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Antibacterial Effects of Quercus Infectoria on Bacteria Isolated from Urinary Tract Infection Patients

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Abstract:- A number of bacteria have now become antibiotic-resistant. This increases the importance of UTI drugs. We report, here, the activity of ethanol extract, of Quercus infectoria galls against UTI pathogens . antibacterial activity of ethanolic extract. Quercus infectoria from Sudan market was macerated with ethanol 85 %, fractionated by colum chromatography using benzene ethyl acetate, ethanol, and 70% ethanol. Phytochemical analysis of the extracts revealed the presence of antimicrobial active agents such as alkaloids, phenols ,glycosides, flavonoids resins, saponins, and tannins and then were subjected to chemical compound analysis Phytochemical screening, total phenolic content, total flavonoids content, and total tannins content were obtained. Eschericia coli shown resistance to Ciprofloxacin except Gentamicin and Proteus mirabilis shown resistance Trimethoprim while Staphylococcus aureus, Eschericia coli , Proteus mirabilis, Bacillus subtilis shown sensitivity to the crude extract and all the fractions except for A3 ,A4 ,A5 tubes of serial dilution, Eschericia coli, Staphylococcus aureus, Proteus mirabilis, Bacillus subtilis the results were exhibited a high activity (MIZD) against Proteus mirabilis (19 mm) and Bacillus subtilis (20 mm)have shown highly sensitivity the Quercus infectoria extract while e . Eschericia coli have shown moderate sensitivity (17 mm) Staphylococcus aureus (12mm) shown low

Keywords:- *Quercus Infectoria, Urinary Tract Infections, Pathogenic Bacteria, Natural Agents, Traditional Medicine*

I. INTRODUCTION

The overall volume of antibiotic consumption in the community is one of the foremost causes of antimicrobial resistance .In developing countries like sudan , pharmacists often dispense 'prescription-only' drugs, like antibiotics, to patients who do not have a prescription . Not much data is available regarding detailed information on behaviour of antibiotic use by community pharmacists which is of particular significance to develop a suitable and sustainable intervention programme to promote rational use of antibiotics,[1] Quercus infectoria or locally known as Alafas The galls arise on young branches of this tree as a result of attacks by gall wasps. Also known as Majuphal in Indian [2] traditional medicine, manjakani has been used as dental powder and in the treatment of toothache and gingivitis. The so-called "Aleppo tannin" is Tannic acid gained from Aleppo oak galls, which displays unique chemical properties essential in the preparation of gold sols (colloids) used as markers in Immunocytochemistry. Nowadays, gallnut extracts are also widely used in pharmaceuticals and Escherichia coli cause of 80–85% of community acquired urinary tract infection [3]

II. MATERIAL AND METHOD

A. Bacterial Strains

The standard organism Bacillus subtilis (Staphylococcus aureus Eschericia coli and were obtained from the Department of Microbiology, Medicinal and Aromatic Plants &Traditional Medicine Research Institute, National Center for research, Khartoum, Sudan. While the identified isolated strains from urine of the same species were obtained from military hospital, Omdurman, Sudan.

B. Solvents and Chemicals

Ethanol, Benzene, Ethyl acetate, Wagner's reagent, Hager'sreagent, Fehling's A & B solutions , alcoholic α-naphthol solution, Hydrochloric acid, Ammonia, Chloroform, Sulphuric acid, Ferric Chloride, Lead acetate, Nitric acid, Copper acetate, sodiumcarbonate, Sodium nitrite, Aluminium chloride solution, Sodium hydroxide, 1% K3Fe (CN)6,Folin ciocalteau reagent, gallic acid, quercetin, and tannic acid. Multi disk for Antimicrobial Susceptibility Testin.

C. Sample Collection

Quercus infectoria fruit in summer season were collected form west sudan plant parts were examined and identified by dr.yahia Specimens were furtherconfirmed with reference to Herbarium sheets available in the Department of Botany

- *Preparation of Extracts*

dried material was ground into coarsely powder this 300 mgs plant were crushed with mortar and pestle and the compounds were extracted by maceration using ethanol 85 % for 48 hours and evaporated by rotary evaporator and left for day remain ethanol [4].

