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Research Paper

Phytochemical screening and antibacterial activity of rosmarinus officinalis l. against Escherichia coli. local isolates

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Abstract

Rosmarinus officinalis is a medicinal plant which belongs to family Lamiaceae. It is an evergreen bush. It was analyzed for its phytochemical constitution and antibacterial activity. For this purpose flowers, leaves and stem of aqueous and methanolic extracts were used. The tests of phytochemical screening showed that flavonoids, terpenoids, reducing sugar and saponins were present in different concentrations, while tannin was absent in all parts of plant. The antibacterial activity was determined against E. coli with different concentrations (i-e 0.5gm, 1gm and 1.5gm) kept for 24 hours and 48 hours duration. The results showed that the highest mean inhibition zone was observed in the methanolic extracts of leaves 1.5gm kept for 48 hours (28.6 ± 4.261mm).In the stem the methanolic extracts showed the highest inhibition zone with the concentration of 1.5gm (13.4 ± 1.624mm) kept for 24 hours. In the flower the highest inhibition zone in methanolic extracts was observed in 1gm (25.4 ± 2.416mm) kept for 48 hours. In the aqueous extracts the highest inhibition zone values were (17.6 ± 4.029mm, 15.8 ± 2.993mm, 14.4 ± 2.33mm) for stem, flower and leaves respectively.

Keywords: *Aqueous Extract. Methonolic Extract; Concentrations; Phytochemical; Antibacterial.*

1. Introduction

Rosmarinus officinalis is a medicinal plant it belongs to the family Lamiaceae [2]. It is an evergreen, perennial bush. Its height is 1-2 meters long [32]. It is a much branched shrub. The branches are cylindrical and the bark of this plant is pale brown fibrous. The leaves of this plant are evergreen opposite, 1-1 inch long, blunt at both ends, densely covered with white stellate hairs. The flower of this plant is shortly stalked; rather large arranged in opposite pair bracts short acute, calyx is tubular and deeply cut into 2 lips. Corolla has a short tube it is also strongly 2 lipped. The upper lip is cut into two segments. The stamens are filamentous [4].

It is native of Mediterranean areas of new East and Europe [27]. It is abundantly found in North Africa, Madeira and Canary Islands. In South France and North Italy the wild plant is gathered for use [5]. This plant showed good adaptability in cold and dry areas [2]. This plant is cultivated in all countries as an ornamental and medicinal plant [27]. It is aromatic and seasoning plant due to which it is used in cosmetic and perfume industries [20]. It is also used as food spices [23]. And also used as a flavoring agents [8].

About 80 % of the world population uses the medicinal plants for the health needs. The inter-relationship among plants, man and drugs derived from the plants which are describing the history of mankind. Plants are the great source of medicines. In plants different phytochemicals were present which are active against different diseases [31]. Plants contain different chemical compounds called secondary metabolites are involved in the growth and developments of plants. Some important secondary metabolites are alkaloids, terpenes, and phenolic compounds [41]. Plants contain different anti-oxidant compounds including phenolic compounds like phenol acid, flavinoids, tannins etc [25], [34].

Rosemary is a medicinal plant and it is used in many ways as a medicinal herb such as it improves the blood circulation [20]. The extract of rosemary relaxes the smooth muscles [14]. The volatile oil of Rosemary is a powerful growth

stimulator. The volatile oil of Rosemary with the combination of other substances is used for the growth of hairs because it promotes the growth of hairs [5]. It has a strong aromatic odour and bitterish taste [24]. It was used in cosmetics and perfume industries [17]. It is commonly used as flavoring agent [18]. The volatile oil of rosemary was a powerful growth stimulator [5].

Different medicinal properties are associated with the Rosemary such as antibacterial, antiviral activity, antitumor activity, due to these activities it is traditionally used medicinally [8], [39].

