International Journal of Pharmaceutical Chemistry
ISSN: 2249-734X (Online)
Journal DOI: 10.7439/ijpc
CODEN: IJPCH3 (American Chemical Society)
Research Article

Antibacterial and Antitubercular Activity of *Gnidia glauca* (Fresn gilg) Root Extracts

Gowrish A[1], Vagdevi H.M[1*] and Rajashekar H[2]

[1]*Department of Chemistry, Sahyadri Science College (Autonomous), Shivamogga-577203, Karnataka, India*
[2]*Department of Chemistry, Tunga Mahavidyalaya, Thirthahalli, Shivamogga-577432, Karnataka, India*

Abstract

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Because of the side effects and the resistance that pathogenic microorganisms that build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from medicinal plants. The intention of this study was to evaluate the antibacterial activity of pet-ether, chloroform and ethanol extracts of *Gnidia glauca* against five Gram-positive bacteria and five Gram-negative bacteria by agar well diffusion method. The zone of inhibition of extracts was compared with standard antibiotics. The results of the study indicated that, the pet-ether and chloroform extracts of the plant were highly effective towards most of the bacterial strains. The ethanol extract showed appreciable activity against *B. subtilis* and showed a moderate growth inhibitory activity towards all other tested organisms. The organisms which were highly susceptible to extracts were selected for the determination of Minimum Inhibitory Concentration (MIC) by Broth dilution method. When compared to chloroform and ethanol extracts, pet-ether extract has showed a lowest MIC of 1.562 mg/ml against *B. subtilis*, *V. cholerae* and 3.125 mg/ml against *S. pyogenes*. The antitubercular activity of all the extracts of *Gnidia glauca* have been evaluated against *Mycobacterium tuberculosis* $H_{73}Rv$ strain using Microplate Alamar Blue Assay (MABA). The activity was documented within MIC range of 0.2 to 100µg/ml. The results of MABA showed that pet-ether extract exhibited excellent antitubercular activity. The chloroform extract is moderately active, whereas ethanol extract is less active against *Mycobacterium tuberculosis*. The present investigation suggests that *Gnidia glauca* possess remarkable antibacterial and antitubercular activity.

Keywords: *Gnidia glauca*, antibacterial activity, antitubercular activity, MIC, MABA

1. Introduction

Medicinal plants represent a rich source of antimicrobial agents [1]. Plants are known to produce a variety of compounds to protect themselves against a variety of their own pathogens and therefore can be considered as potential source of different classes of antimicrobial substances [2]. Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action [3,4]. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials [5].

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection [6,7]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host, including hypersensitivity, immune suppression and allergic reactions [8]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance [9], there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.

* Correspondence Info
Dr. Vagdevi H M
Associate Professor, Department of Chemistry,
Sahyadri Science College (Autonomous),
Shivamogga-577203, Karnataka, India.
E-mail: vagdevihm@gmail.com

Tuberculosis (TB) is one of the leading infectious disease and health burden in the world [10]. It has been estimated that, one third of world's population including 40% from India estimated to be infected with tuberculosis [11]. More than nine million new cases diagnosed and approximately two million people killed annually [12]. There are a number of new factors that make people more susceptible to tuberculosis infection worldwide, the important of which is Human Immunodeficiency Virus (HIV) infection and the corresponding development of AIDS. The association of tuberculosis with HIV infection is so dramatic that in some cases, nearly two third of the patients diagnosed with the tuberculosis are also HIV-1 seropositive [13].

Current tuberculosis treatment is a long course of combination of 3-4 antibiotic drugs, which have one or the other toxic side effects and led to poor patient compliance. Antitubercular drugs such as isoniazid (INH), rifampicin (RIF), pyrazinamide, ethambutol, streptomycin etc have been a mainstay in the treatment of tuberculosis [14]. The global emergence of multidrug trsistance (MDR) and extensively drug resistant (XDR) strains of *M.tuberculosis* and more recently the reports of totally drug resistant tuberculosis [15,16] has become a common phenomenon, which cause drugs to be ineffective.