- *Fractionation*

Fraction of Crude extract by Column chromatography, and stationary phase Silica gel and the moving phase: (Ethanol Ethy acetate,) three fractions of the plant aquatic ethanol85% were collected. The fractions compound were Identified using Thin Layer Chromatography (stationary

phase silica gel and moving toluene : ethy actate 7:3 and nonaquatic ethanol) to use under UV Light

➢ *Identification and Fractionation*

Fraction of extract by Column chromatography, and stationary phase Silica gel and the moving phase (Ethanol, Ethy acetate, ,) result three fraction of each of three plant Crude Extract Identification using Thin Layer Chromatography (TLC)

D. Antibacterial Activity

➢ *Isolation and Identification Bacteria form Patient's urine*

The urine was cultured on blood agar media and put in inoculation for 24 hr and subcultured two different colony into macconkey agar media.

➢ *Preparation of Disc for Antibacterial activity*

1 g of dry extract was added on 9 mml distal water and disc of filter paper was putted to solution, 1ml of solution into 9 mml distal water was added tube 2 ,1ml of solution into 9 mml distal water was added tube 3, 1ml of solution into 9mml distal water for was added tube 4, 1ml of solution into 9 mml distal water was added tube 5, the process resulted 5 tubes each one contain 9 ml [5].

E. Preparation Fractured Compounds

Dices of filter paper were putted into Crude extract (1g/9ml), fractured compound (25mg/9ml) for 24 hrs to saturate . Were lifted to dry inside oven.[5]

F. Qualitative Analysis

➢ *Phytochemaical Screening*

- *Test for Phenol*

To (2 ml) the test solution, a few drops of ferric chloride solution were added. Bluish green or red colour indicates the presence of phenol. [6]

- *Tannin*

✓ *Ferric Chloride Test*

10ml of distilled water was added to 3 ml of ethanol fraction, 3 ml of 5% w/v ferric chloride solution was added. The blue – black colour was noted due to the presence of tannins. [6]

✓ *Gelatin Test*

3ml solution of ethanol fraction, aqueous solution of gelatin and sodium chloride are added. A white buff coloured precipitate is formed indicates the presence of tannins. [6]

- *Flavonoid*

✓ *Lead Acetate Test*

To (3ml) of solution of ethanol extract, 3 ml of lead acetate solution were added. a white precipitate indicates the presence of flavonoid. [6]

- *Alkaline Reagent*

To (3ml) of ethanol extract were treated with few drops of sodium hydroxide solution formation of intense yellow color ,which becomes colorless on addition of dilute HCL indicates the presence flavonoids.. [6]

- *Test for Saponin*

The test solution was shaken with water. Copious lather formation indicates the presence of saponin. [6]

- *Test for Steroid*

✓ *Libermann- Burchard Test*

To 2 ml of the test solution, a few drops of chloroform, 3 - 4 drops of acetic anhydride and one drop of concentrate sulphuric acid were added. Appearance of purple colour, which changes to blue or green colour, shows the presence of steroid. [6]

✓ *Salkowski Test*

To (2 ml) of the test solution, a few drops of chloroform, 3 - 4 drops of acetic anhydride and one drop of concentred sulphuric acid were added. Appearance of purple color, which changes to blue or green color, shows the presence of steroids. [6]

- *Test for Carbohyrate*

✓ *Molisch's Test*

The Molisch's reagent was prepared by dissolving 10 g of α- naphthol in 100 ml of 95 % alcohol. A few mg of the test extract was placed in a test tube containing 0.5 ml of water, and it was mixed with two drops of Molisch's reagent. To this solution, was added 1 ml of concentrated sulphuric acid from the side of the inclined test tube, so that the acid formed a layer beneath the aqueous solution without mixing with it. If a red brown ring appears at the common surface of the liquids, sugars are present. [6]

- *Test for Alkaloids*

✓ *Mayer's Test*

To a few ml of plant sample extract, two drops of Mayer‟s reagent are added along the sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids. [6] 2.6.1.8. 2. Wagner's test.

