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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF LEAF EXTRACT OF MYRTUS COMMUNIS L. (ADESS)

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Abstract

Myrtus communis L. (family - Myrtaceae) is an evergreen aromatic medicinal plant which has been used since ancient times for medicinal, food and spice purposes. The main purpose of this study was to screen out phytochemicals and apply integrated study between methanol extract and solvent fractions on one gram positive bacterial strains (*Staphylococcus aureus*) and one gram negative bacterial strains (*Escherichia coli*) based on four selected bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae*), of preliminary antibacterial test results of *M. communis* L. leaves. The present study was screen out phytochemicals by maceration method and determine qualitatively depend on Phytochemical test (alkaloid, flavonoid, carbohydrate, protein, amino acid, steroid and phytosterols, tannin, terpenoid, phenol, gum and mucilage, fat and oil, anthraquinone, cardiac glycoside, and saponine) and essential oil by hydrodistillation. Then, to assess the antibacterial activity of methanol extracts and its solvent fractions by agar well diffusion methods. During phytochemical screening test, most of phytochemicals (51.2%) had a positive result. Thus; it is a promising candidate for antibacterial agents. The antibacterial activity of *M. communis* L. phytochemicals were studied using agar well diffusion method against *S. aureus* and *E. coli* showed high activity (24 ± 1 mm and 23.6 ± 0.994 mm zone of inhibition in $900 \mu\text{g}/\text{well}$ concentration of crude and n-butanol fractions respectively by comparing with controls of ciprofloxacin and DMSO. Thus, from the present study the plant leaf extracts and its solvent fractions of *Myrtus communis* L. showed an abundant production of phytochemicals as secondary metabolites. These phytochemicals (secondary metabolites) of *Myrtus communis* L. leaves crude extract and solvent fractions which are used for the treatment of various diseases caused by microbes.

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Introduction:-

Medicinal plants are a source of secondary metabolites which used as traditionally for treating mankind (80%) and formulation of modern medicine. Such plants are used as anticancer, antioxidant, antiviral, antifungal, and antibacterial purpose. In addition to plants other terrestrial organisms used as a medicinal purpose^{1,2,3}.

In Ethiopia, approximately 6500 to 7000 species of higher plants are medicinally important to human and animals⁴. 80% of human and 90% of animals is treated by traditionally by medicinal plants. Such uses of plants are transmitted orally from one generation to the other generation by indigenous people depending on heredity. WHO have a reset strategic plan for the development and promotion of traditional medicinal plants for controlling of heredity based transmission of these plants most of in industrialized societies^{5,6,7,8,9,10}. Their people depend and relay on traditional medicinal plants purpose by the reason of medicinal power /immediate solution, accessibility and economically cheapest than modern medicine¹¹.

Myrtuscommunis L. are a medicinal plant (shrub) which used as in traditional and modern way in Europe, Mediterranean and its bordering country and Ethiopia¹², such as decoction huge, anticancer, antioxidant, antiviral, antifungal, and antibacterial purpose. In wollo Ethiopia, peoples are used this plant as a decoction during epiphany, head ache, stomach ache, wound healing, clot protection, dandruff removing purpose hair growth purpose by mixing its powdered leaf with butter in traditional way.

The botanical name of myrtus is *MyrtuscommunisL.* which is from kingdome of plantae, order of myrtales, family of myrtaceae, genus of myrtus, species of *M.communis* which includes approximately 100 genera and 3000 species. The name of *MyrtuscommunisL.* in English-myrtle, German-myrtle, Spanish and Italian-mirto, French-myrtle, latin-myrtus^{13, 14, 15, 16}. It has 1-5 m long and their leaves have 1-5 cm long¹⁷. The other name of *MyrtuscommunisL.* in Ethiopia is (Ades – Amharinya, Gurageniya, Tigrinya; Addisaa, Coddoo – Orominya; Wobattaa– Welaitinya)^{3, 14, 15, 17}. *Myrtuscommunis L.* leaves contain varies compounds such as alkaloids, tannins, flavonoids, saponins, unsaturated fatty acids, phenolic compounds, terpenoids, steroids, cardiac glycosids, aminoacid¹⁸.



Figure 1:-*Myrtuscommunis L.* plant leaf (photo. By kassawyimer).

In different research, different method and solvent on extraction and fractionation have different quality and quantities of the product. In addition to that geographical distribution of myrtus has an effect on it. There is no integrated study on the crude and solvent fractions in antibacterial effect of leave of *myrtuscommunis L.* And also there is no study on geographical effect on the Phytoconstituents in different area.

