Int. J. Pharm. Investigation, 2022; 12(2) : 228-232

Original Article

Antibacterial and Antibiofilm Activities of Natural Herbs against Methicillin Resistant *Staphylococcus aureus* in Pus from Scabies

Smritikana Biswas[1,*], Abhijit Ghosh[1], Biplab Giri[2,*]
[1]Department of Physiology, Krishnath College, Berhampore, Murshidabad, West Bengal, INDIA.
[2]Department of Physiology, University of Gour Banga, Malda, West Bengal, INDIA

ABSTRACT

Introduction: Mite *Sarcoptes scabiei* causes scabies which is a communicable skin infection and it promotes a suitable niche for microorganisms for their growth and development to cause secondary infection. This study was conducted to isolate the prevalent microorganisms collected from the pus of volunteers having scabies only, as well as to detect MRSA strains to study the antimicrobial and antibiofilm activity of *Allium sativum* bulbs, *Olea europaea, Piper nigrum* extracts against these strains. **Materials and Methods:** Pus samples were collected from 50 volunteers having scabies only and antibiotic susceptibility of the prevalent microorganisms as well as biofilm assay for MRSA isolates were also tested. Ethanolic extracts of *Allium sativum* bulbs, *Olea europaea* leaves, *Piper nigrum* berries were prepared and their antimicrobial and antibiofilm activities were determined. **Results:** out of 290, 65% (189) of them are confirmed as *Staphylococcus aureus* and 47 of these *Staphylococcus aureus* isolates were found to be resistant for Methicillin. On the other hand, in the present study *Allium sativum* bulbs and extracts of leaves of *Olea europaea* had potent antimicrobial activity against the MRSA strains isolated from the scabies while *Piper nigrum* extract had least amount of antimicrobial activity. Present study also revealed that extracts of *Allium sativum* bulbs possessed more antimicrobial property than the leaf extract of *Olea europaea* against the said strains. **Conclusion:** It can be concluded that MRSA strains harbouring volunteers with scabies may act as the reservoir for secondary infection and *Allium sativum* bulbs are more effective against the said strain than the *Olea europaea* and *Piper nigrum* extracts.

Keywords: Scabies**,** *Staphylococcus aureus***,** MRSA**,** *Allium sativum, Olea europaea, Piper nigrum***.**

Correspondence

[1]Dr. Smritikana Biswas
Assistant Professor, Department of Physiology, Krishnath College, Berhampore, Murshidabad-742101, West Bengal, INDIA.

Email id: physio.smritikana@gmail.com

[2]Dr. Biplab Giri
Professor, Department of Physiology, University of Gour Banga, Malda-732103, West Bengal, INDIA.

Email id: bgiri.emscrl@gmail.com

DOI: 10.5530/ijpi.2022.2.40

INTRODUCTION

Scabies is a communicable skin infection which is caused by *Sarcoptes scabiei* and has now become a health problem in the developing countries with improper hygiene, overcrowded tropical areas which promote the prevalence and complications of the disease. It is also suggested that Group A *Streptococci* and *Staphylococcus aureus* cause the secondary infection and induces the occurrence of pyoderma among the patients with scabies.[1] On the other hand, biofilms are reported by Costerton *et al.* (1999)[2] to be the basis for persistent of chronic bacterial infections. *Staphylococcus aureus* are also found to form biofilms on biomaterials, damaged tissues, catheter or other devices used for medical purposes causing biofilm-related infections.[3] Biofilm was reported to induce resistance against antimicrobial agents through biofilm formation which confers protection for their survival in the environmental reservoir.[2-5]

Emergence of multidrug resistant bacteria now has become a global problem due to excessive use of antibiotics.[6] It is necessary to search new strategies for fighting against infection. Medicinal values of different plants are well accepted throughout the world, as they contain various types of physiologically important phytochemicals possessing pharmacological activities.[7] Garlic extract (*Allium sativum* L.) has been suggested to inhibit the growth of *Staphylococci*, and is also used to prevent common cold, bacterial and fungal infections.[8,9] Beside this, Olive tree (*Olea europaea*) is a small tree or shrub which is a Mediterranean species.[10] Several reports suggest that Olive leaf possesses high quantities of phenolic substances which are responsible for their antibacterial activity.[10-12]