A few drops of Wagner‟s reagent are added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive. [6]

- *Test for Glycosides*

For 50 mg of extract is hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath, filtered and the hydrolysate is subjected to the following tests. [6]

✓ *Borntrager's test*

To 2 ml of filtered hydrolysate, 3 ml of choloroform is added and shaken, choloroform layer is separated and 10% ammomia solution is added to it. Pink colour indicates presence of glycosides.

- *Diterpenes*

✓ *Copper Acetate Test*

Extract were dissolved in water and treated with 10 drops of copper acetate solution, formation of emerald green colour indicates presence of diterpenes. [6]

G. Determination of Total contents of Tannins , Flavonoids and Phonlic

➢ *Total Phenolic Content*

The total phenolic content was determined adopting the method as described by , 1mg/ml of extract were taken in a 10ml glass tube and made up to a volume of 3ml with distilled water. 0.5ml Folin ciocalteau reagent (1:1 with water) and 4ml Na2Co3 (7.5%) were added sequentially in each tube. A blue colour was developed in each tube and the intensity of the colour is directly proportional to the phenolic content. The blue coloration in the tube is due to the formation of molybdenum blue as a result of complex redox reaction between phenols and phosphomolybdic acid in Folin ciocalteau reagent in alkaline medium. The test solution kept in dark for 30 minutes, cooled and absorbance was measured at 765nm. The total phenolic contents were expressed as gallic acid equivalents (mg/l) using the following equation based on the calibration curve: y=0.0008x+0.0397 where x=concentration of gallic acid (mg/l) corresponding to optical density. A standard and used for calculation of total phenolic compounds [7]

➢ *Total Flavonoids Content*

The total flavonoids content was determined by adopting the method described by . Aliquot of extract was pipette out and volume was made up to 2ml with distilled water, 0.3ml of sodiumnitrite (5%) was added to the tube and incubated for 5min. at room temperature, 0.3ml of aluminium chloride solution (10%) was added and incubated for 5min, 2 ml of sodium hydroxide(1M) were added. Absorbance was measured at 415nm against a reagent blank. Total flavonoids content was expressed as quercetin (mg/l) using the following equation based on the calibrationcurve: y=0.0007x +0.053, where y was the absorbance .A calibration curve was constructed,using quercetin (50-800mg/l) as standard and total flavonoids content of the extracts (mg/l) expressed as quercetin equivalents [7]

➢ *Total Tannins Content*

The tannins content was determined by using FeCl3 and gelatin test with some modification. About 1ml of extract (1mg/ml) was transferred to vials, 1ml of 1% K3Fe (CN)6 and 1ml of FeCl3 were added, and the volume was made up to 10ml with distilled water. After 5min absorbancewas measured at 510nm against a reagent blank. The total tannins content was calculated usingthe following equation y=0.002x +0.083 where x=concentration of tannic acid (mg/l)corresponding to optical density. A calibration curve was constructed, using tannic acid (100-900mg/l) as standard and total tannins content of the extracts (mg/l) expressed as tannic acid [7]

III. RESULTS AND DISCUSSION

A. Qualitative Analysis

Test name	Plant *Quercus infectoria*
Wager's Test & Mayer' s test	+
Modified Borntrager's Test	+
Foam Test	+
Salkowski's Test & Libermann Test	+
Ferric Chloride Test	+
Copper acetate Test	+
Molisch's Test	+
Lead acetate Test & Alkaline Reagent	+
ferric chloride test & Gelatin test	+

Table 1:- Qualitative Analysis of Phytochemicals present in Quercus infectoria (Keys: positive = +)

