ejpmr, 2020,7(2), 587-590

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIBACTERIAL ACTIVITY OF *CASSIA AURICULATA* AND *EUGINEA JAMBOLANA* AGAINST METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*

R. Kanchana[1], Dr. B. Bharathi[2]* and Dr. Deepa C. Philip[3]

[1]Department of Medical Laboratory Technology, MMM College of Health Sciences, Chennai, Tamilnadu.
[2]Associate Professor, Department of Microbiology MMM College of Health Sciences. Chennai, Tamilnadu.
[3]Principal, MMM College of Health Sciences, Mogappair, Chennai, Tamil Nadu.

***Corresponding Author: Dr. B. Bharathi**

Associate Professor, Department of Microbiology MMM College of Health Sciences. Chennai, Tamilnadu.

Article Received on 22/12/2019 **Article Revised on 12/01/2020** **Article Accepted on 02/02/2020**

ABSTRACT

Background: Various medicinal properties have been attributed to natural herbs. The antibiotic resistant microorganisms has increased in past three decades. The Methicillin Resistant *Staphylococcus aureus*(MRSA) is a problematic pathogen in the field of medical science. This organism tends to cause serious infection in the burn patients and causing higher mortality. Thereby our study aims to control these micro-organisms using various plants extracts. Natural products of higher plant may possess a new source of antimicrobial agent with possible novel mechanism of action. **Aim:** The main objective of this study is to evaluate the antibacterial activity of *E.jambolana* and *C.auriculata* against MRSA and to do screening for phytochemical constituents. **Materials and methods:** A prospective study was conducted to analyse the phytochemical components and to compare the efficacy of ethanolic, ethylacetate, diethyl ether, hexane and aqueous extracts of *E.jambolana* and *C.auriculata* on MRSA. **Results:** The highest inhibition zone 20mm was observed in 45 µl ethylacetate and aqueous extracts of *C.auriculata* and 18mm highest zone was observed in 45µl ethylacetate extracts of *E.jambolana.* **Conclusion:** The inhibitory effect of the extracts justified the medicinal use of both plants *Cassia auriculata* and *Eugenia jambolana.* In future after the detailed study these medicinal plants can be used as potent biochemical drugs to overcome the infections caused by these organisms.

KEYWORDS: Methicillin Resistant *Staphylococcus aureus, Eugenia jambolana* and *Cassia auriculata.*

INTRODUCTION

Bacterial and fungal pathogens are the etiological agents of human infection.[1] Bacteria develop resistance towards antibiotics, through overuse or misuse of antibiotics.[2] MRSA had evolved resistance towards the β lactam antibiotic which are previously used antibacterial agent, such as methicillin, penicillin, oxacillin, amoxicillin and cephalosporins.[3] MRSA is a severe pathogen found to be most prevalent pathogen found in hospitalized patients and the second most prevalent pathogen found in healthcare setting outside of the hospital. Methicillin is a narrow spectrum β lactam antibiotics targeting cell wall synthesis and commonly used for gram positive organism such as *Staphylococcus.*[4,5] Plant material remain an important resource to combat serious diseases in the world, the traditional medicinal methods, especially the use of medicinal plants still plays a vital role in cover the basic health needs in the developing countries.[6,7] They are active in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials,[7,8] Extraction and characterization of several active phytocompounds from those green factories have given birth to some high activity profile drugs.[8,9] Phytochemical screening of plant has revealed the presence of numerous chemical including alkaloids, tannins, flavonoids, steroids, glycosides and saponins etc, secondary metabolites of plants serves as secondary mechanisms against predation by many microorganisms, insects and herbivores.[10] *E.jambolana* possess the range of pharmacological properties such as antidiabetic, anti inflammatory, antiulcerogenic ,cardioprotective, antidiarrhoeal, antioxidant and hepatoprotective activity.[11] The antibacterial property of *Cassia auriculata* has been evaluated against *Staphylococcus aureus, Enterococcus faecalis, Bacillus subtilis, Salmonella typhi, Salmonella paratyphi A, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio cholera* and *Shigella dysenteriae.*[12] The main objective of this study is to evaluate the antibacterial activity of *E.jambolana* and *C.auriculata* against drug resistant pathogens and to do screening for phytochemical constituents by using the minimal inhibitory concentration(MIC), the values are used to determine the susceptibility of MRSA to various extracts of the plant by well diffusion method.

MATERIALS AND METHODS

Plant materials: Flowers of *C.auriculata* and seeds of *E.jambolana* were collected from local market, dried in shade for 10 days and grained into fine powder by electrical blender and stored in air tight container.