On the other hand, Black pepper (*Piper nigrum*) is a medicinal plant found in India. Phenolic compounds are reported to be present in Black pepper which has antimicrobial property against several types of bacteria like *Salmonella typhimurium*, *Bacillus*, *Staphylococcus aureus* etc.[13-15] Our study was conducted to detect the prevalent micro-organisms collected from the pus of volunteers having scabies only to detect the presence of MRSA (Methicillin resistant *Staphylococcus aureus*) and also to study the antimicrobial and antibiofilm activity of extracts of *Allium sativum* bulbs, *Olea europaea* leaves, *Piper nigrum* berries against Methicillin resistant *Staphylococcus aureus* isolates.

MATERIALS AND METHODS

Selection of Subject

Inclusion Criteria

Volunteers (age group-10 to 50 years) of both sexes who had scabies only and no addiction for smoking or alcohol were selected for the study.

Exclusion Criteria

Volunteers having the history of smoking or alcohol addiction and also other diseases were excluded. Moreover, volunteers who were too young

or two old (below 10 years or above 50 years) were also excluded for the study.

Collection of Sample

Samples containing pus were collected from the 50 selected volunteers of (age group of 10- 50 years) belonging to low socio-economic condition from the slum areas of Berhampore, Murshidabad, West Bengal, India under the supervision of Dermatologist, Lalbagh Hospital, West Bengal with the informed consent form signed by the selected volunteers during the period of February 2019 to July 2019.

Ethical Statement

Our study was carried out according to the ICMR human ethical guidelines adopted by the Institutional Ethics Committee, University of Gour Banga, Malda, India (Ref.No. UGB/IEC (Human) 0003-21).

Method for Culture of Bacterial Strains

Collected pus samples were allowed to grow on the LB agar media and kept at 37°C for 18 hr and then the bacterial colonies were cultured on the sheep blood agar in incubator at 37°C for 18 hr.

Identification

Gram stain, coagulase test, DNase and heat-stable nuclease tests, Mannitol salt fermentation test were used to detect *Staphylococcus aureus.*[16,17] Positive control strain was *Staphylococcus aureus* ATCC 25923.

Antibiotic Susceptibility

Tryptic soy agar containing NaCl (0.68 mol/L) and Oxacillin (6 mg/ml, w/v) was used to detect Methicillin-resistant *Staphylococcus aureus* (MRSA) [CLSI].[18]

Biofilm Formation Ability

Biofilm formation ability of these isolates was estimated by the standard method[19] with further modification in our laboratory. All strains of Methicillin resistant *Staphylococcus aureus* were allowed to be grown in TSB-glucose (containing 0.25% glucose) (HiMedia, India). 50 µl of each bacterial culture was taken into the sterilized 5 ml TSB and kept at 37°C for 6h. Then, 500 µl of this bacterial culture was inoculated into sterile test tube and kept at 37°C for 18 h. After that 500 µl of sterile phosphate buffered saline (PBS) are added to the test tubes for washing and after washing for three times all the test tubes were kept in an inverted position. 1ml of 0.1% safranin was used to stain them for 30s. After rinsing, the absorbance was measured at 490 nm with the help of spectrophotometer and three separate experiments were conducted for each assy.

Collection of Plants

Garlic (*Allium sativum*) was bought from the vegetable market in Gorabazar, Berhampore. Olive leaves (*Olea europaea*) were brought from Siliguri. Black pepper berries (*Piper nigrum*) were bought from a grocery shop in Berhampore. Authentication No. for *Allium sativum*, *Olea europaea* and *Piper nigrum* were KNC/BOT 30, KNC/BOT 31, KNC/BOT 32 respectively given by the department of Botany, Krishnath College, Berhampore, West Bengal.