Plant extract Fraction Antibiotic Serial dilution	Inhibition zone by mm			
	E.coli	Staphylococcus	Proteus	Bacillus subtilis
Crude extract	17mm	12mm	18mm	19 mm
F A ethylacetate	15mm	10mm	19mm	12 mm
F A thanol	17 mm	11mm	10mm	` 20 mm
A 1	16mm	12mm	16mm	10 mm
A 2	10mm	5mm	11mm	6mm
A 3	-	-	-	-
A 4	-	-	-	-
A 5	-	-	-	-
Ciprofloxacin	-	25mm	25mm	20 mm
Cefixim	-	20mm	35mm	24mm
Gentamicin	14 mm	18mm	15mm	19mm
Trimethoprim	-	20mm	-	23mm

Table 2:- antibacterial activity of phytochemicals compound compare with common antibiotic against isolate strain form patients urine

B. Quantitative Analysis

➢ *Determination of total contents of Tannins , flavonoids and Phonlic*

- *Total Phenolic Content*

RESULT :-
Three reads was obtained at 765 nm are :-
2.3041
2.3046
2.3057

The average is 3.4572

The content obtained by the following equation

y=0.0008x+0.0397
y= absorbance
x = concentration

calculation:-

3.4572=0.0008x+ 0.0397
X= 3.4572-0.0397 = 4,272 mgGAE/mg

0.0008

- *Total Flavonoids Content*

Three reads was obtained at 415 nm are:-
2.7852
2.7872
2.7864

calculation:-
y=0.0007x +0.053
x= 2.7862-0.053 = 3,904.5 mgQE/mg
0.0007

- *Total Tannins Content*

Three reads was obtained at 510 nm are:-
1.1108
1.1151

1.1253 calculation:-
y=0.002x +0.083

x= 1.1170-0.083 = 12.457mgTAE/mg 0.083

✓ *Total Phenolic Content*

By using the method prescribed by [8], the absorbance (y) of extract was obtained and the concentration (x) was calculated using the equation y = 0.0008x+0.0175

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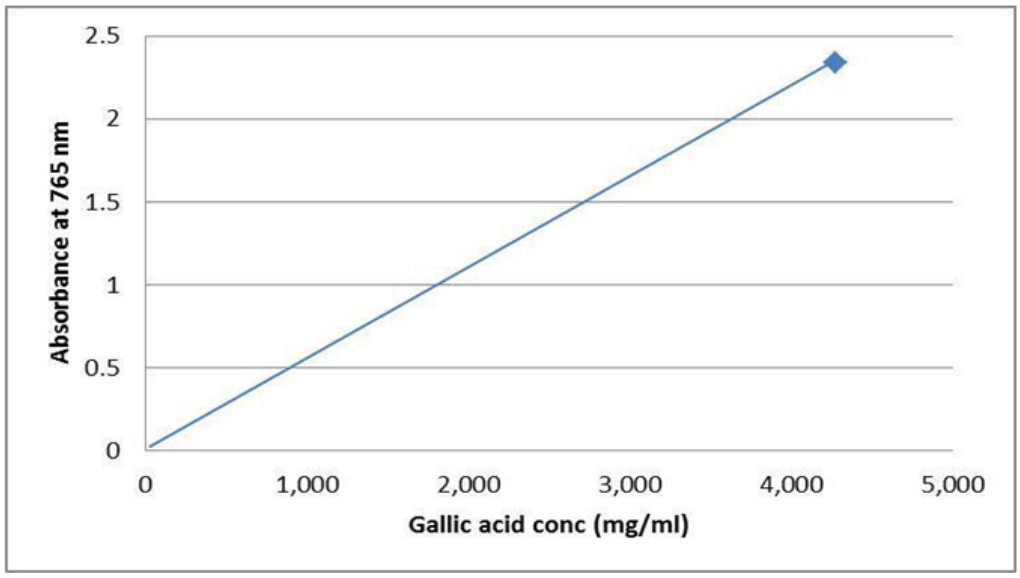

Fig 1:- Total Phenolic content using gallic acid calibration Curve (y) absorbance (x) concentration

