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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF QUERCUS INFECTORIA AND TERMINALIA CHEBULA

***Revathi, M. Ambikapathy, V. Panneerselvam, A. and Senthilkumar, G.**

P.G & Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613503.Thanjavur Dt, Tamilnadu, India

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ABSTRACT

Plants are commonly used as herbal medicine because it contains various secondary metabolites such as alkaloids, flavonoids, saponins and terpenoid etc.*Quercus infectoria* and *Terminalia chebula* Retz. Were examined for their antibacterial activity against Methicillin Resistant *Staphylococcus aureus*. Antibacterial activity of*Quercus infectoria* and*T. chebula* Retz. Was carried out by using the well diffusion method. Ethanol extract of *T. chebula* were more significant activity against MRSA compared with other solvents. Methanol extract of *Q. infectoria* showed highest zone of inhibition than other solvents. Minimum inhibitory Concentration of *T. chebula*were observed at the ranges from <0.24 to 7.81 mg/ml for all extracts. MIC of the*Q. infectoria* were showed at the ranges from <0.24 to3.90 mg/ml. This results indicate that the both *T. chebula* and *Q. infectoria* have found greatest antibacterial activity against Methicillin Resistant *Staphylococcus aureus*.

Key words: Terminalia chebula, Quercus infectoria, MRSA, Antibacterial activity, Minimum Inhibitory Concentration, phytochemical activity.

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INTRODUCTION

Plant medicines are used on a worldwide scale to prevent and treat infectious diseases. They are of great demand both in the developed as well as developing countries for the primary health care needs due to their wide biological and medicinal activities, higher safety margin and lesser costs. Medicinal plants have their values in the substances present in various plant tissues with specific physiological action in human body. The broad definition of medicinal plants has been incorporated in an ancient Indian literature which portrays that "all plant parts to be potential sources of medicinal substances" (Shankar *et al*., 2003). The dried ripe fruit of *Terminalia chebula* Retz. (Combretaceae) has traditionally been used in the treatment of asthma, sore throat, vomiting, cough, diarrhea, bleeding piles,heart and bladder disease. In addition, the plant is commonly used for acidity, chronic lung disease, skin diseases and eye disorders and possesses radio-protective (Gandhi *et al*., 2005), cardioprotective, hepatoprotective, and wound healing activities (Prakash *et al.,* 2012). It has been reported that *T. chebula* fruit exhibits a range of biological properties, including antioxidant (Suchalatha*et al*., 2005), anticancer (Saleem *et al*., 2002), antibacterial (Kumar *et al.,* 2009), antifungal (Srikumar *et al*., 2010) and antiviral (Ma *et al*., 2010) properties.

***Corresponding author:* Revathi,**
P.G & Research Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College (Autonomous), Poondi-613503.Thanjavur Dt, Tamilnadu, India

The galls of *Q. infectoria* have also been pharmacologically documented to possess astringent, antidiabetic, antitremorine, local anesthetic (Hussein *et al*., 2000) antiviral (Fatima*et al*., 2001), antibacterial (Digraki *et al*., 1999), antifungal (Redwane *et al*., 2002), larvicidal (Kaur *et al*., 2004) and anti-inflammatory (Ikram *et al*., 1977) activities. Gargle of hot water extract of *Q. infectoria*galls is very effective to inflamed tonsils, although unswerving application of boiled along with bruised galls on skin effectively cures several swelling or inflammation. It is a widely known plant in Indian traditional medicine has been used as dental powder and in the treatment of toothache and gingivitis (Hwang *et al*., 2000).

MATERIALS AND METHODS

Screening of Methicillin Resistant *Staphylococcus aureus* (MRSA)

A total of 308 pus specimens from chronic wound patients were collected for *S.aureus* screening. Thesamples were obtained from various health care hospitals in Tirupur, Karur and Erode Districts. Wound swabs were streaked onmannitol salt agar and incubated at 37°C for 24 to 48 hours. Growth and fermentation of mannitol on MSA wereexamined. *S. aureus* were identified using gram stain and biochemical tests based on Bergey'smanual of systematic bacteriology (Bergey *et al*., 1994).

