Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(2): 1802-1809. **OPEN ACCESS**

Antimicrobial activities of *Dalbergia sissoo* DC. and *Caralluma tuberculata* (N.E.Br.)

Ijaz Ali[1*], Ghazala Hafeez Rizwani[2], Urooj Zafar[3], Atif Ali Khan Khalil[4], Zahid Farooq[5], Erum Dilshad[6], Abdul Baseer[7], Zahid Khan[8], and Muhammad Romman[9*], Saqibullah[10], Zahoor ul Haq[11]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Lahore, Lahore, **Pakistan**
[2]Department of Eastern Medicine, Hamdard University, **Pakistan**
[3]Department of Microbiology, University of Karachi, **Pakistan**
[4]Department of Biological Sciences, National University of Medical Sciences, Rawalpindi 46000, **Pakistan**
[5]Department of Pharmacology, University of Sargodha, **Pakistan**
[6]Department of Bioinformatics and Biosciences, Faculty of Health and Life Sciences, Capital University of Science and Technology (CUST), Islamabad, **Pakistan**
[7]Department of Pharmacy, Abasyn University, Peshawar, **Pakistan**
[8]Department of Pharmacognosy, Faculty of Pharmacy, Federal Urdu University of Arts, Science and Technology, Karachi 75300, **Pakistan**
[9]Pahrmacognosy Lab, Department of Botany, University of Chitral**,** KP**, Pakistan**
[10]Department of Botany, Qurtuba University of Science and Information Technology, KP, Peshawar, **Pakistan**
[11]Department of Botany, Shaheed Benazir Bhutto University, Sheringal, Dir (U), KP, **Pakistan**

***Correspondence:** dr.romman.uom@gmail.com **Received 03-04-2021, Revised: 23-06-2021, Accepted: 25-06-2021 e-Published: 29-06-2021**

Dalbergia sissoo DC. (Roxb.) and *Caralluma tuberculata* (N.E.Br.) are among the valuable flora of world including Pakistan. Several studies have reported the medicinal uses of these plants in folklore as well as in the ethnobotanical medicinal system. The anti-infective potential of these plants has a profound importance for the treatment of various infectious conditions. This research was aimed at exploration of antimicrobial potential of these important plant species by using agar well diffusion assay. All sample specimens were found active against different strains of bacteria and fungi. Minimum inhibitory concentrations were also determined for significantly active fractions. Significant results against *Proteus* and *S. saprophyticus* spices were observed which can be beneficial to develop new herbal remedy to cure community-acquired and catheter-associated urinary tract infections, cystitis, pyelonephritis, prostatitis, wound infections and burn infections. Furthermore, this study also justifies the traditional use of these two herbs for the treatment of various infectious conditions.

Keywords: Antibacterial, Antifungal, Clinical isolates, *Dalbergia sissoo, Caralluma tuberculata*

INTRODUCTION

A World Health Organization survey showed that 50% of mortalities are due to infections caused by various types of microbes, therefore it is important to have a strong regime against pathogenic microorganisms (Abdallah, 2011) Eradication of pathogens especially bacteria and fungi need prescribing rationale antimicrobial agents. Efficacy, safety and economy are three important aspects to consider for rationality while prescribing any therapeutic especially antimicrobial agent (Tamilarasi and Ananthi, 2012). Plant-based antimicrobials have proven rationality over allopathic drugs in terms of

efficacy, safety and economy (Ahmad and Beg, 2001). Therefore, herbal medicines are gaining importance over allopathic therapies (Cowan, 1999). This trend shift is due to many factors including multiple drug resistance (MDR) developed as a result of the extensive irresponsible use of antimicrobials (Davies, 1994, Service, 1995). Furthermore, adverse effects of antimicrobials on the host are also obvious i.e. hypersensitivity, immuno suppression, hepatotoxicity, nephrotoxicity and allergic reactions (Ahmad et al, 1998.). Similarly, price of allopathic antimicrobials is also an important factor of consideration especially in developing countries like Pakistan. All these issues have compelled the researchers to explore alternative antimicrobial options. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents (Monroe, 2000; Bhavnani and Ballow, 2000). Hence to treat infections successfully herbal antimicrobials are required to explore (Clark, 1996; Cordell, 2000). Extensive antimicrobial screenings are required to achieve this objective. Plenty of evaluations have already been performed throughout the world.