✓ *Total Flavonoids Content*

By using the method prescribed by [8-9],the absorbance (y) of extract was obtained and the concentration (x) was calculated using the equation

$$y=0.0007x +0.053$$

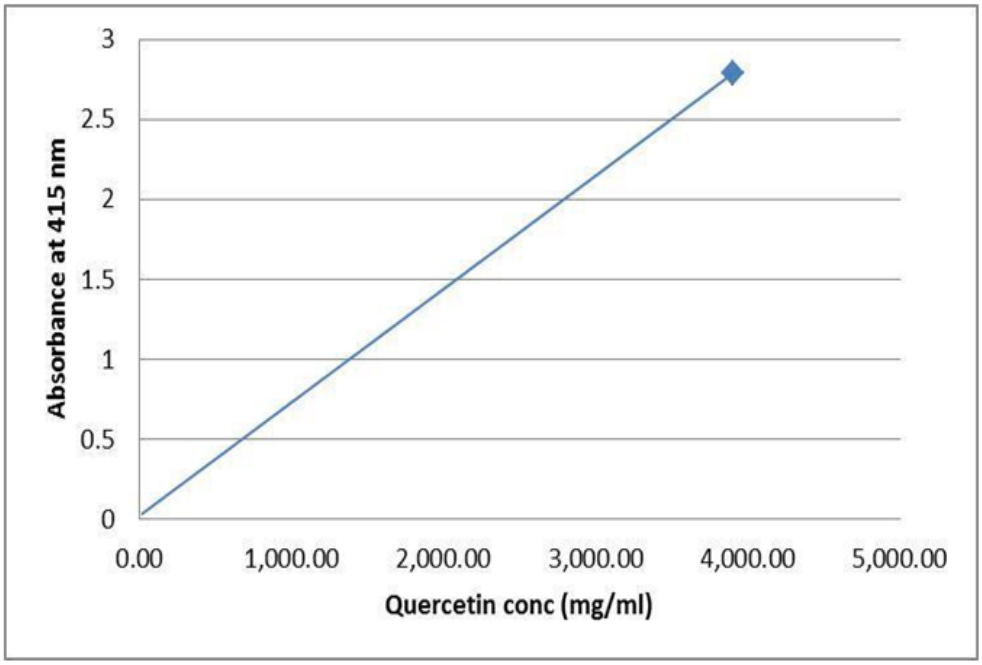

Fig 2:- Total flavonoids content using quercetin calibration Curve (y) absorbance (x) concentration

✓ *Total Tannins Content*

by using the method prescribed by [8],the absorbance (y) of extract was obtained and the concentration (x) was calculated using the equation

$$y=0.002x +0.083$$

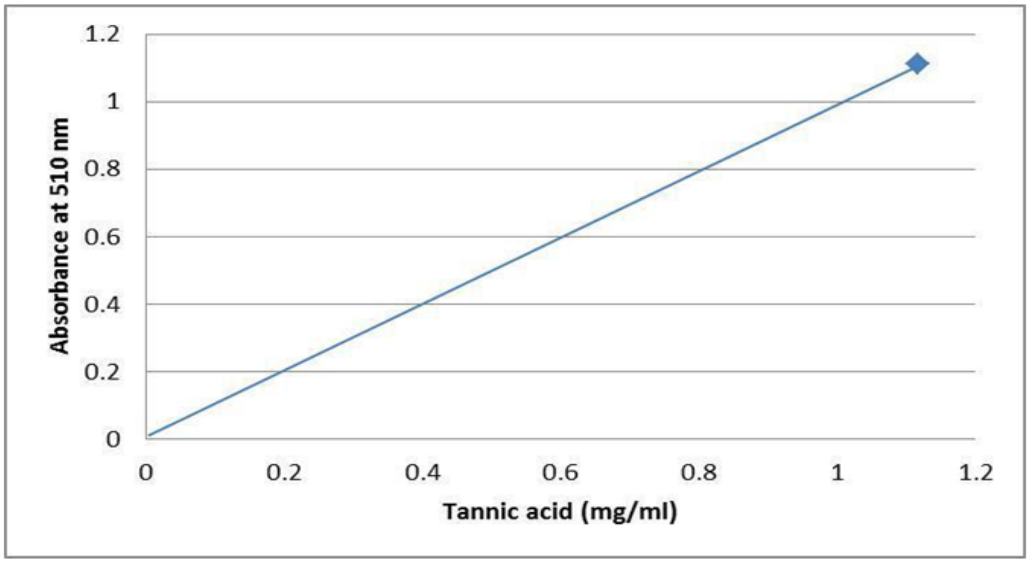

Fig 3:- Total flavonoids content using quercetin calibration Curve (y) absorbance (x) concentration