All the plant extracts were made according to Ramya (2008)[20] with further modification in our laboratory.

Preparation of Plant Extracts

After cleaning with distilled water Leaves of *Olea europaea* as well as Black pepper berries (*Piper nigrum*) were dried in the hot oven at 50°C for 48 hr, separately and then allowedto ground into fine particles with the help of electrical blender. 20 gm of the powdered samples of leaves of *Olea europaea* and *Piper nigrum* berries were allowed to be soaked in 400 ml of 95% ethanol and 100 ml of 95% ethanol respectively.

On the other hand, Garlics (*Allium sativum*) were sliced after skinning. 50 gm of these sliced pieces were squeezed in a blender for 1 min and then 20 gm of it was soaked in 180 ml of 95% ethanol. Then each mixture was kept in standing condition for about 48 hr for extraction and then each of the extracts was passed through Whatman No. 1 filter paper by using Buchner funnel. For getting final extraction, each filtrate was allowed to evaporate in oven at 37°C for 24 hr. The final product was stored at 4°C. The extract was dissolved in DMSO diluted by sterile double distilled water to attain the final concentration of the extract of 1 gm/ml of DMSO for the antimicrobial assay.

Antimicrobial Assay

Antimicrobial activities of the said plant extracts (concentration of each plant extract was 1 gm/ml of DMSO) were determined according the standard method against MRSA isolates.[18] 100 µl of diluted culture (10^5 CFU/ml) of each MRSA isolates was spread on the nutrient agar plates. 40 µl of each of the plant extracts was given to the respective wells which were made in the plate and all of them were incubated at 37°C for 18 to 24 hr. Zone of inhibition (mm) against the MRSA isolates was measured to detect antimicrobial activity by three separate experiments.

Determination of Minimum Inhibitory Concentration (MIC)

The plant extract (having high antimicrobial activity) was used to determine MIC for each strain and diluted concentrations which were used as follows 180, 135, 90, 45, 36, 27, 18, 9, and 1 mg/ml respectively. 100 µl of Methicillin resistant *Staphylococcus aureus* isolates (10^5 CFU/ml) mentioned earlier was taken into tubes containing nutrient broth and plant extracts (1:1v/v) with different concentrations. Then the tubes were kept in incubator at 37°C for one to two days in aerobic condition. Control for media, control for organism and control for extract were used for each strain. The plant's extract which have lowest concentration producing no growth after 2 days and will be considered as MIC. All of them were compared with the control.

Biofilm Formation and Plant Extracts

Biofilm assay was done by standard protocol[19] with further modification in our laboratory. Methicillin resistant *Staphylcoccus aureus* isolates having high biofilm formation ability were grown for 24 hr using tryptone soy broth (TSB) (HiMedia, India) containing glucose (0.25%). 50 µl of each bacterial culture was taken into the 5 ml sterilized TSB containing plant extracts (1:1v/v) (test group) in a sterile test tube while 50µl of same bacterial culture was also transferred to 5 ml of TSB in the sterile test tubes without plant extracts (control group) and incubated at 37°C for 6hr. Then, 500 µl of this bacterial culture of control group was inoculated into sterile test tube and same volume of the inoculums of test group was also taken into another sterile test tube. Then, all test tubes including blank (one test tube containing 5 ml broth only) were kept at 37°C for 18 hr. Biofilm formation was measured which was described in the earlier section.

Screening for Phytochemical

Phytochemicals were analyzed qualitatively according to the standard methods.[21]

Statistical Analysis

Mean and standard deviation (SD) were obtained after repeating each of the quantifications for three times. Data were analyzed by using post hoc Dunn's test for multiple comparisons using SPSS Statistics 21.

P values ≤ 0.05 was used as the level of significance to justify the significant differences between groups.