Dalbergia sissoo DC. and *Caralluma tuberculata* (N.E.Br) are two important medicinal plant species distributed in various parts of the world including Pakistan (Ali et al, 2016; Rizwani, 1991). *D. sissoo* is reported to be a stimulant used in folk medicine and remedies. It is a folk remedy for gonorrhoea and skin ailments. Ayurveda system prescribes various parts of *D. sissoo* to treat a variety of ailments. For example, leafy juice for eye ailments, the woody bark paste as anthelmintic, antipyretic and analgesic. The wood is also used in India for boils, leprosy and nausea. Its oil is reported to have larvicidal and repellant actions against mosquitoes (Ansari, 2000; Shuaib, 2020). Similarly, *C. tuberculata* is also highly beneficial medicinal plant; its aerial parts are used to treat several ailments including diabetes, rheumatism, leprosy, peptic ulcer, inflammation, jaundice, dysentery, constipation, stomach pain, hepatitis B and C. However, these highly valuable and useful plant species need further exploration of their antibacterial and antifungal potential. Therefore, the current study was designed to explore the antimicrobial activities of these plants.

MATERIALS AND METHODS

Plant Material:

Leaves, Pods and Bark of Plant A (*D. sissoo*) were collected from the botanical garden of the University of Karachi and Plant B (*C. tuberculata*) was collected from Kohat District KPK Pakistan. After collection authentication was done by Prof. Dr. Ghazala.H Rizwani, Director Pharmacy, Hamdard University.

Extraction:

Cleaned, garbled plant materials (500 gm) were soaked in clean transparent glass jars for 15 days in 1000 ml methanol. After 15 days filtration was performed with each soaked plant material. The filtered liquid was dried on a rotary evaporator at 40ºC temperature with 100 rpm. 15 g, 12 g, 10 g and 8 g dry crude extracts were obtained from leaves, pods barks of plant A and whole plant B and coded as DSL, DSP, DSB and WTR respectively. Extracts were packed in amber fluted glass vials and kept at 4ºC temperature for further experimentation.

Microorganisms:

The bacterial strains were obtained from the Department of Microbiology, University of Karachi Pakistan. The bacterial strains studied were *Micrococcus luteus, Staphylococcus aureus, S. saprophyticus, Bacillus cereus, Methicillin resistant staphylococcus aureus (MRSA), Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Shigella flexenari, Salmonella typhi, Citrobactor sp.* and *Proteus sp.* Bacterial strains were maintained in nutrient broth, and sub-cultured for 14 days. All the organisms were characterized and confirmed by microscopy as well as biochemical tests (IMVIC). The fungal species studied include *Candida albicans, Trichophyton longifuses, Aspergillus flavus, Microsporum canis, Fusarium solani, Candida glabrata* were obtained from Habib Ebrahim Jamal (HEJ) Research Centre, Karachi University, Karachi Pakistan.

Antibacterial assay:

Agar well diffusion assay:

Plant extracts were screened for in vitro antibacterial and antifungal bioassays using agar well diffusion method. A confluent lawn of the microbial inoculums was seeded over the Mueller Hinton agar surface. Wells, 8 mm in diameter, were made aseptically with a sterile borer, and a volume (40 µl) of extracts dissolved in DMSO (40 mg/ml) was introduced into the well. Agar plates

were incubated at 37°C for 24 hrs. The antimicrobial agent diffused in the agar medium and inhibits the growth of the microbial strain tested.

The results were further verified by the disc diffusion method (data not given). The diameters of the zone of inhibition were measured in mm. For antifungal activity, 24 g of crude extracts were dissolved in 1ml sterile DMSO serving as stock solutions. Saturated dextrose agar was used for the growth of fungus. Media with acidic pH (5.5-5.6) containing 2% glucose was prepared by mixing 32.5g/500ml distilled water.

Minimum inhibitory concentration (MIC):

The test samples showing zones of inhibition of 15mm or more in diameter were considered significant and forwarded for the determination of their MICs by the well diffusion assay. Recent studies suggest that the agar diffusion method is comparable with the broth dilution method for MIC determination. Varying concentrations of samples were prepared by two-fold dilution (from 40 to 0.156 mg/ml). Samples were introduced into wells in Mueller Hinton agar plate seeded with test culture followed by incubation at 37°C for 24 hrs. The minimum inhibition concentration was determined as the lowest concentration of the sample showing the zone of inhibition. For Minimum bactericidal concentration, streaks were transferred from a zone of inhibition to Nutrient agar and growth was observed.