Total phytoconstituent in mg of crude extract	Y (absorbance)	X (concentration)
Total Phenolic	3.4572	4,272 mgGAE/mg extract
Total Flavonoids	2.7862	3,904.5 mgQE/mg extract
Total Tannins	1.1170	12.457mgTAE/mg extract

Table 3:- GAE: Gallic Acid Equivalent,QE:Querstin Equivalent, TAE: Tannic Acid Equivalent [10]

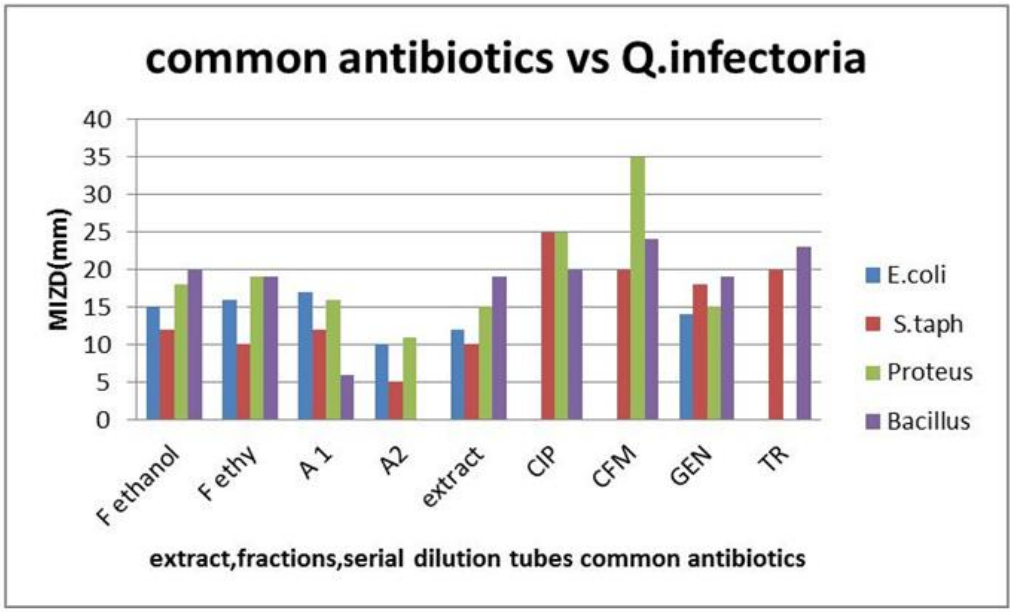

Fig 4:- extract 25 mg/ml and fractions each one 10 (mg/ml)(CIP= Ciprofloxacin 5mg) ,(CFM= Cefixim 10 mg) , (GEN =Gentamicin 10 mg) , (TR = Trimethoprim 5 mg) (E.coli = Eschericia coli),(S.taph= Staphylococcus aureus),(Proteus= Proteus mirabilis),(Bacillus= Bacillus subtilis) A1 and A2 = serial dilution tubes.