The present study would be intended to rationalize the use of this plant leaf against infectious bacterial diseases or improve / aware the use of this plant phytochemicals to the world scientifically. And also it used as a reference to WHO in their application of strategy. It also used as impute for the formulation of modern medicine. Therefore, the present study is aimed to Screening of phytochemicals, in vitro Antibacterial Activity of Leaf Extract and fractions of *Myrtus Communis L.* (Adess)

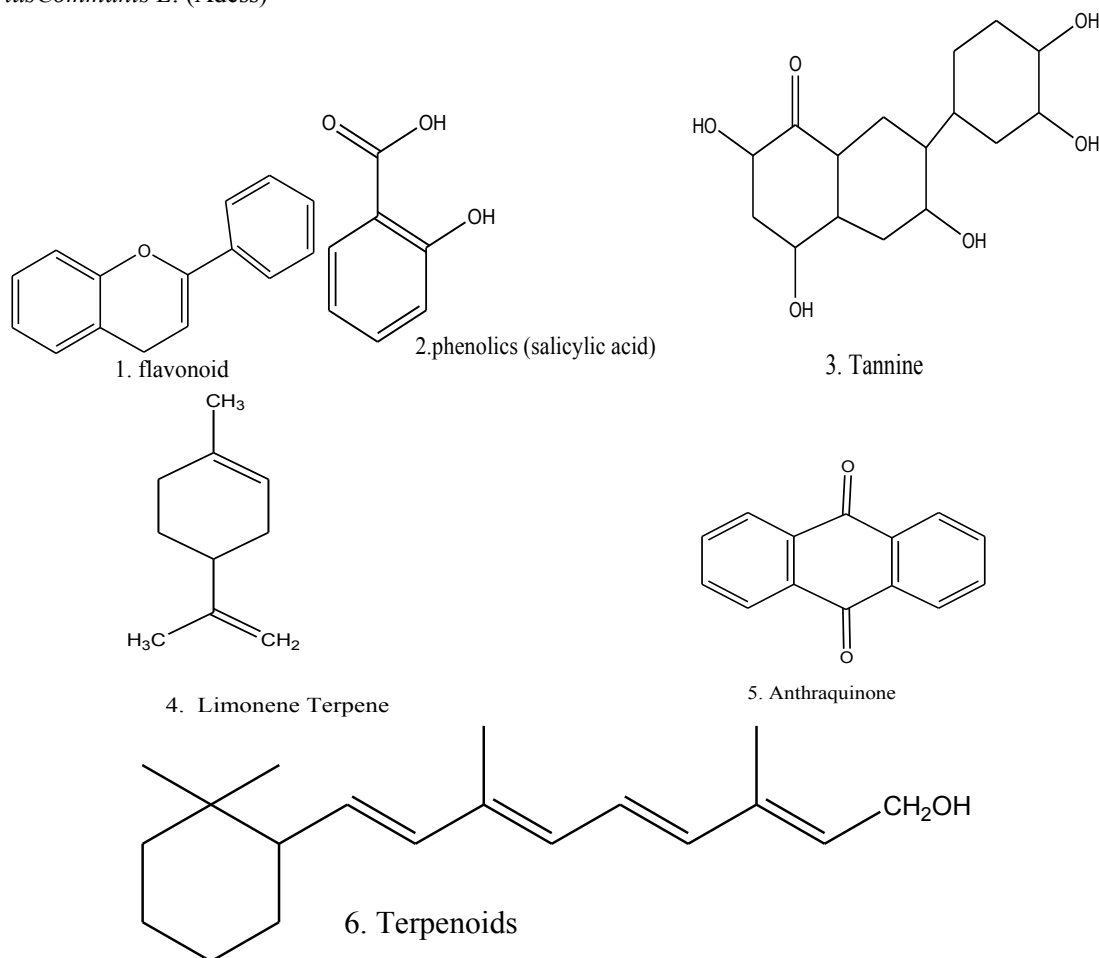


Figure 2:- Some of molecular structure of phytoconstituents of *myrtuscommunisL.*^{15, 16, 25,29}.

Experimental

Apparatus and Reagents

Reagents:

Methanol(99.9%) (Abron exports-133001, India),hydrochloric acid (37%) (LobachemiePVt. Ltd. 1o7), sulphuric acid (98%)(lobachemiePVt. Ltd. 1o7), Wagner's reagent(1.27 g of iodine and 5 g of potassium iodide(Avishkar international PVt. Ltd.Mumbai-2) dissolved in 100 mL distilled H₂O), Fehling solution, nitric acid (lobachemiePVt. Ltd. 1o7), sodium hydroxide (Abron exports-133001, India), chloroform, copper sulphate(rankem industry and tradingindia), glacial acetic acid, ethanol (99.5%), lead acetate, n-hexane, chloroform, ethyl acetate, n-butanol (lobachemiePVt. Ltd. 1o7), diethyl ether and Mueller-Hinton Agar (Hi Media Laboratories Pvt. Ltd., India).