Detection of MRSA by chromogenic agar

The Hi-Media (India) made HiCrome MeReSa Agar Base (M1674) was used for detection of the MRSA among the clinical isolates of *S. aureus*. The medium was prepared by suspending 41.65 g of the medium into 500 ml ofthe distilled water and boiling. The medium was cooled to around 45 to 50°C and MeReSa selective supplement (FD229) reconstituted with 5 ml sterile distilled water into each methicillin vials having 2.0 mg of cefoxitin antibiotic as per the direction of the supplier (HiMedia-India), was added and mixed very well. Soon after, the medium was poured into Petri plates and cooled then checked for sterility by keeping at 37°C overnight. In this study the detection of MRSA was determined by direct culture of each swab on HiCrome medium and by subculture of the identified *S. aureus* strains from mannitol salt agar onto the HiCrome MeReSa agar. Plates were incubated at 35°C for 24 h after which, all cultures showing blue colored growth were taken as MRSA positive strains, while all others are recorded as MSSA strains (Jawad *et al*., 2013).

Collection of Plant Materials

Terminalia chebula and *Quercus infectoria* fruits were collected from herbal shop at Tirupur District, Tamilnadu, India. The plant materials were washed thoroughly two times with purified water and air shade dried. Then the plant materials powdered using electric blender and powder were kept in sealed containers.

Preparation of Plant Extract

10gm of the powdered plant material of *Terminalia chebula* and *Quercus infectoria* were soaked separately in 50ml of ethanol, methanol, acetone and cold water for 24 hours at room temperature. Then mixture was filtered throughWhatmanNo.1 filter paper and boiled at 80°C or 2 hours. The dried crude extracts were stored at 4°C for further use (Roopalatha *et al*., 2013**)**.

Antibacterial Activity

20ml of nutrient agar were poured into the sterile petri plates. Wells of 6 mm in diameter were made on nutrient agar using gel puncture. Fresh overnight culture was swabbed uniformly using sterile cotton swabs, and then 50µl of plant extract solution was loaded into the wells. After incubation at 37°C for 24 hours, the different levels of zone of inhibition were measured (Cappouccino *et al*., 1998)

Minimum Inhibitory Concentration

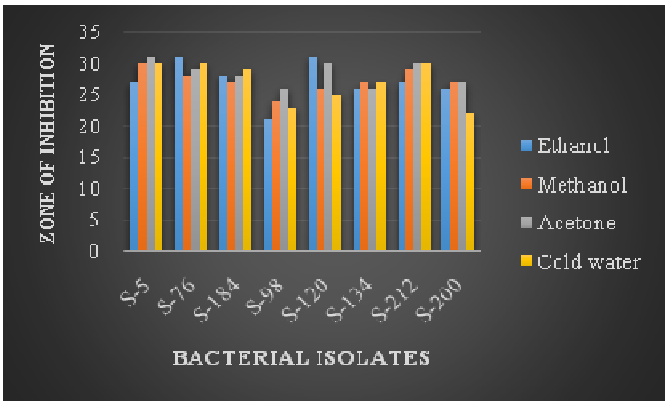
Minimum inhibitory concentrations of the plant extracts were tested by the checkerboard assay method (Kumarasamy *et al*., 2002). The test extracts were dissolved in 5% DMSO to obtain 500mg/ml stock solutions. The 96 well sterile plates were taken and 100µl of stock solution was added to row 1. Fifty microlitres of sterile normal saline was added to row 2 to11. Two fold dilutions were performed by transferring 50µl of extracts from row 1 to 2 using a multichannel pipet. The above process was repeated up to row 12. Forty microlitres of double strength nutrient broth and 10µl of bacterial solutions were added to all the wells, so the final concentrations of inoculum in all the wells were $5x10^6$ CFU/ml. To prevent dehydration, the plates were covered with a sterile plastic cover and then incubated at 37°C or overnight. Bacterial growth determined after addition of 40µl of p-iodonitro tetrazolium violet (0.2mg/ml). The MIC result was determined as the lowest sample concentration at which no red color appeared.

Phytochemical Analysis

Phytochemical analysis of alkaloid, tannin, terpenoid and flavonoid were carried out according to standard methods described by Adetuyi *et al*., 2001; Venkatasan *et al*., 2009.