Pathogens: Methicillin resistant *Staphylococcus aureus* obtained from ATCC were subcultured in nutrient agar plates and incubated at 37^{o} C for 24 hours. Fresh cultures were employed for assessing MIC and antibacterial activity of plant extracts.

Extract preparation:[13]

The plant samples were washed under tap water, rinsed with distilled water and dried in shadow, then grinded into powder form. Each plant sample was weighed and soaked in respective solvent (distilled water, ethyl acetate, diethyl ether and hexane) at a ratio of 5:50 (w/v) for 72 hours. Extracts were then filtered through whattman No: 1 filter paper to separate the plant residues. Thus, the obtained plant extracts were stored at 4^{0}C until further use for phytochemical and antimicrobial activity screening.

Phytochemical screening:[14,15]

Phytochemical examinations were carried out for all the extracts for tannins, terepenoids, alkaloids, flavonoids, saponins, phenols and glycosides as per the standard methods.

Determination of Minimum inhibitory concentration (MIC)

The broth dilution is a qualitative technique for determining MIC of antimicrobial agent (µg or units/ml), which will inhibit the growth of the organism by in vitro.Broth dilution method is the simple procedure for testing a small number of isolates, even a single isolate. It has added advantage that the same tube can be used for MBC (minimum bactericidal concentration) test also. The antimicrobial agent is serially diluted in Nutrient broth by doubling dilution, then a standard amount of 1/100 diluted 4 hours broth culture is added to each of the antibiotic dilution and control tube. The MIC is the highest dilution of the antimicrobial agent, which shows with clear fluid with no development of turbid. Dilution of plant extract 1 ml = 1000 µl =1000 µg i.e, 1 µl = 1 µg.

Assay for antibacterial activity

Antimicrobial activity of the plant extracts was determined by the agar well diffusion method (Holder *et al* 1994), with modifications. 200 µL of overnight NB culture were added to 15 ml of molten MUELLER-HINTON Agar (Hi-Media M-173), mixed well, poured into a sterile PETRI dish and allowed to set. A well of about 3mm, 4mm, 5mm and 6mm diameter with sterile cork borer was aseptically punch on each agar plate. The diameter of the well was selected based on the filling capacity of the plant extract. Antibacterial activity of the *Eugenia jambolana* plant extract (aqueous, ethyl acetate, diethyl ether and hexane) was introduced into the well with different concentration (20 µl, 35 µl, 50 µl), for *Cassia auriculata* the different extract was given above was introduced in agar plate with different concentrations (15 µl,30 µl, 45 µl). The concentration of plant extract was selected based on the results obtained by MIC. A negative control well also made without extract on the agar plate. Plates were kept in laminar air flow for 30 minutes for diffusion of extract to occur and then incubated at 37^{0} C for 24 hours. Resulting of zone of inhibition was measured in millimetres. The disk diffusion method outlined by the National Committee for Clinical Laboratory Standards (NCCLS) was used with a 1 µg oxacillin disk (Oxoid). Zone sizes were read after incubation at 35°C for 24 h. Isolates with zone sizes 10 mm were considered methicillin resistant. Broth dilution method (Diagnostic manual)

RESULTS

1. Result of phytochemical analysis – *Eugenia jambolana* (Table 1)

Eugenia jambolana seed extracts were subjected to synthetic phytochemical screening with aqueous, ethyl acetate, Diethyl Ether and hexane extracts. The aqueous extract was found to be containing alkaloid, carbohydrates, flavonoid, tannins and phenols. Ethylacetate extract was positive for carbohydrates, flavonoids and phenols. The phytochemicals triterpenes, carbohydrates, flavonoid, phenols and glycosides were found in diethyl ether extract and finally hexane extract contained triterpenes, alkaloid, carbohydrates, flavonoid, phenols and glycosides. Terpenoides were absent in any of the extracts.

Table 1

S. No	Phytochemical constituents	Aqueous	Ethyl acetate	Diethyl ether	Hexane
1	Terpenoids	-	-	-	-
2	Triterpens	-	-	+	+
3	Alkaloid	+	-	-	+
4	Carbohydrates	+	+	+	+
5	Flavonoid	+	+	+	+
6	Tannins	+	-	-	-
7	Phenols	+	+	+	+
8	Glycosides	-	-	+	+
9	Saponin	-	-	-	-

2. Result of phytochemical analysis – *Cassia auriculata* (Table 2)

Cassia auriculata flower extracts were subjected to synthetic phytochemical screening by aqueous, ethylacete, Diethylether and hexane. The aqueous extract found to be contain terpenoids, alkaloid, carbohydrates, tannins, phenols, glycosides and saponin. Ethylacetate extract contains terpenoids, carbohydrates, phenols, glycosides and saponin. Diethyl ether extract contain triterpenes, carbohydrates ,tannins, phenols, glycosides and saponins and finally hexane extract contain terpenoids, alkaloid, carbohydrates, phenols and saponin.