RESULTS

The results of the present study are encouraging as methanolic extracts of selected parts of Plant A, abbreviated as (DSL Leaves, DSP Pods and DSB Bark) and aerial parts of Plant B denoted as (WTR) showed marked activities against several selected pathological strains of bacteria and fungi. No single plant was found to be equally effective against all the bacteria tested. All the samples showed considerable growth inhibition against *S. saprophyticus* and *B. cereus* with zones of inhibition ranging from 14 mm-30 mm (Table 1). The extracts of *D. sissoo* were also found bactericidal against *Proteus vulgaris* and *Citrobactor* with significant zones of inhibition as shown in Table 1 (13 mm-27 mm). Whereas for *M. luteus* and *K. aeruginosa* two parts (DSL and DSB) were found active with a zone of inhibition around 14mm for almost all samples. Only methanolic extract of *D. sissoo* showed activity against *S. typhi* having 17 mm zone of inhibition. On contrary *Escherichia coli, S. flexenari* and *P. aeruginosa* showed no noticeable response for all selected parts of both plants (Table 1).

The antifungal potential of plants understudy were also evaluated by performing the antifungal assay. For the antifungal profiling, *T. longifuses, A. flavus, M. canis, F. solani, C. glabrata* and *C. albicans* were used. Miconazole and Amphotericin B were used as a standard antifungal agent and positive control for comparison. In this evaluation, all selected parts of Plant A (DSL, DSP and DSB) were found active showing inhibition range 65-80%. Similarly plant B (WTR) also showed inhibition range 65-80% (table 2, 3) against all tested fungal strains.

Minimum inhibitory concentrations were also determined for antibacterial and antifungal assays (Table 4, 5). In antibacterial testing, DSL showed Minimum Inhibitory Concentrations (MIC) values 5, 10 and 20 mg/ml against *B. cereus, Proteus sp. and S. saprophyticus* respectively.While for DSB values were 20, 10, 20 mg/ml respectively. MIC for DSP was only calculated for *S. saprophyticus* which was 10 mg/ml.Same was performed for WTR with 20 mg/ml MIC.

The MIC evaluations for antifungal was conducted, it was found that both DSP and DSL show 16mm zone of inhibition at 1.25 µg/ml concentration whereas DSB exhibited 13mm zone on just 0.3 µg/ml concentrations (Table 5). On the other hand, WTR showed 19mm zone of inhibition on 0.75mg/ml minimum concentration. For in-depth explorations, minimum bactericidal studies were also conducted and results were recorded. For this purpose, three bacterial species *B. cereus, Proteus sp.* and *S. saprophyticus* were selected (Table 6).

DSP, DSB and DSL showed strong activity against *C. albicans*, with a minimum inhibitory concentration of 1.25, 0.15 and 1.25 mg/ml. While DSB with least MIC was found a better antifungal agent. Among bacteria, DSL showed the least minimum inhibitory concentration of 5 mg/ml showing the highest activity. None of the extracts showed detectable activity against *S. aureus*, MRSA, *E. coli, S. flexeneri* and *P. aeruginosa*. The results obtained were further confirmed by the disc diffusion method and the results obtained were comparable. Antifungal effects of all parts of *D. sissoo* were significant.

Minimum Bacterial Concentrations (MBCs) were calculated for *C. tuberculata*. It showed 20 mg/ml MBC against *S. saprophyticus.* The MBC is complementary to the MIC whereas the MIC test demonstrated the lowest level of extract that

greatly inhibited growth, the MBC demonstrated the lowest level of extract resulting in microbial death. Antibacterial agents are regarded as bactericidal if the MBC is no more than four times the MIC. MBC testing can be a good and relatively inexpensive tool to simultaneously evaluate multiple antimicrobial agents for potency.

In this research, MBC values for methanolic extracts of leaves, bark and pods of *D. sissoo* and vegetative part of *C. tuberculata* were found excellent and all extracts were bactericidal.

Gentamicin, Clarithromycin, Enrofloxacin, Ciprofloxacin, and Cefizox were used as standard drugs for culture sensitivity testing.