Apparatus:

Materials used in this research were rotary evaporator, miller, refrigerator,water bath, test tube(borosilicate glass 3.3),separatory glass(borosilicate glass 3.3),, round bottomed flask(borosilicate glass 3.3),, measuring cylinder(borosilicate glass 27°C) wathman no. 1filter paper, beaker(borosilicate glass 3.3),, and thermometer, cheeler (karlsruheGmbh)

Plant material collection and preparation

Fresh leaves of *Myrtus communis* L. were collected from Kombolchatown around Dessie (10 km far from Dessie) from a farmer by checking of cutting time.

The leaves were washed under running tap water followed by distilled water to eliminate dust and other foreign particles and dried in the shade for a week to a constant weight at room temperature. Dried leaves were blended to powder in order to increase the surface area for extraction.

Extraction and Fractionation

The powdered leaf material (100.0g) was macerated in 100% methanol (850mL) in 2000 ml conical flask for eight days and then agitation was done every 12 h for 15 min.

The extract was filtered through what man No.1 filter paper and concentrated by using rotary evaporator at 42°C. The crude extract was transferred in to vial and stored in freezer at 4°C for the further fractionate work^{2, 18}.

The crude extract of (36.8g) *Myrtus communis* L. is fractionated sequentially by liquid-liquid extraction in a separatory funnel in n-hexane, diethyl ether, chloroform, dichloromethane, ethyl acetate and n-butanol^{20, 28}.

The solvent fractionation was done by suspending the crude extract (15.0g) in 100mL of distilled water, adding each solvent (115mL) and shaking well. The mixture was then allowed to stand for 24 h before separating the organic and aqueous layers. The organic fraction was collected in a round bottom flask. Solvent was separated using a rotary evaporator. The filtrate was transferred into vials and placed in an air until dried then stored in refrigerator at 4°C until further use.

Phytochemical screening

The crude methanolic extract and fractions of n-hexane, diethyl ether, chloroform, ethyl acetate, and n-butanol were qualitatively tested for the presence of alkaloids, phenols, flavonoids, cardiac glycosides, fixed oils and fats, tannins, steroids and phytosterols, terpenoids, amino acids, anthraquinones, fixed oils and fats, gum and mucilage, carbohydrate and protein^{2, 4, 24, 18}.

Test of Alkaloids**Wagner's test:**

The extracts (0.5g) and small amount of each all solvent fractions were stirred with 5mL of 1% aqueous hydrochloric acid on a water bath for 10 minutes and filtered. To 1mL of filtrate, few drops of Wagner's reagents were added by the side of the test tub. There was a formation of Reddish brown or creamish colored solution, which indicates the presence of alkaloids

Carbohydrate test**Fehling's test:**

The extract (1mL) and small amount of each all solvent fractions on water bath boiled with 1mL each of Fehling's solution. There was no formation of red colored solution, which indicates the absence of carbohydrate.

Test of proteins

The extract and small amount of each all solvent fractions filtered through Whatmann No.1 filter paper and the filtrate was subjected to test for proteins.

Xanthoproteic test:

The extracted test solutions (5mL) and small amount of each all solvent fractions was added, in to 1mL concentrated nitric acid and boiled the content. After cooling excess 40% sodium hydroxide (w/v) was added on it. On adding some drops of HCl acid and sodium hydroxide different color was developed. When addition of HCl gives yellow, in addition of NaOH gives orange, which indicates the presence of proteins.

Test of Anthraquinones

Few drops of 2% hydrochloric acid (v/v) were added in to leave extract (0.5 mL) and small amount of each all solvent fractions. There was no formation of reddish colored solution, which indicates the absence of anthraquinone.

Test of Steroids and Phytosteroids

The plant extracts (0.5 mL) and small amount of each all solvent fractions were added in to equal volume of chloroform and few drops of concentrated sulphuric acid was added into it. There was a formation of brown ring color, which indicates the presence of Steroids and Phytosteroids.

Tannin test

Small quantities of extracts and solvent fractions were dissolved in distilled water (2mL) and tested the presence of tannins by 5%-FeCl₃(w/v). There was a formation of darkvioletcoloredsolution, which indicates the presence of tannin.

Amino acid test

The extracts (5 drops) and small amount of solvent fractions were dissolved with water (2mL).

Biuret test: -1mL of equal volume of 5% NaOH solution (w/v) and 1% of CuSO₄ solutions (w/v) were mixed with each other. There was a formation of darkpink coloredsolution, which indicates the presence of amino acid.

Flavonoid test

NaOH(10% (w/v)) was added in to the extract (0.02g) and small amount of solvent fractions. There was a change of orange to crimson, which indicates the presence of flavonoid.