The plant contains different phyto chemicals such as flavinoids, phenols, volatile oil, terpenoids, caffic, rosemarinic, labiaticacids. The volatile oil contains different components composed of mainly monoterpenes and hydrocarbons which include alpha and beta pinenes, camphene and limonine. Some natural pigments were reported in this plant which is capsaicin and curcumin. Due to which this plant as a great value in medicines, aromatic and as an ornamental plant [8], [29].

Due to the presence of phytochemical compounds different medicinal properties are associated with this plant including antibacterial, antiviral, anti-tumor and antioxidant activities [43]. The extracts of rosemary contain bioactive properties such as phenolic compound which inhibit the growth of gram negative and gram positive bacteria [32]. Rosemary oil has an effective antimicrobial agent it inhibit the growth of molds and many types of bacteria. Carnosolic acid which was present in Rosemary had activity against human immunodeficiency virus type 1 (HIV-1) [18].

Escherichia coli are gram negative bacteria and are a symbiotic bacillus of colon of human beings and vertebrates. This bacterium is easy to grow in the artificial medium at 37˚c under optimal conditions it divides after every 20 minutes [32].

2. Material and methods

Plant material was collected from Sardar Bahadur Khan Women's University Quetta botanical garden (botany department) on 26th of March 2011. Plant material was dried for a week at room temperature and grained. The material was used for different phytochemical tests and antibacterial activity.

For the determination of phytochemistry 25 g of dried powder of plant material was mixed in 100 ml of methanol and kept for 24 hours then filtered [9], [15], [22], [38].

2.1. Antibacterial activity of Rosemarinus officinalis

The experiment was conducted to determine the antibacterial activity of the extracts of Rosemarinus officianalis against E. coli. For this purpose the stem, leaves and flowers of Rosemarinus officianalis collected from SBK Women's University Quetta were used. The following steps were employed during the laboratory work.

The key step for the successful determination of antibacterial activity of an organism is sterilization. Sterilization was followed by two methods: dry and wet sterilization. All The glassware (Petri-plates) were covered with aluminium foil and then kept into the Oven at 90˚C for two hours.

2.1.1. Preparation of extracts

a) 0.5gm flower extracts of Rosemarinus officianalis for 24 and 48 hrs:

Weight four samples of 0.5 gm of flower powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

b) 1gm flower extract of Rosemarinus officianalis for 24 and 48 hrs:

Weight four samples of 1gm of flower powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

c) 1.5gm flower extract of Rosemarinus officianalis for 24 and 48 hrs:

Weight four samples of 1.5 gm of flower powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

d) 0.5gm leaves extract of Rosemarinus officianalis for 24 hrs and 48 hrs:

Weight four samples of 0.5 gm of leaf powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

e) 1gm leaves extract of Rosemarinusofficianalis for 24 hrs and 48 hrs:

Weight four samples of 1gm of leaf powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

f) 1.5 gm leaves extracts of Rosemarinus officianalis for 24 hrs and 48 hrs:

Weight four samples of 1.5 gm of leaf powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

g) 0.5 gm stem extracts of Rosemarinus officianalis for 24 hrs and 48 hrs:

Weight four samples of 0.5 gm of stem powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

h) 1gm stem extracts of Rosemarinus officianalis for 24 hrs and 48 hrs:

Weight four samples of 1gm of stem powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

i) 1.5 gm stem extracts of Rosemarinus officianalis for 24 hrs and 48 hrs:

Weight four samples of 1.5 gm of stem powder and put it separately into four 100 ml conical flasks and mark them as 24 hrs and 48 hrs. Add 100 ml distilled water in two flasks and 100 ml of Methanol in two flasks shake it well and leave it for 24 hrs and 48 hrs. After the completion of time filter the solution and soak the Watmann's filter paper discs into the filtrate.

2.1.2. Preperation of solid medium of agar

28 gm of agar nutrient was dissolved in 1000 ml of distilled water. Then the solution was kept on hot plate for some time. Than autoclaved for 45 minutes.

2.1.3. Agar in petri-plates

The prepared agar was poured in the Petri plates in front of flame under Laminar flow. Before use the floor of Laminar flow was wiped with 70% ethanol. After pouring the Petri dishes were kept in the Incubator for 24 hrs in inverted position.