RESULT AND DISCUSSION

Out of 308 wound pus swab samples, 214 *Staphylococcus aureus* were identified by using MSA agar plate technique, out ofthese 148 (69.16%) cultures were found to be MRSA based on CHROM agar mixed with cefoxitin.Cefoxitin, a cephamycin is a more potent inducer of the PBP2a and several groups of investigator have reported that the results of cefoxitin disc diffusion correlate better with the presence of mec gene responsible for methicillin resistance (Skov *et al*., 2006).Anand *et al*., (2009) found cefoxitin disc method to be 100% in concordance with PCR. Harleen Kaur *et al*., (2012) also reported cefoxitin disc test results were in 98.5% concordance with PCR results. Based on MDR calculation 8 strains were selected for further studies.



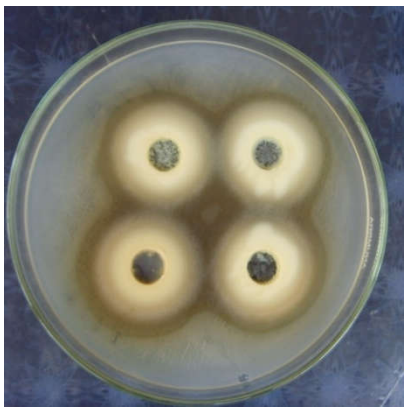
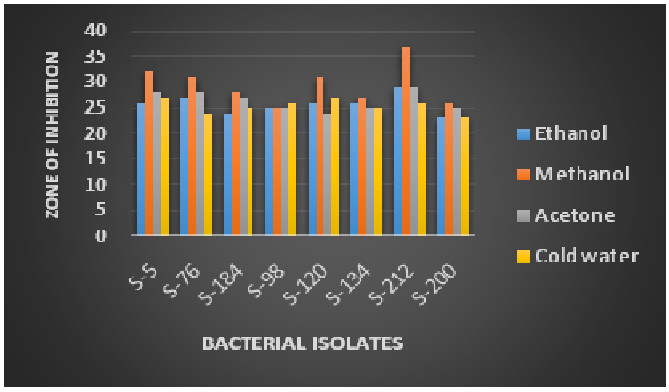


Figure 1. Antibacterial activity of *Terminalia chebula* against Methicillin Resistant *Staphylococcus aureus*

All the Crude extract of *T. chebula* showed inhibitory response against Methicillin Resistant*S. aureus*(Figure: 1). Ethanol extract was found to be effective zone of inhibition (26mm to 31mm) followed by methanol (24mm to 30mm), Cold water (22mm to 30mm) and acetone (21mm to 31mm) respectively. Bag *et al*., (2009) showed all the test extracts (cold aqueous, hot aqueous and ethanol) found varying degrees of strain

specific inhibitory action for *S. aureus* strains IZD ranged from 12 – 20 mm and for *E. coli* strains it was 13 – 21 mm.Prabhat *et al.*,(2010) reported that the maximum zone of inhibition against *S. aureus* was 27mm in methanolic extract of *T. chebula.*



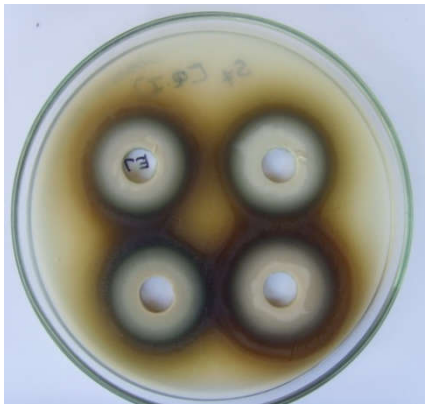


Figure 2. Antibacterial activity of *Quercus infectoria* against Methicillin Resistant *Staphylococcus aureus*