Table.1: Antibacterial Activity Profile of Methanolic extracts of different parts of *D. sissoo* and *C. tuberculata.*

S.No.	Bacterial Group	Zone of Inhibition (mm)			
		DSL[a]	DSP[b]	DSB[c]	WTR[d]
1	*Micrococcus luteus*	15	14	-	-
2	*Bacillus cereus*	19	14	18	14
3	*Staphylococcus saprophyticus*	30	21	17	22
4	*Methicillin Resistant Staph Aureus*	-	-	-	-
5	*Citrobacter sp.*	25	27	19	-
6	*Staphylococcus aureus*	-	-	-	-
7	*Proteus sp.*	22	13	18	-
8	*Klebsiella pneumoniae*	15	15	-	-
9	*Escherichia coli*	-	-	-	-
10	*Salmonella typhi*	17	-	-	-
11	*Shigella flexenari*	-	-	-	-
12	*Pseudomonas aeruginosa*	-	-	-	-

DSL methanolic extract of *Dalbergia sissoo* Leaves., b. DSB methanolic extract of *Dalbergia sissoo* Bark. c. DSP methanolic extract of *Dalbergia sissoo* Pod. d. WTR methanolic extract of the vegetative part of *Caralluma tuberculata*

Table 2: Anti- Fungal activity profile of parts (Leaves, Pods and Bark) of *D. sissoo*

S. No.	Name of Fungus	Percentage inhibition %							Standard drug MIC µg/ml
		DSP	DSL	DSB	Control	DSP	DSL	DSB	
1	*Trichophyton longifuses*	35	20	20	100	65	80	80	Miconazole
2	*Aspergillus flavus*	28	30	28	100	72	70	72	Amphoterecin B
3	*Microsporum canis*	24	22	22	100	76	78	78	Miconazole
4	*Fusarium solani*	20	20	30	100	80	80	70	Miconazole
5	*Candida glabrata*	30	24	24	100	70	76	76	Miconazole
6	*Candida Albicans*	30	26	22	100	70	74	70	Miconazole

Table 3: Anti- Fungal activity profile of vegetative parts of *C. tuberculata*

S.No	Name of Fungus	WTR (Linear Growth)	Control (Linear Growth)	Inhibition WTR %	Standard drug MIC µg/ml
1	*Trichophyton longifuses*	35	100	65	Miconazole
2	*Aspergillusflavus*	28	100	72	Amphoterecin B
3	*Microsporum canis*	24	100	76	Miconazole
4	*Fusarium solani*	20	100	80	Miconazole
5	*Candida glabrata*	30	100	70	Miconazole
6	*Candida albicans*	24	100	76	Miconazole

Table 4: Minimum Inhibitory concentration (MIC) of crude Extracts of *D. sissoo* (Leaves, Pods, Bark) and *C. tuberculata* (vegetative part) against bacterial species.

S.No.	Samples	Bacterial Species	MIC(mg/ml)	Zone of Inhibition(mm)
1	DSL	*B. cereus*	5	14
2	DSB	*B. cereus*	20	13
3	DSL	*Proteus sp.*	10	18
4	DSB	*Proteus sp.*	10	21
5	DSL	*S. saprophyticus*	20	14
6	DSB	*S. saprophyticus*	20	17
7	DSP	*S. saprophyticus*	10	18
8	WTR	*S. saprophyticus*	20	15

Table 5: Minimum inhibitory concentrations (MIC mg/ml) of crude Extracts of *D. sissoo* (Leaves, Pods, Bark) and *C. tuberculata* (vegetative part) against selected fungal species

Sr.N0.	Samples	Fungal Specie	Concentration (mg/ml)	Zone Of Inhibition(mm)
1	DSP	*Candida albicans*	1.25	16
2	DSL	*Candida albicans*	1.25	16
3	DSB	*Candida albicans*	0.15	13
4	WTR	*Candida albicans*	0.75	19

Table 6: Minimum bactericidal concentrations (MBC) of crude Extracts of *D. sissoo* (Leaves, Pods, Bark) and *C. tuberculata* (vegetative part) against selected fungal species

S.No.	Sample code	Bacterial Species	MBC(mg/ml)
1	DSL	*B. cereus*	10
2	DSB	*B. cereus*	20
3	DSL	*Proteus sp.*	10
4	DSB	*Proteus sp.*	20
5	DSL	*S. saprophyticus*	20
6	DSB	*S. saprophyticus*	20
7	DSP	*S. saprophyticus*	10
8	WTR	*S. saprophyticus*	20

Table 7: Culture Sensitivity test results

Antibiotics	Cultures									
	S. aureus	C. xerosis	VRE	Para typhi A	Para typhi B	E.coli	Enterobacter	P. mirabilis	K. pneumonia	P. aeruginosa
Gentamicin (GN)	-	20 mm	-	16 mm	16 mm	-	10 mm	-	-	12 mm
Clarithromycin (CLR)	-	-	-	-	-	-	-	-	-	-
Enrofloxacin (ENR)	-	29 mm	-	26 mm	26 mm	-	28 mm	-	-	17 mm
Ciprofloxacin (CIP)	-	35 mm	-	27 mm	26 mm	-	30 mm	-	-	26 mm

The methanolic extract of leaves of *D. sissoo* showed significant activities (15 mm) against *M. luteus* which was greater than Cefizox (5 mm) showing its better efficacy to treat *M. luteus* infections. DSP showed (14 mm) zone of inhibition again better than Cefizox (5 mm) and lesser than other antibiotics while DSB and CT did not show any detectable effects against *M. luteus*.