Test of Cardiac glycosides

Glacial acetic acid (2 mL) and 5% ferric chloride (w/v) (5 drops) were added into 0.5% of the extract and small amount of solvent fractions. Then concentrated sulphuric acid (1 mL) was added in to it. The formation of brown ring at the interface indicates the presence of cardiac glycoside.

Test of Saponins

Distilled water (2 mL) was added in to plant extract (4 drops) and small amount of solvent fractions and then shaken for 15 minutes. The formation of 1 cm foam indicates the presence of saponin.

Test of Terpenoids**Salkowski's test:**

The extracts (10 mL) and small amount of solvent fractions were mixed in chloroform (4 mL), and concentrated H₂SO₄ (6 mL) carefully added to form a layer. The formation of reddish brown colored solution indicates the presence of terpenoids.

Test of Phenols: Lead acetate test: The extract (5mg) and small amount of solvent fractions were dissolved in distilled water and 10% lead acetate solution (w/v) (3 mL) was added in to it.. The formation of bulky white precipitate indicates the presence of phenol.

In-vitro antibacterial activity test**Test organisms**

The test organisms used in this study were found in Amhara regional laboratory are Staphylococcus aureus(ATCC 25923) as a representative organism for gram positive bacteria and Escherichia coli (ATCC 25922) as a representative organism for gram positive bacteria. The organisms were growth in nutrient broth.

Measurement of inhibition zone

The determination of inhibition zone of the extracts and fractions were tested using agar well diffusion method by taking one gram positive bacterial strain; Staphylococcus aureus and one gram negative bacterial strain; Escherichia coli. The zone of inhibition was compared against reference antibiotics, ciprofloxacin^{26,27,28}.

Freshly prepared sterile Mueller Hinton Agar media (70 mL) was poured in to 150 mm diameter agar plate and allowed to cool at room temperature. Within 15 min of adjusting the turbidity of the inoculums suspension to 0.5 McFarland standard, a sterile cotton swab was dipped in to adjusted microbial suspension, rotated gently and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculums from the swab. The swab was streaked to the entire surface of the MHA plate three times by rotating approximately 60° each time to ensure even distribution of the inoculums. Petri-plates were left for 5 minutes at room temperature.

Equal distance hole with a diameter of 6 mm was punched aseptically using sterile cork borer tip. Methanolic extracts (0.5 g) and solvent fractions were dissolved in 1mL DMSO to get 0.5 g/mL stock solution different concentrations (45µg/well– 900 µg/well) of methanol extract and solvent fractions at concentration of (45 µg/well, 90 µg/well and 900µg/well) were prepared by dilution of the stock solutions in DMSO. Each extracts (90µL) were introduced to fill the wells using micropipette. Similarly, Positive and negative controls were added in to prepared place of a plate. After placement of the plant extracts and controls, the plates were placed undisturbed at room temperature for 2 hours and were incubated in an incubator at 37 for 24 hours. After 24 hours of incubation, complete zone of inhibition measured in millimeter as judged by the naked eye using ruler²³. The mean zone of inhibition and standard error of the mean (Mean ± SD) were calculated for the methanol extract and solvent fractions as well as for standard antibacterial. All the tests were performed triplicates.

Data analysis

The yield of the sample was calculated by ratio of weight of extract after evaporation of solvent and the powdered and dry weight of the sample multiplied by 100.

$$\text{Yield (\%)} = \frac{W_2 \times 100}{W_1}$$

w₁=weight of powdered and dried sample, w₂= weight of extract after evaporation of solvent.

Result and Discussion:-

The yield of the crude extract from 100.0g of *Myrtus communis* L. leave powder was 36.8% (W/W). But, in the previous study from 150g of powder was get 16.33%. The %yield of the extracts was less than to the present study % yield result²⁰. This difference was existed may be due to the quantity and concentration of methanol solvent. Different yields were obtained from the fractionation of the crude extract (15g) in 5 solvents for the purpose of extraction power. The %yield of fraction in ethyl acetate fraction was the highest while that of n-hexane was the least. This is due to the polarity of the *Myrtus communis* L. leave phytoconstituents are beyond to polar characteristics. So, ethyl acetate separated like polar phytoconstituents that have around 4.4 polarities.