RESULTS

In the present study samples were collected from 50 selected volunteers having scabies from the slum areas of Berhampore, West Bengal, India. About 290 bacterial isolates were isolated and 65% (189) of them were confirmed as *Staphylococcus aureus* (Table 1). Moreover, it was found that 47 of these *Staphylococcus aureus* isolates were resistant for Methicillin (Table 1) and it was also found that out of 50 volunteers, samples of 37 volunteers with chronic infection (74%) possessed MRSA. Moreover, 42 MRSA isolates were found to have moderate to high biofilm formation ability (Figure 1)

On the other hand, in the present study antimicrobial activities of ethanolic extracts of *Olea europaea* leaves, *Allium sativum* bulbs, *Piper nigrum* berries, against the MRSA isolates of scabies were determined. It was also observed that zone of inhibition produced by the extract of *Allium sativum* against 37 (78.72%) MRSA strains was significantly higher ($p \leq 0.05$) than the zone of inhibition created by the extracts of *Olea europaea* and *Piper nigrum* while extract of *Olea europaea* produced significantly higher ($p \leq 0.05$) zone of inhibition than *Allium sativum*, *Piper nigrum*, against 3 (6.38%) MRSA isolates (SBV5, SBV11 and SBV15) (Figure 2, Figure 3). But antimicrobial activity of *Piper nigrum* was negligible against the MRSA isolates in this study (Figure 2). Range of the MIC values of *Allium sativum* and *Olea europaea* were 4.5 mg/ml to 9.0 mg/ml and 9 mg/ml to 18 mg/ml respectively against 93.62% of the MRSA isolates (Table 2) while MIC values of *Allium sativum* and *Olea europaea* were 18 mg/ml and 4.5 mg/ml respectively against 6.38% of the MRSA isolates (Table 2). Whether the said plant extracts have any antibiofilm activity, biofilm formations by the MRSA strains having high biofilm formation ability were tested. Biofilm formation by these strains was found to be significantly ($p \leq 0.05$) reduced by three folds and two folds after the treatment of extracts of *Allium sativum* bulb, *Olea europaea* leaf respectively while there was no significant reduction ($p \leq 0.05$) in the biofilm production by these said strains after the treatment of the extract of *Piper nigrum* berry in comparison to the control (Figure 4). Phytochemical screening was performed to detect secondary metabolites and it was found that saponin, tannins, alkaloids, flavonoids, cardiac glycosides and phenolic compounds were present in the ethanolic extract of both *Allium sativum* bulb and *Olea europaea* leaf while the important secondary metabolites like saponin, flavonoids and phenolic compounds were absent in the ethanolic extract of *Piper nigrum* berries (Table 3).

Table 1: Prevalence of *Staphylococcus aureus* and antibiotic susceptibility.

No. of bacterial isolates	*Staphylococcus aureus* isolates	MRSA
290	189 (65%)	62

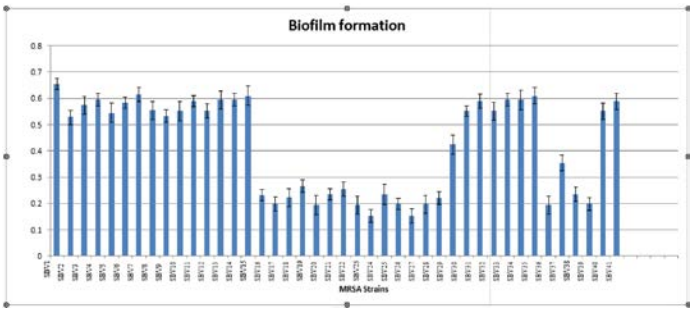

Figure 1: Biofilm formation by the MRSA isolates. Values are Mean ± SD. Biofilm formation at 37°C for 18 hr in MRSA isolates is presented. The strains which do not produce any biofilm are not shown in the figure. OD490 values are indicator of the amount of biofilm produced. "OD"-Optical density.