Synthetic antibiotics showed (5-25 mm) zone of inhibitions when applied on *B. cereus*. DSL (19 mm) showed better efficacy for treating *B. cereus* infections than Cefizox and equal efficacy in comparison with Gentamicin (19 mm), lesser than other three antibiotics while DSP (14 mm) and DSB (18mm) also exhibited a similar spectrum of activities. DSL showed the highest efficacy against *S. saprophyticus* i.e. (30 mm) as compared to all antibiotics which showed (16 mm), (21 mm), (21 mm), (26.5 mm), (23.5 mm) ZOI respectively. DSP (21 mm) showed higher effect than Gentamicin equal to Clarithromycin and Enrofloxacin and lower than Ciprofloxacin while DSB (17 mm) showed higher efficacy than Gentamicin and lower than all other antibiotics whereas the effectiveness of CT (22 mm) extract was higher than Gentamicin, Clarithromycin and Enrofloxacin and lower than Ciprofloxacin and Cefizox.

Citrobactor sp. showed (20 mm), (5 mm), (29 mm), (35 mm) and (5 mm) zone of inhibitions for Gentamicin, Clarithromycin, Enrofloxacin, Ciprofloxacin and Cefizox respectively.The susceptibility of *Citrobactor sp.* to DSL (25 mm) and DSP (27 mm) was higher than Gentamicin, Clarithromycin and Cefizox while lower than Enrofloxacin and Ciprofloxacin while DSB (19 mm) showed higher efficacy than Clarithromycin and Cefizox and lower than all other used antibiotics (Table-7).

DISCUSSION

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population. In the present work, the methanolic extracts obtained from *D. sissoo* plant (leave, pod and bark) strong antimicrobial activities were observed against most of the tested bacterial and fungal strains. The methanolic extract of leaves of *D. sissoo*, showed the significant effect against *M. luteus, B. cereus, S. saprophyticus, Proteus sp., Citrobactor, K. pneumoniae* and *salmonella typhi.* Methanolic extract of pods of *D. sissoo* showed significant activity against *M. luteus, B. cereus, S. saprophyticus, Proteus sp., Citrobactor sp.* and *K. pneumoniae*. Methanolic extract of barks of *D. sissoo* exhibited significant antibacterial activities against *B. cereus, S. saprophyticus, Proteus sp.* and *Citrobactor* sp. (Bauer, 1996; Hussain et al. 2014). Methanolic extract of *C. tuberculata* exhibited significant activity against *B. cereus, S. saprophyteicu*s.DSP, WTR, DSB and DSL showed strong activity against *C. albicans*, with minimum inhibitory concentration of 1.25, 0.75, 0.15 and 1.25 mg/ml. Among bacteria, DSL showed the least minimum inhibitory concentration of 5 mg/ml. None of the extracts showed detectable activity against *S. aureus*, MRSA, *E. coli, S. flexeneri* and *P. aeruginosa* and reported strong activity of leaf extracts against *E. coli* (Hussain et al. 2014; Burt, 2004). The significant antimicrobial actions of methanolic extracts of plant A (*D. sissoo*) are possibly due to the presence of bioactive compounds (Behera, 2013). The longevity of this plant in strenuous conditions is also associated with its diverse phytochemical nature. It possesses several valuable compounds which are responsible for its antimicrobial actions (Al-Snafi, 2017; Romman et al. 2015). Further the presence of inorganic constituents especially calcium both in the intracellular form in the phloem, pith and parenchymatous tissues and extracellular form as calcium oxalate crystals (Thite et al. 2013). Presence of these organic and inorganic compounds might has enabled this plant long-lasting with strengthening properties for human beings as well. Presence of calcium might be a reason of folkloric use of this plant in Dysmenorrheal and bone disorders and as a safe tonic. The folkloric use of woody products made up of this plant-like drinking pots can also be justified with the same evidence. Furthermore, the nutrient-rich extract strengthens the immune system which helps us to combat infectious conditions more successfully. Similarly, the presence of tannins also potentiates the antimicrobial effects of methanolic extracts of this plant (Al-Snafi, 2017; Nowsheen et al. 2020). According to folklore, its decoction is said to be tonic within safe limits and utilized for the same purpose. The present study justified the use of various parts of *D. sissoo* in the traditional system of medicine to treat various infectious diseases caused by different microbes. Methanolic extract of the vegetative part of *C. tuberculata* (N.E.Br.) was tested for its antibacterial effects. Antifungal