Phytochemical screening results

Table 2 Shows the result of qualitative chemical screening that of secondary metabolites in the *Myrtus communis* L. from the Phytochemical screening the researcher observed that the methanolic extract gave a positive result with alkaloid, flavonoid, cardiac glycosides, saponines, steroids and phytosteroids, tannins, proteins, amino acids, gum and mucilage, terpenoids, and phenol which indicated the presence of saponines in the *Myrtus communis* L. leave extract. Carbohydrate, anthraquinone, gum and mucilage, and fixed oil and fats, were found to be absent from the extract in this test. The previous researcher said that, results indicated that the highest polar and non-polar solvents used for fractionation of the least number of compounds when separated by all the solvent systems used for the separation¹⁸. Similarly, in present study the result indicated that the highest fractions separated from the crude by intermediate and both polar and non-polar properties of solvents.

Table 3:- Phytochemical screening test results of Methanol extract and solvent fractionations extract and solvent fractionations.

No	Phytochemical constituents	crude extracts	Fractions				
			n-hexane	Diethyl ether	Chloroform	Ethyl acetate	n-butanol
1	Alkaloid	+	+	+	+	—	+
2	Flavonoid	+	+	+	+	+	—
3	Carbohydrate	—	—	—	—	—	—
4	Cardiac Glycosides	+	—	—	+	+	+
5	Saponines	+	—	—	—	+	—
6	Steroids and Phytosteroids	+	+	+	+	—	—
7	Tannins	+	+	—	—	+	+
8	Proteins	+	+	—	+	+	+
9	Anthraquinone	—	—	—	—	—	—
10	Amino acid	+	—	—	+	—	+

11	terpenoid	+	+	-	+	+	+
12	phenol	+	+	-	+	-	+

From the above table, + represents present, - represents absence of that phytochemical in *Myrtuscommunis* plant leaf.

The alkaloid phytochemicals of *Myrtuscommunis* L. leaf which had a positive result in some area research. This phytochemical was checked by previous researcher (NegeroGemedetal... 2011) and present research study which existed in this plant leaf but in ZenebeHagos et al...2017 Study, this is not found in the crude extracts. This is may be due to geographical distribution of medicinal plants phytochemical constituent.

In vitro antibacterial susceptibility assay

Table: 4. shows the result of antibacterial activity on the selected bacterial strains in selected solvent fractions and methanol extracts in triplicate test based on the result of preliminary bacterial test.

The results obtained in the present study revealed that tested phytochemicals of *Myrtuscommunis* L. leaf possess potential antibacterial activity against two selected bacterial strains (*Staphylococcus aureus* and *Escherichia coli*) indicated on Table: 4.

The antibacterial activity of *Myrtuscommunis* is influenced predominantly by both bacterial strains of extract and solvent fractionations¹⁸.

The solvent fractions, which indicated less antibacterial inhibition activity than methanol extract against *S.aureus* and *E.coli* strains with the whole three fractions. This may be due to the substract of antibacterial phytoconstituents in to their like polarity solvents by separated from methanol extracts. So, the fraction is weak than methanol extracts.

The test results were compared with standard antibiotics of ciprofloxacin (5.0µg/disc) which was not more suppress of inhibition zone from the methanol extract of 900 µg/well.

Previously, majhenicetal.reported methanol extracts were better antibacterial activity of medicinal plants phytochemicals. Therefore, the results indicated in this study are supported by previous studies^{26,27}.

Previously, the result indicated that, different solvents have different extraction and separation potential of biological active phytoconstituents. This previous study was in agreement with this study^{10,19}.

There was no previous study (report) on integrated evaluation between methanol extract and solvent fractions against these set of microorganisms.

Based on the given result (table 4), this plant leave of crude and solvent fractions indicated significant value in zone inhibition of these most different concentrations of extracts and fractions. This is may be due to the presence of high concentration of antibiotic phytochemicals which have high antibacterial effect.