IV. CONCLUSION

The results of phytochemical screening, as shown in Table 1 indicated that the Quercus infectoria contain alkaloids, carbohydrates, glycosides, Phytosterols, phenols, Flavonoids, saponins. Table 3 shows the total phenolic content studied in one milligram of crude extract, which was found to be(4,272 mgGAE/mg extract). Total flavonoids content(3,904.5 mgQE/mg extract) and total tannins content(12.457mgTAE/mg extract). As reported in figure 4 Quercus infectoria crude extract , fractions and serial dilution have antibacterial activity against UTI Pathogenesis bacteria and Eschericia coli shown resistance to Ciprofloxacin except Gentamicin and Proteus mirabilis shown resistance Trimethoprim while Staphylococcus aureus, Eschericia coli , Proteusmirabilis, Bacillus subtilis shown sensitivity to to the crude extract and all the fractions except for A3 ,A4 ,A5 tubes of serial dilution, as fig 4 and table 2 Bacillus subtilis shown highly sensitivity to both fraction of ethanolic and ethanolic extract (ethanol 85%) , Bacillus subtilis (20 mm) whileshown fraction of ethanolic (19 mm) and moderate sensitivity to both ethyl acetate fraction (12 mm) , A1 tube number 1 (10mm) , while shown low sensitivity to A2 (6 mm) and no sensitivity to remain tubes, A3,A4,A5 . Proteus mirabilis have shown highly sensitivity to crude extract (18 mm) ,and ethyl acetate fraction (19 mm) while shown moderate to A1 of serial dilution 16 mm and have shown low both to ethanol fraction (10mm) and A2 (11mm) of serial dilutions and no sensitivity to remains tubes of serial dilutions A3,A4,A5. while Staphylococcus have shown highly sensitivity to both crude extract(12mm)and A1of serial dilutions (12 mm) and have shown moderate to both ethanol fraction (11 mm) and ethyl acetate (10 mm) and low to A2 of serial dilutions (5 mm) while no sensitivity to remain A3 , A3 , A4,A5 . at last Eschericia coli have shown highly to both crude extract (17 mm) and ethanol fraction (17 mm) and have shown moderate to both ethyl acetate (15 mm) and A1 of serial dilutions (16 mm) while shown low to A2 of serial dilutions (10

And no sensitivity to remain A3 ,A4 , A4 , A5 . Proteus mirabilis and Bacillus subtilis have shown highly sensitivity the Quercus infectoria extract while Eschericia coli have shown moderate sensitivity Staphylococcus aureus shown low

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REFERENCES

[1]. J Clin Pharm Ther. 2012 Jun;37(3):308-12. doi: 10.1111/j.1365-2710.2011.01293.x. Epub2011 Aug 23
[2]. Stashia Eleaness; Rosland Abel (2013). "1". In Universiti Teknologi Malaysia. The extraction of essential oil from Quercus infectoria (Manjakani) galls using supercritical carbon dioxide pressure swing technique. Faculty of Chemical Engineering.
[3]. School of Chemical and Biotechnology, SASTRA University, India received: April 24, 2014 | Published: May 15, 2014
[4]. International Journal of Advanced Research in Chemical Science (IJARCS)Volume 2, Issue 4, April 2015, PP 25-32
[5]. Mohanty, A. (2010): Physiochemical and antimicrobial study of polyherbal. Pharmacie globale. Vol. 4(4) 1-3., library.med.utah.edu/WebPath/EXAM/LabMedCurric/ LabMed05_06.html
[6]. International Journal of Advanced Research in Chemical Science (IJARCS) Volume 2, Issue 4, April 2015, PP 25-32
[7]. abdealsiede, missa mohammed saleh. Isolation And Identification Of Some Biologically Active Compounds From Some Sudanese Medicinal Plants. B.Sc (Hons.) Chemistry, M.Sc. Chemistry, Sudan University of Science and Technology. 2016:37-39.
[8]. Marijana kosanic Branislav Rankovic SCREENING OF ANTIMICROBIAL ACTIVITY OF SOME LICHEN SPECIES IN VITRO .kragujevac J.Sci 2010;32:67
[9]. parveen M, Ghalid ,R M,Khanam, Z,Mehdi,S.H. and Ali M. Anovel antimicrobial agent from the leaves of peltophprum vogelianum (benth) .Nat.Prod .Res 2010;24:24:1268
[10]. Safaa Abdelaziz Eltayed Elhassan et Department of Pharmacognosy .al International Journal of InnovativePharmaceutical Sciences and Research / IJIPSR / 5 (02), 2017, 1-11 ISSN (online) 2347-2154 DOI: 10.21276/IJIPSR.2017.05.02.512