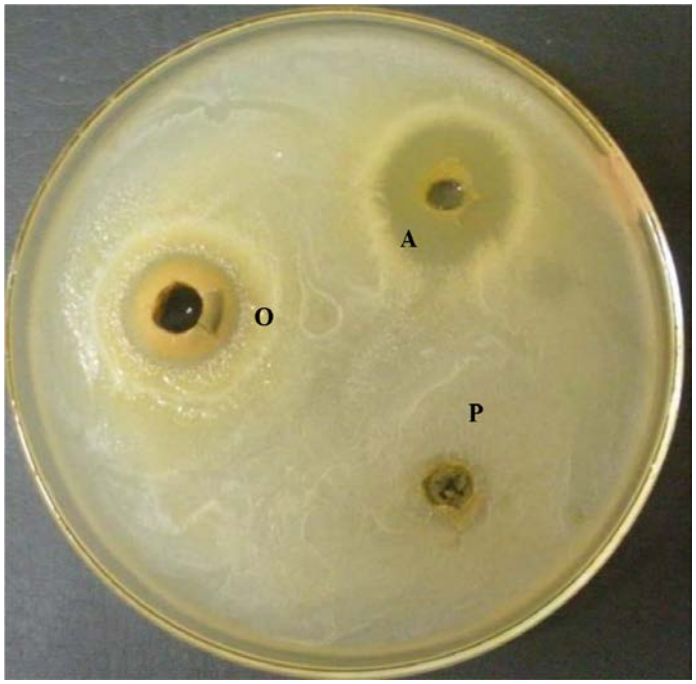

Figure 2: Methicillin resistant *Staphylococcus aureus* isolate on LB agar plate and Antibiotic susceptibility of *Allium sativum* bulb (A) extract, *Olea europaea* (O) leaf extract and *Piper nigrum* (P) berry extract against Methicillin resistant *Staphylococcus aureus*.

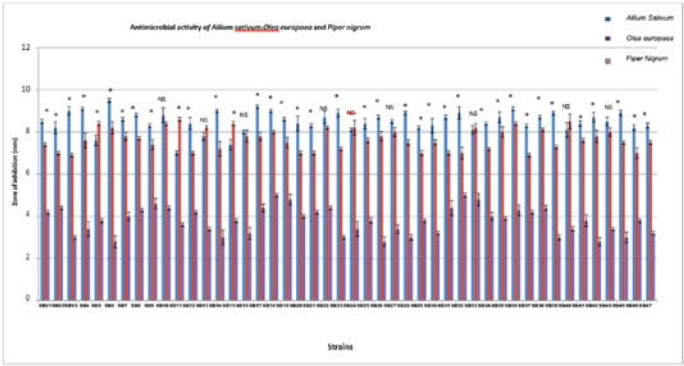

Figure 3: Comparison of Zone of inhibition produced by extracts of *Allium Sativum*, *Olea europaea*, *Piper Nigrum*

DISCUSSION

Communicable and incurable itching skin disease, scabies is caused by mites who impair the complement mediated defence system in humans.[22-23] In the present study samples were collected from 50 selected volunteers having scabies from the slum areas of Berhampore, West Bengal, India. About 290 bacterial isolates were isolated and 65% (189) of them were confirmed as *Staphylococcus aureus*. Findings of this study indicate that scabies mites may create a suitable niche for microorganisms for their establishment of burrows and this may augment the growth

Table 2: MIC values of extracts of selected plants against Methicillin resistant *Staphylococcus aureus* isolates.