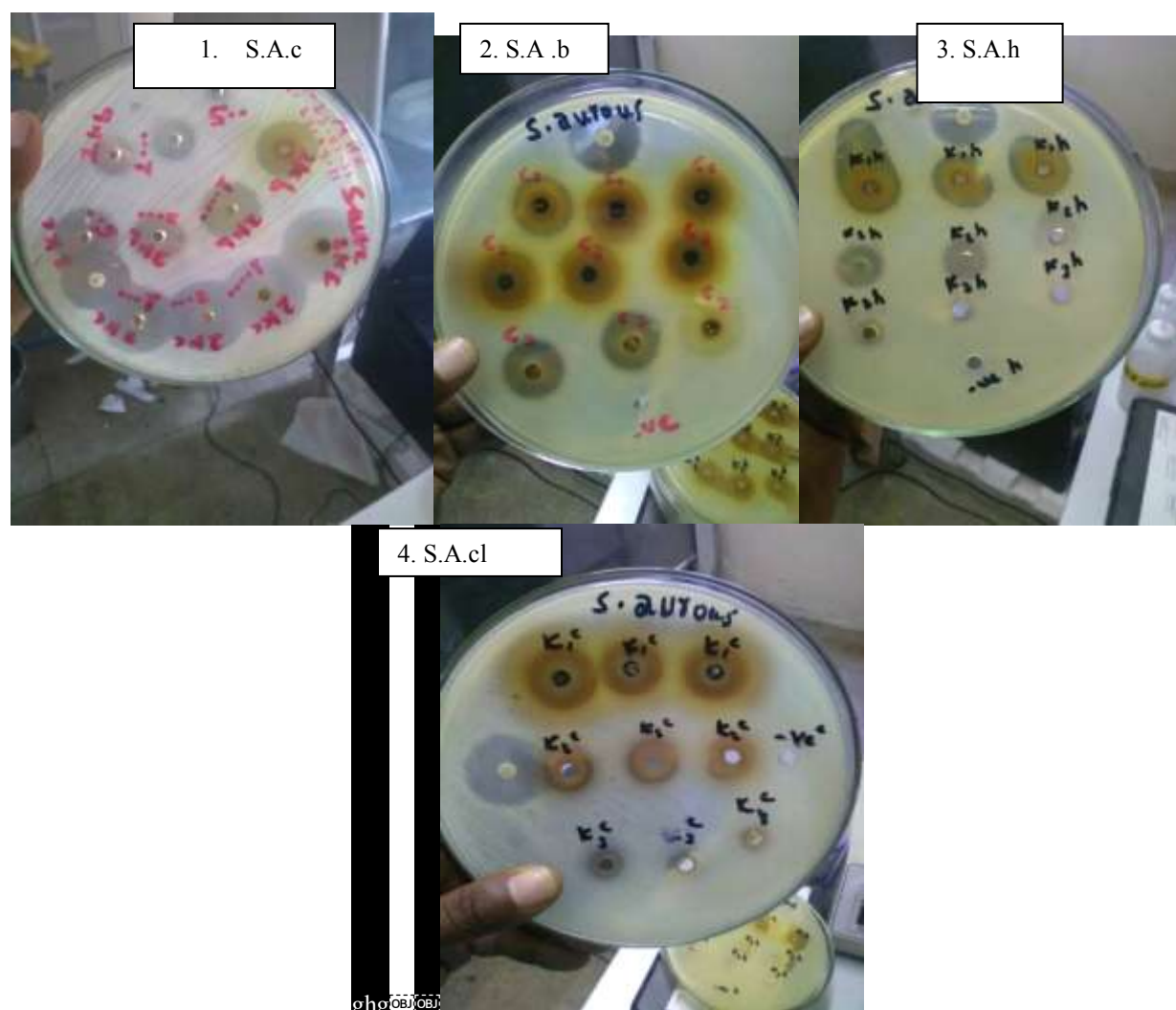
In previous and presentresearch report, the results suggest that methanolic extracts and n-hexane solvent fractions of *Myrtuscommunis* can be used in the treatment of infection caused by *Escherichia coli* and *Staphylococcus aureus*strains¹². Additionally in present research report, chloroform and n-butanol solvent fractions of *Myrtuscommunis* could be used in the treatment of infection caused by *E.coli*and *Staphylococcus aureus*strains in higher concentration of crude and solvent fractions.

Table 4:- Means of inhibition growth diameter obtained by agar well diffusion method (well and disc variants) using different concentrations of *Myrtuscommunis* against *Staphylococcus aurous* and *Escherichia coli*.

No	Name of selected bacterial strains	Mean ± SD in mm											
		solvent fractions											
		crude extract			n-hexane			Chloroform			n-butanol		
		900µg/	90	45	900	90	45	900	90	45	900	90	45

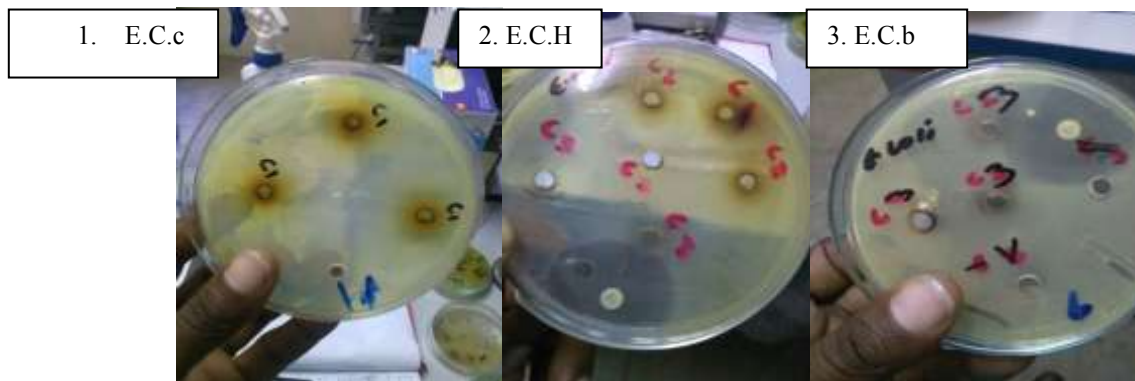
		well	µg/well	µg/well	µg/well	µg/well	µg/well	µg/well	µg/well	µg/well	µg/well	µg/well	µg/well
1	Staphylococcus aureus	24.7±1	24 ± 09	23.7 ± 0.9	20.3 ± 0.9	17.8 ± 0.9	11.7 ± 0.8	21.5 ± 0.5	17.8 ± 0.9	13 ± 0.4	20.3 ± 0.9	19.2 ± 0.3	17 ± 0.9
2	Escherichia coli	17.3 ± 0.9	16 ± 0.3	14.3 ± 0.9	17.3 ± 0.9	18.3 ± 0.5	15.3 ± 0.4	16 ± 0.3	6 ± 0.	9.8± 0.4	23.3 ± 0.9	18.3 ± 0.5	16.7 ± 0.8