Figure: 2 showed the Crude extract of *Q. infectoria* showed inhibitory response against *S. aureus*. Methanolic extract of gall of *Quercus infectoria* showed maximum zone of inhibition (25 - 37mm) against *Staphylococcus aureus*, followed by acetone (24 - 29mm), ethanol (24 - 29mm) and cold water (23 - 27mm) respectively. Leela *et al*., (2011) reported, methanol extract displayed excellent activity against gram positive *B. subtilis* (25.3mm), *S. aureus* (22.0mm). Sucilathangam *et al*., (2012) showed that galls had higher antimicrobial activity against methicillin resistant *Staphylococcus aureus*. The range of minimum inhibitory concentration of *Terminalia chebula* extracts recorded was<0.24 to 7.81 mg/ml. The MIC value of this extract was 0.25 mg/ml for six MRSA strains and 0.5 mg/ml for the remaining 2 MRSA strains (Table: 1).Bag *et al*., (2009) have reported MIC values for cold aqueous, hot aqueous and ethanol extracts of *Terminalia chebula* against the test strains were ranged from 6.25 - 25.00 mg/ml; 3.12 - 12.50 mg/ml and 3.12 -12.50 mg/ml respectively. Dharmaratne *et al*., (2013) reported the lowest MIC values for the aqueous extract of *T.chebula* obtained by the reflux method. Their MIC value ranges from 0.25 mg/ml to 0.5 mg/ml. In the present investigation minimum inhibitory concentration of *Quercus infectoria* showed at the ranges from <0.24 to3.90 mg/ml (Table: 2). Khder *et al*., (2010) recorded activity of ethanolic extracts of galls against *E. coli* where MIC was 1200 µg/ml. Mekseepralard *et al*., (2010) recorded the MIC values of *Q. infectoria* extract against *Staphylococcus aureus* to be 0.41 mg/ml and 6.25 mg/ml for *P. aeruginosa*. Basri *et al*.,(2005) reported that the aqueous and acetone extracts from the galls of *Q. infectoria* showed MIC value ranging between 0.0781 mg/mL and 1.25mg/mL. The phytochemical screening of *Terminalia chebula* extract (Table:3) were found to tannins, flavonoid, terpenoid, alkaloid and sterols. The phytochemical analysis *T. chebula* showed the presence of terpenoids, resins and saponin where as it showed absence of alkaloids which is omparable to the works of Kathirvel*et al*., (2012) who investigated on bioactive compounds of *T. chebula* fruits and concluded that, it contains classes of compounds like phenol, flavonol, flavonoid, ascorbic acid, and proteins, carbohydrates. The phytochemical analysis of *Quercus infectoria* (Table: 3) showed the presence of tannins, flavonoid, terpenoid, alkaloid and sterols. Sreekanth *et al*., (2013) reported extracts of Gall of *Quercus infectoria* revealed the presence of alkaloids, tannins, phenolic compounds, Fat and Lipids, Resins, proteins, glycosides and flavanoids.

Table 1. Minimum Inhibition Concentration of *Terminalia chebula*:

S. No	Bacterial isolates	MIC of *T. chebula* (mg/ml)			
		Ethanol	Methanol	Acetone	Cold water
1	S-5	3.90	7.81	7.81	3.90
2	S-76	3.90	3.90	3.90	3.90
3	S-184	3.90	3.90	3.90	3.90
4	S-98	<0.24	3.90	3.90	<0.24
5	S-120	0.97	7.81	7.81	0.97
6	S-134	1.95	7.81	7.81	1.95
7	S-212	1.95	3.90	3.90	1.95
8	S-200	3.90	1.95	1.95	3.90

Table 2. Minimum Inhibition Concentration of *Quercus infectoria*

S. No	Bacterial isolates	MIC of *Q. infectoria* (mg/ml)			
		Ethanol	Methanol	Acetone	Cold water
1	S-5	<0.24	3.90	<0.24	0.48
2	S-76	<0.24	3.90	<0.24	0.48
3	S-184	<0.24	3.90	<0.24	0.48
4	S-98	<0.24	0.48	<0.24	0.48
5	S-120	<0.24	3.90	<0.24	1.95
6	S-134	<0.24	3.90	<0.24	<0.24
7	S-212	<0.24	1.95	<0.24	0.97
8	S-200	<0.24	3.90	<0.24	0.97

Table 3. Phytochemical analysis of *Terminalia chebula* and *Quercus infectoria*dry seedpowder

S. No	Name of the phytochemicals	Color indicates	Result	
			Terminalia chebula	*Quercus infectoria*
1	Carotenoids	Blue color ring	-	-
2	Flavonoids	Yellow color formed	+	+
3	Terpenoids	Reddish brown precipitate	+	+
4	Tannins	Green color	+	+
5	Saponins	Foam formed	+	+
6	Sterols	Red color	+	+

+ = Present; - = Absent

REFERENCE

Adetuyi AO, Popoola AV. Extraction and dyes ability potential studies of the colourant in *Zanthoxylum zanthoxyloides* plant on cotton fabric. *J Sci Eng Tech*. 2001; 8 (2), 3291-3299.

Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar and PCR for mecA gene for detection of MRSA. *Indian Journal of Medical Microbiology*. 2009; 27(1), 27-9.

Anwesa Bag, Subir Kumar Bhattacharyya, Premananda Bharati Nishith Kumar Pal, Rabi Ranjan Chattopadhyay. Evaluation of antibacterial properties of Chebulic myrobalan (fruit of *T chebula* Retz.) extracts against methicillin resistant *Staphylococcus aureus* and trimethoprim-sulphamethoxazole resistant uropathogenic *Escherichia coli*. *African Journal of Plant Science*. 2009; 3 (2), 25-29.

Basri DF, Fan SH. The potential of aqueous and acetone extracts of galls of *Q. infectoria* as antibacterial agents. *Ind J Pharmacol*. 2005; 37, 26-29.

Bergey DH, Holt JG. Bergey's Manual of Determinative Bacteriology 9th Edition.Lippincott Williams and Wilkins. 1994; 1268.

Dharmaratne MPJ, Manoraj A, Thevanesam V, Bandara BMR,Ekanayake EWMA, Kumar NS. Antibacterial Activity of Aqueous Extracts of *Terminalia chebula* Fruit against Some Multidrug-Resistant Human Pathogens.*International Journal of Research in Pharmaceutical and Biomedical Sciences*. 2013; 4 (4).

Digraki M, Alma MH, Ilcim A, Sen S. Antibacterial and antifungal effects of various commercial plant extracts. *Pharm Biol*. 1999; 37, 216-20.

Fatima S, Farooqi AHA, Kumar R, Kumar TRS, Khanuja SPS. Antibacterial activity possessed by medicinal plants used in tooth powders. *J Med Aromatic Plant Sci*. 2001; 22, 187-9.

Gandhi NM, Nayar CK. Radiation protection by *Terminalia chebula*: some mechanistic aspects. *J. Molecular and Cellular Biochemistry*. 2005; 48, 277-283.

Harleen Kaur, Archana Saini, Shashank Purwar, Harbhajan Kaur,Kardesai SG, Sanjiva D. Kholkute, Subarna Roy. Susceptibility testing and resistance phenotypic detection in Staphylococcus aureus by conventional and molecular methods: Importance of automated (Vitek 2) system. *International Journal of Engineering Research and Development*. 2012; 3 (10), 68-74.

Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N, Shimotohno K. Inhibitory effects of Sudanese medicinal plant extracts on hepatitis C virus protease. *Phytother Res*. 2000; 14, 510- 516.

Hwang JK, Kong TW, Baek NI, Pyun YR. α- Glycosidase Inhibitory Activity of hexagalloylglucose from the galls of *Quercus infectoria*. *Planta Med*. 2000; 66, 273- 4.

Ikram M, Nowshad F. Constituents of *Quercus infectoria*. *Planta Med*. 1977; 31, 286-7.

Jawad R. Al-Zaidi and Amin A. Al-Sulami. Comparison of chromogenic agar medium and diffusion disk test for detection of hospital acquired Methicillin Resistant *Staphylococcus aureus* (HA-MRSA) from patients and hospital environment in Nasiriyah, Iraq. *African J of Microbiology Research*. 2013; 7 (19), 1888-1895.

Kathirvel A, Sujatha V. In vitro assessment of antioxidant and antibacterial properties of *Terminalia chebula* Retz. Leaves. *Asian Pacific Journal of Tropical Bio medicine*. 2012; 788- 795 (2012).

Kaur G, Hamid H, Ali A, Alam MS, Athar M. Anti-inflammatory evaluation of alcoholic extract of galls of *Quercus infectoria*. *J Ethnopharmacol*. 2004; 90, 285-92.