effects of all parts of *D. sissoo* are significant (table-2) while vegetative parts of *C. tuberculata* extract also showed significant results (table-3). Minimum inhibitory concentrations are presented in table-4 & Table-5 respectively against bacterial and fungal strains. The ability to compare the results for antimicrobial plant compounds or extracts from different studies is limited because of differences in the methodologies used and different definitions of MIC. The present study justified the use of various parts of *D. sissoo* and vegetative parts of *C. tuberculata* in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Antifungal effects of all parts of *D. sissoo* are significant (table-2) while vegetative parts of *C. tuberculata* extract also showed significant results (table-3). Minimum inhibitory concentrations are presented in table-4 & Table-5 respectively against bacterial and fungal strains.

CONCLUSION

The present study highlights the use of various parts of *D. sissoo* and vegetative parts of *C. tuberculata* in the traditional system of medicine to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest. It is part of a PhD thesis and is submitted to the HEC repository directory.

ACKNOWLEGEMENT

Department of Pharmacognosy, Faculty of Pharmacy Ziauddin University, Pakistan and Pharmacognosy Lab, Department of Botany, University of Chitral, KP, Pakistan are conceding for support.

AUTHOR CONTRIBUTIONS

IA designed and performed the experiments and also wrote the manuscript. IA performed animal treatments and data analysis. GHR, designed experiments UZ, AAKK, ZF, ED, AB, ZK, MR, SU and ZH reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Abdallah EM, 2011. J Appl Pharm Sci 1:16-20.

Ahmad I, Mehmood Z, Mohammad F, 1998. J Ethnopharmacol 62:183-193.

Ahmad I, Beg AZ, 2001. J Ethnopharmacol 74:113-123.

Ali I, Rizwani GH, Shareef H, Khan S, 2016. Int J Pharm Pharm Sci 8:48-53.

Al-Snafi AE, 2017. Chemical constituents and pharmacological effects of Dalbergia sissoo-A review. IOSR J Pharm *7*: 59-71.

Ansari MA, 2000. Bio resource Technology. 73.3. 207-211.

Bauer A, 1996. Am J clin pathol. 45:149-158

Bhavnani SM, Ballow CH, 2000. Curr Opin Microbiol 3:528-534.

Burt S, 2004. Int J Food Microbiol. 94:223-253

Behera PC, Verma SM, Kumar PM, Das NB, Mishra PM, Baliarsingh S, 2013. American Journal of Phytomedicine and Clinical Therapeutics 1: 186-194

Clark AM, 1996. Pharm Res 13:1133-1141

Cordell GA, 2000. Phytochemistry 55:463-480 Cowan MM, 1999. Clin Microbiol Rev. 12:564-582

Davies, 1994. J Science 264:375-382

Hussain M, Ullah Khan M, Raza SM, Aziz A, Bakhsh H, MAJEED A, 2014. Int J Pharm Sci. 4:702-706Monroe S, Polk R, 2000. Curr Opin Microbiol. 3:496-501

Nowsheen T, Sayed WAS, Ali H, Gul R, Abdul K, Ikram I, … Afshan B, 2020. Phytochemical, antioxidant and antibacterial screening of *Artemisia Absinthium* from Dir Lower Pakistan. Bioscience Research. 17: 2233-2241.

Rizwani GH, 1991. University of Karachi, Karachi
Romman M, Samin J, Muhammad H, Izhar A, Sher W, 2015. In vitro Antioxidant activity of stem and leaves extracts of *Hedera nepalensis* K. Koch. Int J Biosciences 7: 19-24.Service RF, 1995. Science 270:724-727.
Shuaib M, Naila A, Saraj B, Muhammad R, Qian Y, Chang X. 2020. Variation and succession of microbial communities under the conditions of persistent heavy metal and their survival mechanism. Microbial Pathogenesis 150; 1-9.
Tamilarasi T, Ananthi T, 2012. Research Journal of Chemical Sciences 2: 72-74.
Thite SV, Aparadh VT, Kore BA, 2013. Int. J. Res. Pharm. and Biomed. Sci *4*: 841-6.