In the above Table 4, Positive control (ciprophloxacin) = 28 µg/disc in S.aureus and 30 µg/disc in E.coli. Negative control (DMSO) = 0 in S.aureus and 0 in E.coli. This data were represented as mean ± SD of three replicates.



1. S.A.b- *staphylococcus aureus* in crude extract fraction.
2. S.A.h- *staphylococcus aureus* in n-hexane fraction.
3. S.A.cl- *staphylococcus aureus* in chloroform fraction.
4. S.A.c- *staphylococcus aureus* in n-butanol fraction.

Figure 3:- anti-bacterial activity test of M.communis extract and solvent fractions on S.aureus bacterial strain.



1. E.C. c-*Escherichia coli* in crude extract
2. E.C.h-*Escherichia coli* in n-hexane fraction
3. E.C.b-*Escherichia coli* in n-butanol fraction

Figure.4:- Anti-bacterial activity of *M.communis* extract and solvent fractions on *E.coli* bacterial strain.

Conclusion:-

Anti-bacterial activity on *E.coli* bacterial strain thus, from the present study the plant leaf extracts and its solvent fractions of *M. communis* L. showed an abundant production of phytochemicals as secondary metabolites and they can be used in the antibacterial activity against selected bacterial strains of *S.aureus* and *E. coli*. So it has anti-infective phytochemicals used as prevention and treatment of bacterial infectious diseases.

Reference:-

1. Anees, A.S.; Farah, I.; Seemi, S.; Kapendra, S.; Role of Natural Products in Drug Discovery Process. International journal of drug development and research, **2014**,6.
2. Zenebe, H.; Afework, M.; Gopalakrishnan, V.K.; Krishna, C. K.; Nagaraju, B. Phytochemical screening, in vitro antioxidant and antibacterial activities of essential oil from *Myrtus communis* L. Journal of Pharmacy Research. **2017**, 11(6), 747-752.
3. Mesfin, T.; Brook, M. A review of selected plants used in the maintenance of health and wellness in Ethiopia. Health Issue. **2010**, 2 (1), 85-102.
4. Giday, M. An ethno botanical study of medicinal plants used by the Zay people in Ethiopia., Journal of Ethnobiology/Ethnomedicine. **2001**, 3:81-99
5. Alemayehu, K.; Zemede, A.; Ensermu, K. Journal of Ethnobiology and Ethnomedicine, [online], **2015**, 11, 25
6. Rafieian-Kopaei, M. Medicinal plants and the human needs. J HerbMed Pharmacol, [online], **2012**, 1 1–2.
7. Teklay, G.; Girmay, Y.; Zeyede, T.; Teshome. Assessment of Traditional Medicinal Plants used to treat human and livestock ailments and their threatening factors in Gulomekeda District, Northern Ethiopia. International Journal of Emerging Trends in Science and Technology. [Online], **2017**, Vol.04|Issue|04|Pages 5061-5070|.
8. John Wiley, & Sons. Review of pharmacological effects of *Myrtus communis* L. and its active constituents. **2014**
9. Margaret, C., Geneva WHO. World Health Organization-Strategy for Traditional Medicine. Publication. **2014**
10. Tamer, M.; Sanjay, G. Traditional knowledge and use of medicinal plants in the Eastern Desert of Egypt: a case study from Wadi El-Gemal National Park. Journal of Medicinal Plants Studies. [Online], **2013**, 6(1)10-17.
11. Fassil, H. Ethiopia, a qualitative understanding of local traditional knowledge and medicinal plant use. In IK Notes. [Online], **2003**, 52, 1–4.
12. Besufekad, S.Y.; Mekdes, M.; Abebech, M.; Delesa, D.; Tekalign, D.; Demitu, K.; Birtukan, B. The Antimicrobial Activity of Leaf Extracts of *Myrtus communis*. Journal of Microbial & Biochemical Technology. [Online] **2017**.
13. Gemechu, A.; Aseer, M.; Behailu, M. In vitro Antibacterial Activity and Phytochemical Analysis of *Nicotiana glauca* L. Extracted in Different Organic Solvents Published [online] **2017**, 10.2174
14. Mendes, M.M.; Rodrigues, M.L. Acclimation of *Myrtus communis* to contrasting Mediterranean light environments: effects on structure and chemical composition of foliage and plant water relations. Environ Exp Bot. [online], **2004**, 5: 165-178. 7