Plant extracts	MIC values (mg/ml) of the plant extracts against 47 Meth^R *Staphylococcus aureus* isolates		
	SBV1,SBV2, SBV3, SBV4, SBV6, SBV7, SBV8, SBV9, SBV10, SBV12, SBV13, SBV14, SBV16, SBV17, SBV18, SBV19, SBV20, SBV21, SBV22, SBV23, SBV24, SBV25, SBV26, SBV27, SBV28, SBV29, SBV30, SBV31, SBV33, SBV34, SBV35, SBV38, SBV45, SBV46 (72.34%)	SBV5, SBV11 SBV15 (6.38%)	SBV32, SBV36, SBV37, SBV39, SBV40, SBV41, SBV42, SBV43, SBV44, SBV47,(21.28%)
Allium sativum	4.5 mg/ml	18 mg/ml	9 mg/ml
Olea europaea	9 mg/ml	4.5 mg/ml	18 mg/ml

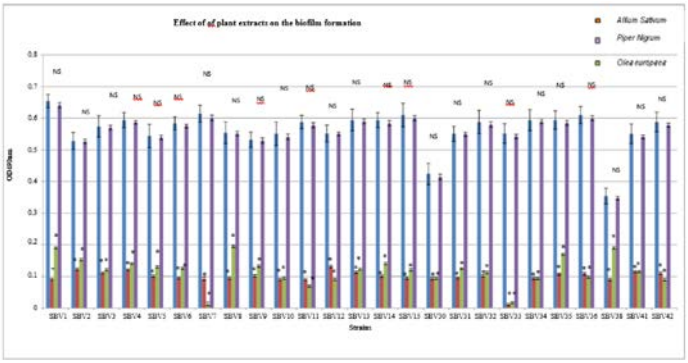

Figure 4: Comparison of biofilm formation by 42 MRSA isolates (having moderate to high biofilm formation ability) after the treatment with control, extracts of *Allium Sativum*, *Olea europaea, Piper nigrum* respectively.

Table 3: Phytochemical screening of the ethanolic extracts.

Sl. No.	Phytochemical Constituent	*Allium Sativum*	*Olea europaea*	*Piper nigrum*
1.	Saponins	+	+	-
2.	Tannins	+	+	+
3.	Alkaloids	+	+	+
4.	Flavonoids	+	+	-
5.	Cardiac glycosides	+	+	+
6.	Phenolic compounds	+	+	-

of *Staphylococcus aureus* which are also consistent with the several reports.[1,22-23] Andrews *et al.* (2009)[1] had shown that Group A *Streptococci* and *Staphylococcus aureus* were responsible for the secondary infection of the patient with scabies and complicacy of disease. Moreover, *Staphylococcus aureus* were found to be the predominating organism in this study and similar observation was shown by Swe *et al.* (2014).[24]

MRSA isolates of this study had moderate to high biofilm formation ability which was also shown by Otto (2008).[3] Beside this, findings of this study suggest that presence of MRSA among the volunteers with scabies may be the reason for their secondary infection.[3,25] Additionally it can be said that scabies of volunteers harbouring MRSA with biofilm formation ability may act as the reservoir for the future infection of the microorganisms by conferring protection and also inducing resistance against antimicrobial agents. Because biofilm formed by MRSA is more resistant to most available antimicrobial agents, leading to the treatment failures and increased survival in the environmental reservoir.[2,4-5] On the other hand, by assessing antimicrobial activities of ethanolic extracts of *Olea europaea* leaves, *Allium sativum* bulbs, *Piper nigrum* berries, against the MRSA isolates of scabies it was observed that *Olea europaea* leaf and *Allium sativum* bulb extracts have potent antimicrobial activity which were also reported earlier.[11-16] But antimicrobial activity of *Piper nigrum* was negligible against the MRSA isolates in this study while it was previously reported that *Piper nigrum* extract had potent antimicrobial activity against the MRSA strain.[15,16] These observations of the study indicate that *Piper nigrum* is less effective against the said strains. Beside this by comparing the observations of this study related to the zone of inhibition and range of MIC values it is suggested that *Allium sativum* bulb extract has more antimicrobial activity than the leaf extract of *Olea europaea* against the most of MRSA strains (78.72%) in the study. Additionally extract of *Allium sativum* bulb, *Olea europaea* leaf have antibiofilm activity as biofilm formation by the said strains was found to be significantly ($p \leq 0.05$) reduced after the treatment of extracts of *Allium sativum* bulb, *Olea europaea* leaf respectively while there was no significant reduction ($p \leq 0.05$) in the biofilm production by these said strains after the treatment of the extract of *Piper nigrum* berry in comparison to the control. On the other hand, phytochemical screening showed the presence of saponin, tannins,alkaloids, flavonoids, cardiac glycosides and phenolic compounds in the ethanolic extract of both *Allium sativum* bulb and *Olea europaea* leaf which was also reported by Nazir *et al* (2019)[26] and Ahmad *et al.* (2015)[12] respectively. Presence of these bioactive compounds may be responsible for conferring potent antimicrobial and antibiofilm activity. On other hand, the important secondary metabolites like saponin, flavonoids and phenolic compounds were absent in the ethanolic extract of *Piper nigrum* berries which have been shown by Ganesh *et al.* (2014).[15] This result suggests that absence of saponin, flavonoids and phenolic compounds in the ethanolic extract of *Piper nigrum* berries may be responsible for its low antimicrobial (not significant) and antibiofilm activity (not significant) against the said strains than the ethanolic extracts of *Allium sativum* bulbs, *Olea europaea* leaves. Further studies are required to find out the detailed underlying reasons for such observations.

