC and TFC determination, we have seen that the extracts have measurable flavonoids and thus antibacterial flavonoids (e g. Catechins) might be having multiple cellular targets, rather than one specific site of action. One of their molecular actions is to form complex with proteins through nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation. Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and so forth. Lipophilic flavonoids may also disrupt microbial membranes.

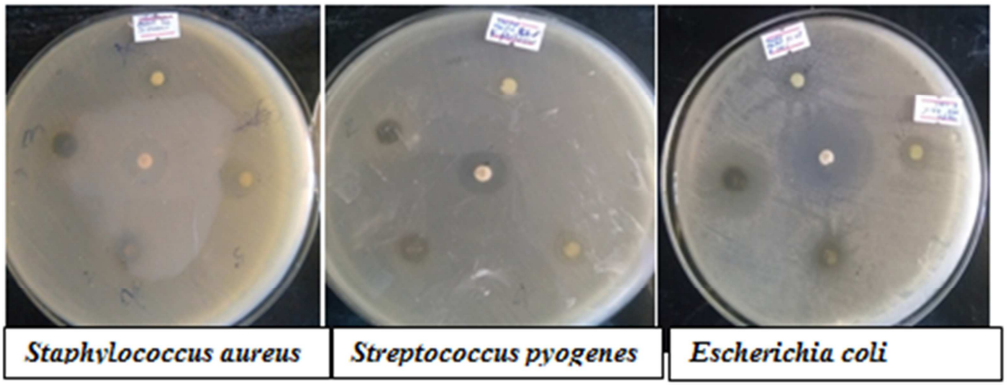


Figure 5. *Escherichia coli, Staphylococcus aureus, and pyrogen aerogenes inhibition of methanol extracts of Salvia Rosmarinus.*

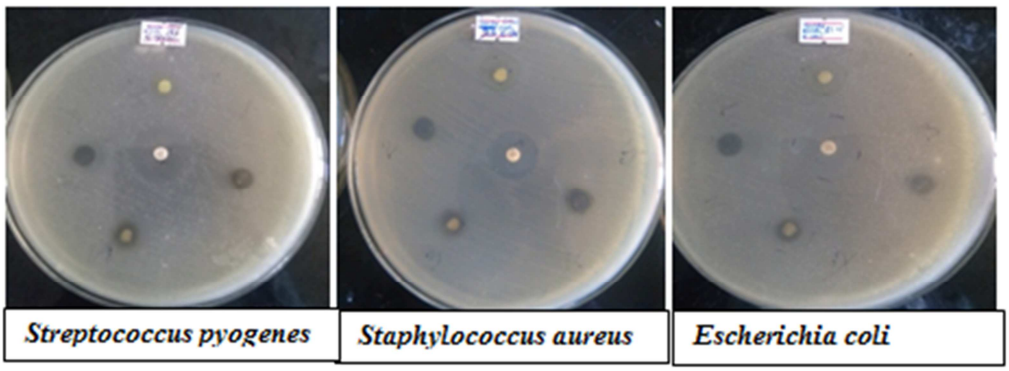


Figure 6. *Staphylococcus aureus, Escherichia coli, and pyrogen aerogenes inhibition of ethyl acetate extract of Salvia Rosmarinus.*

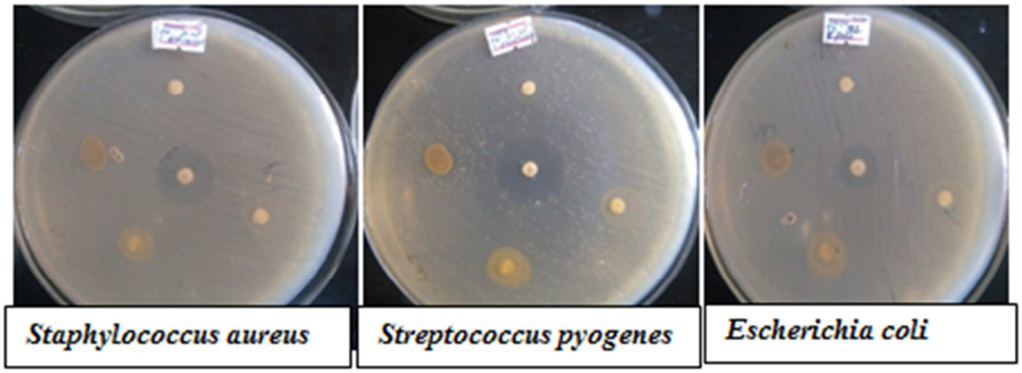


Figure 7. *Escherichia coli, Staphylococcus aureus, and pyrogen aerogenes inhibition zone of petroleum ether extract of Salvia Rosmarinus.*

4. Conclusion and Recommendation

The result of this study clearly indicated the all investigated *Salvia Rosmarinus* leaves extracts have anti-diabetic activity against various anti diabetic assays, α-amylase inhibitions is one of the assays of in vitro colorimetric method using DNSA reagent. In all anti-diabetic activity measurement, methanol, as extraction solvent, showed highest reducing capacity and contained maximum bioactive compounds than ethyl acetate and petroleum ether extracts. Both methanol and ethyl acetate extracts showed a significant anti-diabetic capacity in α-amylase assays. These two extracts also showed higher α-amylase potential with respect to concentration. In addition, this study showed that *Salvia Rosmarinus* leaves extracts had noticeable amount of total phenols, and flavonoids which play major role in controlling the disease caused by oxidation. Even we have to do the correlation results from statistical analyses, the anti-diabetic activity was significantly correlated with TPC and TFC based on α-amylase assays. Moreover, the anti-diabetic activity of *Salvia Rosmarinus* is not only due to phenols and flavonoids but also other secondary metabolites. Thus, flavonoid and phenols are the most important substances that contribute to the anti-diabetic of *Salvia Rosmarinus*. Except petroleum ether extract, most methanol and ethyl acetate extracts had antibacterial activities which were concentration dependent. As the concentration increases antimicrobial activities of methanol and ethyl acetate extracts had more zone of inhibition. From its phytochemical and antibacterial activity results the plant might have high level of medicinal values.

Recommendation

This is a work in progress. This study showed that the leaves extracts of *Salvia Rosmarinus* had good indication for anti-diabetic and antibacterial activities. We can say that this research has been begun but it is not enough and not completed. It has to done with another different assay and different pharmacological application even its edibility (physicochemical characteristics). Moreover, further investigations will be needed to isolate and characterize the individual compounds with different solvent. And further similar studies should be conducted on other parts of the plant. Studies need to be conducted to identify flavonoid and phenolic compounds that are correlated with the anti-diabetic activity of *Salvia Rosmarinus* as well as their synergistic interactions.

Novelty Statement

These leaves are frequently used to cook meat in Ethiopia. We are attempting to analyze the phytochemical component, anti-diabetic, and quantitative antioxidant characteristics of the Salvia Rosmarinus herb's leaves in this research piece. Although there is a wealth of knowledge describing the long-term, uneventful local use of this plant, there is less scientific evidence supporting its effectiveness in a variety of biological activities.

Competing Interests

The authors have declared no conflict of interest.

Author’s Contribution

Material preparation, collecting material data collection and drafted the work were performed by Lomi Abayneh and Tangut Masreshaw. Analysis and interpretation of data were written by Alebachew Molla. Conceptualizing of the findings were written by Hailemariam Assefa. Generating idea, doing experimental work, analysis and overall control of the work were performed by Tesfahun Dagnaw and All authors read and approved the final manuscript.

Availability of Data and Materials

All the data in this research are included in the manuscript. The data and materials of the study are available on request.

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