15. Baytop. T.; Therapy with medicinal plants in Turkey (past and present). Istanbul: Nobel Medical Press. [online], **1999**.
16. Kefalew.; Zemedu, A.; Ensermu, K. Journal of Ethnobiology and Ethnomedicine. [Online], **2015**.
17. Twaij, H.A.; Ali, H.M.; Al-Zohyri, A.M. Phytochemical and antimicrobial studies of *Myrtus communis*. J Biol Sci Res. [online] **1988**, 19, 29-39
18. Negero, G.; Kelbessa, U.; Messay, G.; Kissi, M.; Frehiwot, T.; Ashenif, T.; Hirut, L.; Mulugeta, G. Antibacterial activity of extracts from *Myrtus communis* L. (ades) and *dodonea angustifolia* f. (kitkita) using bioautography method. Ethiop. J. Biol. Sci. **2011**, **10(1)**:57-72,
19. Tadesse, B.; Dereje, A.; Eyasu, E. Ethnobotanical Study of Medicinal Plants in Selected Horro Guduru Woredas, Western Ethiopia. Journal of Biology, Agriculture and Healthcare. [Online]. **2015**, 5, 1.
20. Mekonnen, S.; Ephrem, E.; Workineh, S. Evaluation of the antidiarrheal activity of the leaf extracts of *Myrtus communis* L. in (Myrtaceae) in mice model. Journal of Evidence based complementary and alternative medicine. [online], **2017**, 1035-1043 17: 103
21. Praveen, R.P.; Ashalatha, S.; Nair. Preliminary Phytochemical Screening of Root Extract of *Myxopyrum milacifolium* Blume. Asian Journal of Biomedical and Pharmaceutical Sciences. **2014**, 4(38), 41-45.
22. Andrei, M.; Dan, C. V.; Laurian, V.; Ovidiu, Crişan.; Ana-Maria.; Gheldiu.; Gianina C. Phytochemical Characterization of *Veronica officinalis* L., *V. teucrium* L. and *V. orchidea* Crantz from Romania and Their Antioxidant and Antimicrobial Properties. **2017**
23. Kuta, F.A.; Tsado, D.J.; Garba, S.A.; Saidu, A.N. Antibacterial activity of the leaf and stem bark crude extracts of *Khayasenegalensis*. Med Aromat Plant Res J. [online], **2015**, 3, 9±15.
24. Radwan, I.N.; Randa, B.; Hend, A.N.; Camilia, G. Evaluation of antimicrobial efficacy of four medicinal plants extracts used as root canal irrigant on *Enterococcus faecalis*: An in-vitro study. Int Dent Med J Adv Res. [online], **2015**, 1, 1±8
25. Chalchat, J.C.; Garry, R.F.; Michet, A. Essential oils of Myrtle (*Myrtus communis* L.) of the Mediterranean littoral. J Essential Oil Res. [online], **1998**, 10, 613-617.
26. Majhenic, L.; Kerget, M.S.; Knez, Z. Antioxidant and antimicrobial of guarana seed extracts. Food Chem. **2007** 104:1258-1268.
27. Meryem, S.; Nacéra B.; Chahrazed, B.; Nassima, B. Antioxidant and Antimicrobial Activity of Aqueous and Methanolic Extracts of *Mentha rotundifolia* L. from Algeria. Int. J. Pharm. Sci. Rev. Res. **2014**, 26(1) 228-234
28. Jae-Young, Kim.; Shin Young, Park.; Jae Hoon Lee.; In-Gyu Choi.; Joon Weon, C. Sequential solvent fractionation of lignin for selective production of monoaromatics by Ru Catalyzed ethanolysis. **2017**, 10.1039/c7ra11541e
29. Marianna, U.; Mauro, Marchetti.; Nicola C.; Maurizio, M. Chemical Composition of Myrtle (*Myrtus communis* L.) leaves Essential Oils as Observed in a Collection of Genotypes. **2018**, 23(10):2502.