Khder AK, Muhammed S.Potential ofAqueous and alcohol Extracts of *Quercus infectoria*, *Linusm usitatissium* and *Cinnamomum Zexlanicium* as Antimicrobials and Curing of Antibiotic Resistance of *E. coli*.*Current Research Journal of Biology Sciences*. 2010; 2(5), 333-337.

Kumar M, Agarwal RC, Dey S, Rai VK, Johnson B. Antimicrobial activity of aqueous extract of *T. chebula* Retz. On Gram positive and Gram negative microorganisms. *International Journal of Current Pharmaceutical Research*. 2009; 1(1), 56-60.

Kumarasamy Y, Philip JC, Marcel J, Lutfun N, Satyajit DS. Screening seeds of Scottish plants for antibacterial activity. *J Ethnopharmacol*. 2002; 83(1-2), 73-77.

Leela TM, Satirapipathkul C. Studies on the Antibacterial Activity of *Quercus infectoria* Galls. *International Conference on Bioscience, Biochemistry and Bioinformatics*. 2011; 410 – 414.

Ma H, Diao Y, Zhao D, Li K, Kang T. A new alternative to treat swine influenza A virus infection; *Terminalia chebula* Retz. *African Journal of Microbiology Research*. 2010; 4(6), 497-499.

Mekseepralard C, Kamkaen N,Wilkinson JM. Antimicrobial and antioxidantactivities of traditional Thai herbal remedies for aphthous ulcers. *Phytotherapy Research*. 2010; 24:1514-1519.

Prabhat, Ajaybhan, Navneet, Chauhan A. *Report and Opinion*.2010; 2(6), 37-42.

Prakash DVS, Satya NS, Avanigadda S, Vangalapati M. Pharmacological review on *T. chebula*. *International Journal of Research in Pharmaceutical and Bio-medical Sciences*. 2012; 3(2), 679-683.

Redwane A, Lazrek HB, Bouallam S, Markouk M, Amarouch H, Jana M. Larvicidal activity of extracts from *Quercus lusitania var. infectoria* galls (Oliv.). *J Ethnopharmacol*. 2002; 79, 261-3.

Roopalatha UC, Vijayamala (Grover) Nair. The phytochemical screening of the pericarp of fruits of *Terminalia chebula* Retz. *Int J of Pharma & Biosciences*. 2013; 4 (3), 550-559.

Saleem A, Husheem M, Harkonen P, Pihlaja K. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* Retz fruit. *Journal of Ethnopharmacology*. 2002; 81(3), 327-335.

Shankar D, Ved DK. Indian Medicinal and Aromatic Plants. *The Indian Forester*. 2003; 129, 275-288.

Skov R, Smyt R, Larsen AR. Phenotypic detection of methicillin resistance in *Staphylococcus aureus* by disc diffusion testing and E test on Muller Hinton agar. *J Clin Microbiol*. 2006: 33(12); 4395-4399.

Sreekanth SK, Ganesan V, Kannappan M, Deepa T Vasudevan. Preliminary phytochemical and antimicrobial evaluation of galls of *Quercus infectoria*. *International Journal of Research in Pharmaceutical and Nano Sciences.* 2013; 20`2(1), 101-106.

Srikumar R, Devi RS, Ayyappan SR, Thangaraj R, Jagadeesh R, Hariprasath L. Antifungal activity of aqueous and ethanolic extracts of Triphala and its individual fruit components. *International Journal of Pharma. Research and Development*-online. 2010; 2(8).

Suchalatha S, Devi CS. Antioxidant activity of Ethanolic extract of *Terminalia chebula* fruit against isoproterenol-induced oxidative stress in rats. *Indian Journal of Biochemistry and Biophysics*. 2005; 42, 246-252.

Sucilathangam G, Nithya Gomatheswari S, VelvizhiG, Pauline Vincent, Palaniappan N. Detection of anti-bacterial activity of Medicinal plant *Quercus infectoria*against MRSA isolates in clinical samples.*Journal of pharmaceutical and bio medical sciences*. 2012; 14(08), 1-5.

Venkatesan D, Karunakan CM, Kumar SS, Swamy PTP. Identification of phytochemical constituents of Aeglemarmelos responsiblefor antimicrobial activity against selected pathogenic organism.*Ethano botancial leaflets*. 2009; 13, 1362-1372.

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