Table 2:

S. no.	Phytochemical constituents	Aqueous	Ethyl acetate	Diethyl ether	Hexane
1	Terpenoids	+	+	+	+
2	Triterpens	-	-	-	-
3	Alkaloid	+	-	-	+
4	Carbohydrates	+	+	+	+
5	Flavonoid	-	-	-	-
6	Tannins	+	-	+	-
7	Phenols	+	+	+	+
8	Glycosides	+	+	+	-
9	Saponin	+	+	+	+

3. Antibacterial effect of different extracts of *Eugenia jambolana* on MRSA (Table 3).

The aqueous extract showed the highest zone of inhibition was 18 mm in 50 µl followed by 16 mm and 12 mm in 35 µl and 20 µl respectively. The ethyl acetate extract showed the highest zone of inhibition was 18 mm in 50 µl followed by 16 mm and 11 mm in 35 µl and 20 µl extracts. Ethyl acetate extract showed the highest zone of inhibition for MRSA. Diethyl ether showed the inhibition zones only for MRSA, maximum zone of inhibition was 13 mm in 50 µl followed by 12 mm and 13 mm for 35 µl and 20 µl extracts respectively.

Antibacterial effect of different extract of *Cassia auriculata* on MRSA (Table 3)

Aqueous extract showed zone formation about 5 mm, 12 mm and 15 mm in 15 µl, 30 µl and 45 µl respectively. In ethyl acetate extract, the zone formation was 12 mm, 16 mm and 18 mm in 15 µl, 30 µl and 45 µl respectively followed by in diethyl ether extract showed the inhibition zones 13 mm, 12 mm and 10 mm in 15 µl, 30 µl and 45 µl respectively.

Table 3: Statistical analysis of zones of inhibition of *C.auriculata* and *E.jambolana* against MRSA.

Plants	Bacteria	Aqueous			Ethylacetate			Diethyl ether			Hexane		
	Conc.	15µl	30µl	45µl	15µl	30µl	45µl	15µl	30µl	45µl	15µl	30µl	15µl
C.auriculata	MRSA	5±1.7	12±2.9	15±2.0	12±2.3	16±3.3	18±1.7	10±2.7	12±3.3	15±3.1	NI	NI	NI
E.jambolana	MRSA	12±1.4	16±1.1	18±1.3	***11±0.9**	***16±0.9**	***18±1.7**	***10±2.7**	***12±3.3**	***15±3.1**	NI	NI	NI

*****Significant

The comparison made between the aqueous extracts of *C.auriculata* and *E.jambolana* with other extracts of ethyl acetate and diethyl extract, in that analysis, 5 values shows significant results for *E.jambolana*.

Minimum inhibitory concentration (MIC) of various extracts of *Cassia auriculata* and *Eugenia jambolana* against MRSA (Table 4)

Extracts of *E.jambolana* and *C.auriculata* showed maximum antimicrobial activity was taken for MIC assay. The result of MIC assay showed in table 4.

Cassia auriculata	Aqueous	62.5 mg/ml
	Ethyl acetate	7.8 mg/ml
	Diethyl ether	15.6 mg/ml
	Hexane	3.9 mg/ml
Eugenia jambolana	Aqueous	62.5 mg/ml
	Ethyl acetate	15.6 mg/ml
	Diethyl ether	125 mg/ml
	Hexane	15.6 mg/ml

CONCLUSION

The present study exhibited the antibacterial effects of various effects of *C.auriculata* and *E.jambolana* on MRSA. Aqueous extract of *C.auriculata* the highest inhibition zone (18mm) was observed in 45 µl ethylacetate extract. *E.jambolana* extracts shown the highest inhibition zone (18mm) was observed on 45 µl ethylacetate ant aqueous The inhibitory effect of the

extract justified the medicinal use of both plant *Cassia auriculata* and *Eugenia jambolana*. Further study is required to find out the active component of medicinal value. The comparison was made between the aqueous extract of *C.auriculata* and *E.jambolana* with other (ethyl acetate and diethyl ether) extract by using biostatistical tool mean and standard deviation, in the analysis, 5 values shows significant (P value) results for *E.jambolana*. Both of these plants showed many phytochemical constituents.