2.1.4. Preparation of filter paper discs

Watmann's filter paper was used to prepare filter paper discs. Several discs were made with the help of punching machine. The discs were soaked in the filtrates and then placed in the Petri dishes.

2.1.5. Inoculation of E.coli in the petri-plates

Under the Laminar flow in front of flame inoculate the E. coli in the medium. With the help of inoculating loop the colonies of E. coli were mixed in the distilled water and then with the help of cotton transferred into the Petri-plates. At the same time filter paper discs soaked into the solution were also placed into the Petri-plates.

2.2. Phytochemical screening

a) Test for reducing sugar:

0.5ml of plant extract was mixed with 5ml of boiling Fehling solution (A and B) in a test tube.

Fehling solution A:

Take 6.93g $CuSO_4$and dissolve in 100ml water.

Fehling solution B:

Take 34.6g KNaCu + 10g NaOH and dissolve in 100 ml water.

b) Test for flavinoids:

0.5ml plant extract was added with 5ml of water, 5ml of dilute ammonia and 1ml of conc.H2SO4.

c) Test for saponins:

0.5ml plant extract was added with 5ml of water in a test tube and shake it vigoursly. A stable forthning was formed then add few drops of olive oil and shake. An emulsion was formed.

d) Test for terpenoids:

0.5ml of plant extract was added with 2ml of chloroform and 3ml of conc. H2SO4.

e) Test for tannins:

0.5ml of plant extract was boiled with 10ml of water then filtered. Few drops of 0.1% ferric chloride were added.

3. Results

3.1. Antibacterial activity of Rosemarinus officinalis

This study was conducted to analyze the antibacterial activity of the aqueous and methanolic extracts of Rosemarinus officinalis against E. coli. The results were:

a) 0.5gm leaves aqueous extract of Rosemarinus officinalisfor 24 hrs and 48 hrs:

It was observed that when 0.5gm aqueous leaf extract of Rosemarinus officinalis was kept for 24 and 48 hrs, it exhibit high value of mean inhibition in 48 hrs (11.8mm) as compared to 24 hrs extract (11.4mm).

b) 0.5gm leaves methanolic extracts of Rosemarinus officinalis for 24hrs and 48 hrs

The methanolic leaves extract kept for 24hrs and 48 hrs showed the high mean of inhibition in 48 hrs extract that was 17.2mm as compared to 24 hrs extract 14.6mm.

c) 1gm leaves aqueous extract of Rosemarinus officinalisfor 24 hrs and 48 hrs:

For 1gm of aqueous leave extracts of Rosemarinus officinalis the high value of inhibition was found in 24 hrs extract (12.8mm) while in 48 hrs extract it was (12mm).

d) 1gm leaves methanolic extracts of Rosemarinus officinalisfor 24 hrs and 48 hrs:

For1gm methanolic extracts of leaves the high mean value of inhibition was showed by 24 hrs extract (22.8mm) and for 48 hrs extract it was (13.6mm).

e) 1.5gm leaves aqueous extracts of Rosemarinus officinalisfor 24 hrs and 48 hrs:

1.5gm aqueous leaf extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 24 hrs extract (14.4mm) and for 48 hrs extract it was (13mm).

f) 1.5gm methanolic leaves extracts of Rosemarinus officinalisfor 24 hrs and 48 hrs:

It was observed that when 1.5gm of methanolic leaves extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 48 hrs extract (28.8mm) as compare to 24 hrs extract (23.6mm).

g) 0.5gm stem aqueous extract of Rosemarinus officinalisfor 24 hrs and 48 hrs:

It was observed that when 0.5gm aqueous stem extract of Rosemarinus officinalis was kept for 24 and 48 hrs, it exhibit highest value of mean inhibition in 24 hrs(16.8mm) as compared to 48 hrs extract (10mm).

h) 0.5gm stems methanolic extracts of Rosemarinus officinalisfor 24hrs and 48 hrs:

The methanolic stem extract kept for 24hrs and 48 hrs showed the high mean of inhibition in 48 hrs extract that was 11.8mm as compared to 24 hrs extract 10.4mm.

i) 1gm stem aqueous extract of Rosemarinus officinalisfor 24 hrs and 48 hrs:

For 1gm of aqueous stem extracts of Rosemarinus officinalis the high value of inhibition was found in 24 hrs extract (12.2mm) while in 48 hrs extract it was (12mm).

j) 1gm stem methanolic extracts of Rosemarinus officinalisfor 24 hrs and 48 hrs:

For1gm methanolic extracts of stem the high mean value of inhibition was showed by 48 hrs extract (9.4mm) and for 24 hrs extract it was (8.8mm).

k) 1.5gm stem aqueous extracts of Rosemarinus officinalisfor 24 hrs and 48 hrs:

1.5gm aqueous stem extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 48 hrs extract (17.6mm) and for 24 hrs extract it was (12.6mm).

l) 1.5gm methanolic stem extracts of Rosemarinus officinalisfor 24 hrs and 48 hrs:

It was observed that when 1.5gm of methanolic stem extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 24 hrs extract (13.4mm) as compare to 48 hrs extract (8.6mm).

m) 0.5gm flower aqueous extract of Rosemarinus officinalis for 24 hrs and 48 hrs:

It was observed that when 0.5gm of methanolic flower extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 24 hrs extract (15.4mm) as compare to 48 hrs extract (10mm).

n) 0.5gm flower methanolic extracts of Rosemarinus officinalis for 24hrs and 48 hrs:

The methanolic flower extract of 0.5gm, kept for 24hrs and 48 hrs showed the high mean of inhibition in 48 hrs extract that was 15.4mm as compared to 24 hrs extract 13.4mm.

o) 1gm flower aqueous extracts of Rosemarinus officinalis for 24 hrs and 48 hrs: It was observed that when 1gm of methanolic flower extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 24 hrs extract (15.8mm) as compare to 48 hrs extract (12.2mm).

p) 1gm flower methanolic extracts of Rosemarinus officinalis for 24 hrs and 48 hrs:

The methanolic flower extract of 1gm, kept for 24hrs and 48 hrs showed the high mean of inhibition in 48 hrs extract that was 25.4mm as compared to 24 hrs extract 19.4mm.

q) 1.5gm flower aqueous extracts of Rosemarinus officinalis for 24 hrs and 48 hrs:

It was observed that when 1.5gm of methanolic flower extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 24 hrs extract (13.2mm) as compare to 48 hrs extract (12.8mm).

r) 1.5gm flower methanolic extracts of Rosemarinus officinalis for 24 hrs and 48 hrs:

1.5gm methanolic flower extracts of Rosemarinus officinalis kept for 24 hrs and 48 hrs, it exhibit high value of mean inhibition in 48 hrs extract (25mm) and for 24 hrs extract it was (15.2mm).

Table 1: Showing Mean and Standard Error of Leaves Aqueous and Methanolic Extracts of Rosemarinus Officinalis 24 Hours (0.5g, 1g and 1.5g) Against E. coli

Concentration In Grams.	Water	Methanol
	$\bar{x} \pm \partial$	$\bar{x} \pm \partial$
0.5g	11.4 ± 2.33	14.6 ± 1.496
1g	12.8 ± 3.682	22.8 ± 3.969
1.5g	14.4 ± 2.33	23.6 ± 2.416

Table 2: Showing Mean and Standard Error of Leaves Aqueous and Methanolic Extracts of Rosemarinus Officinalis 48 Hours (0.5g, 1g and 1.5g) Against E. coli:

Concentration In Grams.	Water	Methanol
	$\bar{x} \pm \partial$	$\bar{x} \pm \partial$
0.5g	11.8 ± 1.6	17.2± 3.815
1g	12 ± 2.280	13.6 ± 3.498
1.5g	13 ± 3.464	28.8 ± 4.261