CONCLUSION

The results of this study had shown the presence of *Staphylococcus aureus* as a predominating organism and most of the volunteers with scabies had MRSA isolates. Moreover, the findings of this study indicate that extract of *Allium sativum* bulbs possess more antimicrobial and antibiofilm properties than the leaf extract of *Olea europaea* against the MRSA isolates while extract of *Piper nigrum* is less effective in respect of antimicrobial and antibiofilm properties.

ACKNOWLEDGEMENT

The authors are grateful to all the volunteers who participated in this study.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Andrews RM, McCarthy J, Carapetis JR, Currie BJ. Skin disorders, including pyoderma, scabies, and tinea infections. Pediatr Clin North Am. 2009;56(6):1421-40. doi: 10.1016/j.pcl.2009.09.002, PMID 19962029.
2. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: A common cause of persistent infections. Science. 1999;284(5418):1318-22. doi: 10.1126/science.284.5418.1318, PMID 10334980.
3. Otto M. Staphylococcal biofilms. Bacterial biofilms. 2008;207-28.
4. Donlan RM, Costerton JW. Biofilms: Survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15(2):167-93. doi: 10.1128/CMR.15.2.167-193.2002, PMID 11932229.
5. Hall-Stoodley L, Costerton JW, Stoodley P. Bacterial biofilms: From the natural environment to infectious diseases. Nat Rev Microbiol. 2004;2(2):95-108. doi: 10.1038/nrmicro821, PMID 15040259.
6. Cunha BA. Antibiotic side effects. Med Clin North Am. 2001;85(1):149-85. doi: 10.1016/s0025-7125(05)70309-6, PMID 11190350.
7. Abdallah EM. Plants: An alternative source for antimicrobials. J Appl Pharm Sci. 2011;1(6):16-20.
8. Tsao SM, Yin MC. *In-vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oils. J Med microbial. 2001;50(7):646-9.
9. Houshmand B, Mahjour F, Dianat O. Antibacterial effect of different concentrations of garlic (*Allium sativum*) extract on dental plaque bacteria. Indian J Dent Res. 2013;24(1):71-5. doi: 10.4103/0970-9290.114957, PMID 23852236.
10. Sudjana AN, D'Orazio CD, Ryan V, Rasool N, Ng J, Islam N, *et al*. Antimicrobial activity of commercial *Olea europaea* (olive) leaf extract. Int J Antimicrob Agents. 2009;33(5):461-3. doi: 10.1016/j.ijantimicag.2008.10.026, PMID 19135874.
11. Brahmi F, Mechri B, Dabbou S, Dhibi M, Hammami M. The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. Ind Crops Prod. 2012;38:146-52. doi: 10.1016/j.indcrop.2012.01.023.
12. Ahmad W. Phytochemical profile, antimicrobial potential and GC-MS analysis of wild variety of *Olea europaea* (Olive) cultivated in Pakistan. Pure Appl Biol. 2017;6(1):337-45. doi: 10.19045/bspab.2017.60032.
13. Pradhan KJ, Variyar PS, Bandekar JR. Antimicrobial activity of novel phenolic compounds from green pepper (*Piper nigrum* L.). LWT Food Sci Technol. 1999;32(2):121-3. doi: 10.1006/fstl.1998.0508.