This study concludes that, Plant extracts have many bioactive materials that is phytochemicals which act effectively as bactericidal agents against drug resistant pathogens. In the upcoming years, pathogens are continuously raise resistance against the antibiotics which we are using now a days. So we had selected the plants(*E.jambolana* and *C. auriculata*) which are used by the ancient people to treat many health issues particularly skin problems. The detailed research on phytochemicals and bioactive products will help the development of health care. This study compares the aqueous extract of two plant materials plant against the common drug resistant pathogens – MRSA.

Zone of inhibition formed by the extracts of plants show the significant effect on drug resistant organisms. This study proved the herbal plants can be used instead of commercially available antibiotics for the control of drug resistant bacterial pathogens.

ACKNOWLEDGEMENT

We would like to show our gratitude to our Principal Dr. Deepa C. Philip, MMM College of Health Sciences, Mogappair, Chennai who provided insight and expertise that greatly assisted the project. We thank our friends and colleagues for the great support and ideas.

REFERENCES

1. Negi. B. S., Dave, BP., and Agarwal, Y.K Evaluation of antimicrobial activity of *Bauhinia purpurea* leaves under in-vitro condition. Indian Journal of Microbiology, 2012; 52: 360-365.
2. Micheal CA, Dominey- HowesD and Labbate M. The antimicrobial resistance crisis causes, consequences, and management. Public Health, 2014; 2: 142.
3. Appelbaum PC. Microbiology of antibiotic resistance in *Staphylococcus aureus*. Clinical infectious Diseases, 2007; 45 (Supplement 3): 165-70.
4. Okesola A. Community acquired methicillin resistant S*taphylococcus aureus. A* review of literature. African Journal of Medical Science, 2011; 40: 97-107.
5. Uttley A.H.C.l, RC George, J Naidoo, N Woodfod, A.P. Johnson, CH Collins, D.Morrison, AJ Gilfillan; L. E Fitch and J Heptostall., – High level Vacomin resistant *Enterobacter* causing hospital infection. Epidemiological Infection, 1989; 103: 173-181.
6. Edeogal HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerial medicinal plants. African Journal of Biotechnology, 2005; 4: 685-688.
7. Iwu MW, Ducan AR, Okunji W New antimicrobial of plant origin In perspective on new crops and new uses, eds J Janick, American society horticulture sciences press, Alexandria VA, 1999; 457-462.
8. Ivanova D., D.Gerova. T. Chervenkov and T Yankova Polyphenols and antioxidants capacity of Bulgarian medicinal plants. Journal of Ethnopharmacology, 2005; 96: 146-150.
9. Mandal Y Mohan and S Hemalatha Microwave assisted extraction: An innovative and promising extraction tool for medicinal plant. Research Pharmacognosy Review, 2007; 1: 7-18.
10. Cowan, M.M, plant products as antimicrobial agents.Clinical Microbiology review, 1999; 12: 564-582.
11. Mukherjee PK Sahak, Murugesan T. MandalSc Pal M Saha BP Screening of the antibacterial profile of some plants extract of a specific regin of West Bengal India. Journal of Ethnopharmacology, 1998; 60(1): 85.69.
12. Maneemegalai S and T. Naveen Evaluation of Antibacterial Activity of Flower Extracts of *Cassia auriculata*. Ethnobotanical Leaflets, 2010; 14: 8-20.
13. Chua L E.L, Z.A Zakaria, Z. suhaili, S.Abubasker, M.N.M Desa Antimicrobial activity of plant extract against methicillin susceptible *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus*. Journal of Microbiology research, 2014; 4(1): 6-13.
14. PrashantTiwari, Bimlesh Kumar, Mandeepkaur, GurpreetKaur, HarleenKaur Phytochemical screening and extraction: A Review. International Pharmaceutical Sciences, 2011; 1(1).
15. Hossinejahagirian, MD JelasHaron, HO DS Halim shah ismail, Roshanak Rafiee, Moghaddam, Leili Afsah- Nejiri, Yadollah Abdollahi, Majid Rezeyi , Nazonin Vafaei - Well diffusion method for evaluation of antibacterial activity of copper phenyl fatty hydroxamate synthesized from Canola and palm kernel oil. Digest journal of nanomaterial and biostructure, 2013; 8(3): 1263- 1270.