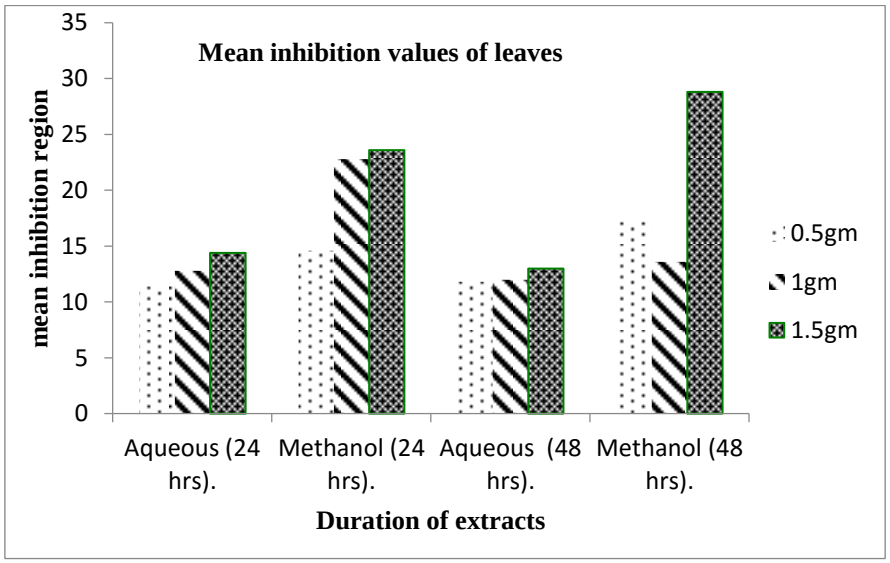


Fig.1: Showing the mean inhibition region of the extracts of leaves of Rosemarinus officinalis.

Table 3: Showing Mean and Standard Error of Stem Aqueous and Methanolic Extracts of Rosemarinus Officinalis 24 Hours (0.5g, 1g and 1.5g) Against E. coli

Concentration In Grams.	Water	Methanol
	$\bar{x} \pm \partial$	$\bar{x} \pm \partial$
0.5g	16.8 ± 2.315	10.4 ± 2.24
1g	12.2 ± 1.939	8.8 ± 1.939
1.5g	12.6 ± 1.362	13.4 ± 1.624

Table 4: Showing Mean and Standard Error of Stem Aqueous and Methanolic Extracts of Rosemarinus Officinalis 48 Hours (0.5g, 1g and 1.5g) Against E. coli

Concentration In Grams.	Water	Methanol
	$\bar{x} \pm \partial$	$\bar{x} \pm \partial$
0.5g	10 ± 1.095	11.8 ± 1.833
1g	12 ± 2.280	9.4 ± 0.8
1.5g	17.6 ± 4.029	8.6 ± 1.854

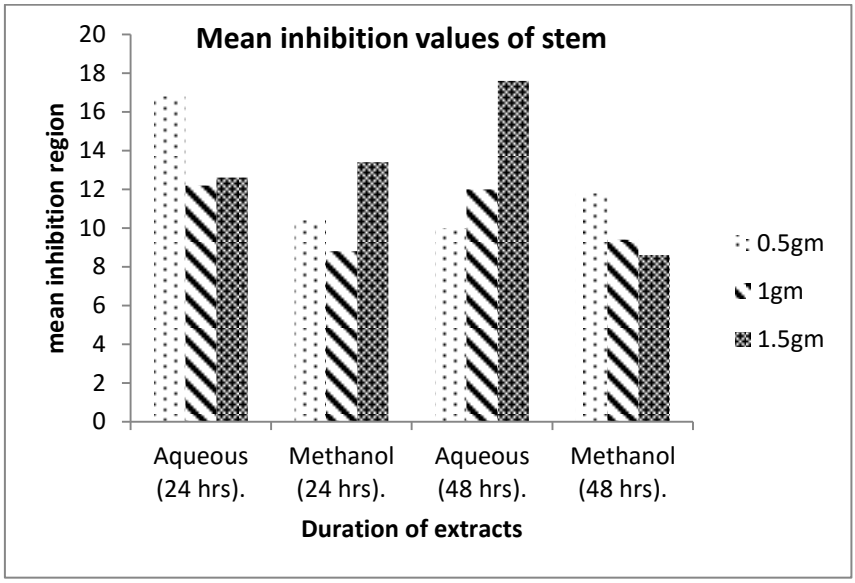


Fig.2: Showing the mean inhibition value of the extracts of stem of Rosemarinus officinalis.