14. Zou L, Hu YY, Chen WX. Antibacterial mechanism and activities of black pepper chloroform extract. J Food Sci Technol. 2015;52(12):8196-203. doi: 10.1007/s13197-015-1914-0, PMID 26604394.
15. Ganesh P, Kumar RS, Saranraj P. Phytochemical analysis and antibacterial activity of Pepper (*Piper nigrum* L.) against some human pathogens. Eur J Exp Biol. 2014;3(2):36-41.
16. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, *et al*. Joint Working Party of the British Society for Antimicrobial Chemotherapy, Hospital Infection Society, Infection Control Nurses Association. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin- resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother. 2005;56(6):1000-18. doi: 10.1093/jac/dki372, PMID 16293678.
17. Quinn PJ, Markey BK, Carter ME, Donnelly WJC, Leonard FC, Maguire D. Veterinary microbiology and microbial disease. Blackwell Science Ltd.; 2002.
18. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 26th ed. Wayne, PA: CLSI; 2016.
19. Heilmann C, Gerke C, Perdreau-Remington F, Götz F. Characterization of Tn917 insertion mutants of *Staphylococcus epidermidis* affected in biofilm formation. Infect Immun. 1996;64(1):277-82. doi: 10.1128/iai.64.1.277-282.1996, PMID 8557351.
20. Ramya S. Antimicrobial activity of aqueous extracts of bark, root, leaves and fruits of *Terminalia arjuna*. Ethnobot leafl. 2008;1:158.
21. Swadhini SP, Santhosh R, Uma C, Mythili S, Sathiavelu A. Phytochemical screening and antimicrobial activity of five medicinal plants against Myrothecium sp. Int J Pharm Biol Sci. 2011;2(1):115-20.
22. Bergström FC, Reynolds S, Johnstone M, Pike RN, Buckle AM, Kemp DJ, *et al*. Scabies mite inactivated serine protease paralogs inhibit the human complement system. J Immunol. 2009;182(12):7809-17. doi: 10.4049/jimmunol.0804205, PMID 19494305.
23. Mika A, Reynolds SL, Mohlin FC, Willis C, Swe PM, Pickering DA, *et al*. Novel scabies mite serpins inhibit the three pathways of the human complement system. PLOS ONE. 2012;7(7):e40489. doi: 10.1371/journal.pone.0040489, PMID 22792350.
24. Swe PM, Fischer K. A scabies mite serpin interferes with complement-mediated neutrophil functions and promotes staphylococcal growth. PLOS Negl Trop Dis. 2014;8(6):e2928. doi: 10.1371/journal.pntd.0002928, PMID 24945501.
25. Whitehall J, Kuzulugil D, Sheldrick K, Wood A. Burden of paediatric pyoderma and scabies in North West Queensland. J Paediatr Child Health. 2013;49(2):141-3. doi: 10.1111/jpc.12095, PMID 23347222.
26. Nazir I, Chauhan R. Qualitative phytochemical analysis of *Allium sativum* (Garlic) and *Curcuma longa* (Turmeric). J Entomol Zool Stud. 2019;7(1):545-7.

Article History: Submission Date : 17-03-2022; Revised Date : 03-04-2022; Acceptance Date : 29-04-2022.
Cite this article: Biswas S, Ghosh A, Giri B. Antibacterial and Antibiofilm Activities of Natural Herbs against Methicillin Resistant *Staphylococcus aureus* in Pus from Scabies. Int. J. Pharm. Investigation. 2021;12(2):228-32.