Table 5: Showing Mean and Standard Error of Flower Aqueous and Methanolic Extracts of Rosemarinus Officinalis 24 Hours (0.5g, 1g and 1.5g) Against E. coli

Concentration In Grams.	Water	Methanol
	$\bar{x} \pm \partial$	$\bar{x} \pm \partial$
0.5	15.4 ± 3.555	13.4 ± 2.332
1	15.8 ± 2.993	19.4 ± 5.748
1.5	13.2 ± 1.166	15.2 ± 3.762

Table 6: Showing Mean and Standard Error of Flower Aqueous and Methanolic Extracts of Rosemarinus Officinalis 48 Hours (0.5g, 1g and 1.5g) Against E. coli

Concentration In Grams.	Water	Methanol
	$\bar{x} \pm \partial$	$\bar{x} \pm \partial$
0.5	10 ± 1.673	15.4 ± 5.388
1	12.2 ± 1.720	25.4 ± 2.416
1.5	12.8 ± 2.315	25 ± 4.816

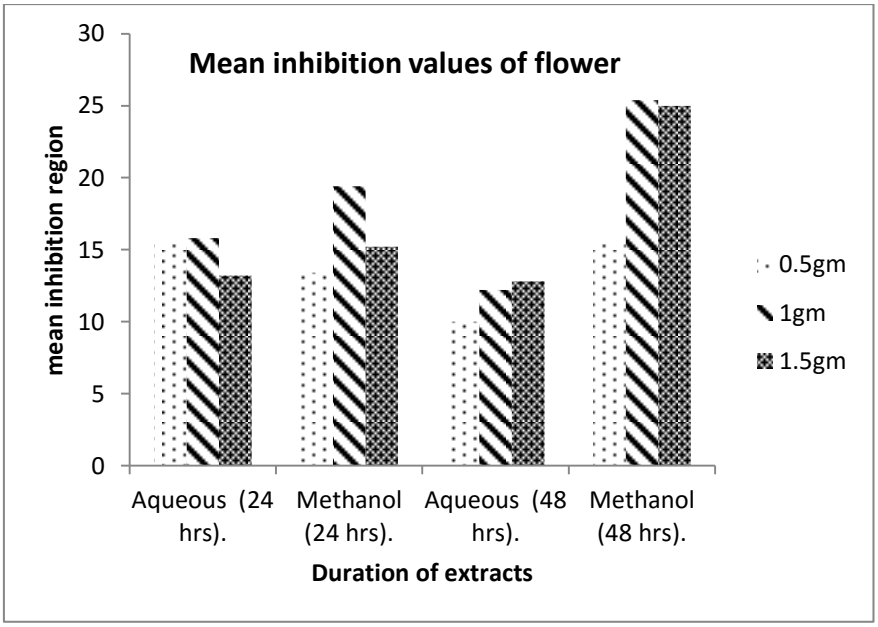


Fig.3: Showing the mean inhibition value of the extracts of flowers of Rosemarinus officinalis.

3.2. Phytochemical analysis of Rosemarinus officinalis

TESTS.	PROCEDURE.	LEAVES.	FLOWERS.	STEM.	RESULT.
Reducing sugar.	0.5 ml plant extract +Fehling solution A and B +boiled.	+ Yellowish green color appeared.	+ Dark green color appeared.	+ Dark green color appeared.	Low conc. in leaves while high in stem and flowers.
Terpenoid.	0.5 ml plant extracts +2ml chloroform +3 ml conc.H2SO4.	+ Reddish brown color appeared.	+ Reddish brown color appeared in the form of rings.	+ Reddish brown color appeared.	High conc. In flowers while low in stem and leaves.
Flavinoid.	0.5 ml plant extract + 5ml ammonia + 1ml conc. Sulphioric acid.	+ Yellow color appeared.	– No reaction.	+ Light yellow color appeared.	Absent in flowers while high conc. in stem.
Tannin.	0.5 ml plant extract + 10ml water+ boiled+ 0.1 % ferric chloride.	– Light yellow color appeared.	– Light yellow color appeared.	– No reaction.	Absent.
Saponins.	0.5 ml plant extract+ 5ml water+ 3 drops of olive oil.	+ Oil emulsion is formed on the top of solution.	+ Oil emulsion is formed.	+ Oil emulsion is formed.	High conc. in leaves while low in stem and flower.

(+) = Present
(-) = Absent

4. Discussion

The Rosemarinus officinalis was investigated for its phytochemical constitution and anti-bacterial activity. The plant material was collected from Sardar Bahadur Khan Women's University Quetta. Stem, leaves and flowers of Rosemarinus officinalis were used for this purpose. Different phytochemical compounds were found in different concentrations. Flavinoids, phenols, volatile oil, terpenoids and tennic acid were the principle constituents of Rosemary. It showed good inhibitory effects against E. coli [21].

The in vitro antibacterial activity of methanolic and aqueous extracts of Rosemary at different concentrations was done against E. coli.

The methanolic extracts of leaves 1.5gm showed highest value of mean inhibition zone that was 28.6mm [10]. Our results agree with the findings of [16].

The second highest inhibition zone was observed in the methanoilc extracts of flower kept for 48 hours with the concentration of 1gm (25.4 ± 2.416mm).

In the aqueous extracts the highest inhibition zone (17.6 ± 4.029mm) was observed in the stem with the concentration of 1.5gm for 48 hrs extract [34].

The test of reducing sugar was done, yellowish green color appeared which was changed in dark green color and indicates the presence of reducing sugar [7].

Terpenoids were present in high concentration in all the parts including flower, leaves and the stem. In the test of flower 3 rings of different colors appeared on the test tube. The top ring was brown in color, in middle it was green and the upper ring was brown colored. These results agree with the work of [36], [6]

The test of flavinods showed different concentrations in the leaves and stem while it was absent in the flowers. In the stem it was present in high concentration while in the leaves it was found in medium concentration. Aeyni & Yahya reported the same results [6].

Tannins were absent in all the parts of Rosemarinus officinalis.

Saponins were present in high concentration in the leaves, stem and flower. An oil emulsion was formed on the top of the solution which indicates the presence of saponins. Our results agree with the results of [1].

The presence of these phyto-chemical compounds indicate that various medicinal properties were associated with Rosemary which supports the traditional and medicinal uses of this plant [3].

5. Conclusion

Rosemarinus officinalis was analyzed for its anti-bacterial activity against E. coli and phytochemical composition. The plant material was collected from Sardar Bahadur Khan Women's University Quetta. To investigate the anti-bacterial activity of Rosemarinus officinalis aqueous and methanolic extracts of the flowers, leaves and stem were prepared and kept for 24 hours and 48 hours with the concentration of 0.5gm, 1gm and 1.5gm.

Highest concentrations of extracts revealed more inhibition against E. coli then the lower concentrations.
Highest inhibition zone was observed in the leaves methanolic extracts of 1.5gm kept for 48 hours (i-e. 28.8 ± 4.261mm). The second highest inhibition zone was observed in the flowers methanolic extract of 48 hours with the concentration 1gm the mean value was (25.4 ± 2.416mm).In the aqueous extracts the highest inhibition zone was observed in the stem extracts of 1.5gm kept for 48 hours. The mean value was (17.6 ± 4.029mm).
Different tests including reducing sugar, flavonoids, terpenoids, tannins and saponins were practiced for phytochemical screening. All the tests were positive except tannins. Our results showed best inhibition against E. coli.

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