Gnidia glauca (Fresn) gilg (Syn: *Lasiosiphon eriocephalus*) belongs to Thymeliaceae family. It is a shrub growing about three meter height and widely distributed in India, Srilanka and Africa. It is used in traditional African medicine for cancer, sore throat, abdominal pain, wounds, burns and snake bites [17]. Leaves have been applied to treat contusions, swelling, back ache and joint aches [18]. It is considered as a power full vesicant. The roots of this plant are used as antiviral agent against rabies in Ethiopia [19]. It also has agrochemical application as a molluscicide, insecticide, pesticide and even larvicidal agents [20-22]. It has been shown that several Gnidian species possess remarkable antineoplastic activity [23].

The objective of this study was to evaluate antimicrobial potential of pet-ether, chloroform and ethanol extracts from roots of *G. glauca* against bacterial and fungal pathogens by agar diffusion method and broth dilution method. In addition the antitubercular activity of the extracts against *M.tuberculosis* H_{37} Rv-ATCC 27294 was also examined by Microplate Alamar Blue Assay (MABA) technique.

2. Materials and Methods

2.1 Collection and Identification of Plant Material

G. glauca was collected in Tunga river basin of Central Western Ghats of Karnataka. The plant was authenticated in Dept. of Studies and Research in Applied Botany, Kuvempu University, Jnana Sahyadri, Shankaraghatta and voucher specimen (KU/AB/KSV/237) was deposited in the department for future reference.

2.2 Extraction of Plant Material

The roots of *G. glauca* were washed thoroughly 2-3 times with running tap water and once with sterile water. The material was shade dried, coarsely powdered and used for extraction. Weighed amount (500g) of the material was successively extracted using solvents of varying polarity namely, petroleum ether (pet-ether; 60-80^{0}C), chloroform and ethanol using soxhlet extractor [24]. Each extraction was carried out nearly 48 cycles. The extracts were filtered and concentrated using rotary flash evaporator under reduced pressure and at controlled temperature. The extracts obtained were dried, packed and stored at 4^{0}C in refrigerator.

2.3 Phytochemical analysis

All the extracts were subjected to preliminary phytochemical analysis using standard procedure to identify the various phytoconstituents [25].

2.4 Test Microorganisms

All the microorganisms used were produced from National Collection of Industrial Microorganisms (NCIM), Pune, India. *Staphylococcus aureus* (NCIM 2079), *Bacillus cereus* (NCIM 2106), *Streptococcus pyogenes* (NCIM 2608), *Staphylococcus epidermidis* (NCIM 2493), *Bacillus subtilis* (NCIM 2699) were the five Gram-positive bacteria while *Proteus mirabilis* (NCIM 224), *Klebsiella pneumoniae* (NCIM 7427), *Enterobacter aerogenes* (NCIM 2692), *Shigella flexneri* (NCIM 5265) and *Vibrio cholerae* (NCIM 5316) were five Gram-negative bacteria used for the study.

2.5 *In-vitro* Antibacterial Assay

The antibacterial activity of the extracts was determined by agar well diffusion method. The 24 hour old nutrient broth cultures of test bacteria were swab inoculated on the surface of solidified nutrient agar plates. The agar wells of 8mm diameter were made by using sterile cork borer. About 100-200 µl of 10% solution (100mg/ml)

of each extracts in Dimethyl Sulphoxide (DMSO) were dispensed in separate well with the help of a micropipette. The plates are then incubated at 37^0C for 24 hours. Penicillin and methicillin (1mg/ml in DMSO) were used as positive controls for Gram-positive bacteria while streptomycin and tetracycline (1mg/ml in DMSO) were used as positive controls for Gram negative bacteria. The zone of inhibition was measured in millimeters by millimeter scale after incubation [26]. The experiments were conducted in triplicates and the results were represented as mean± standard deviation.

2.6 Determination of Minimum Inhibitory Concentration (MIC)

To assess the Minimum Inhibitory Concentration (MIC) of all the extracts, broth dilution test was carried out with the concentration range 0.781, 1.562, 3.125, 6.25, 12.5 and 25 mg/ml in DMSO respectively [27]. Series of 5ml nutrient broth tubes were inoculated separately with 1ml of broth cultures of test organisms. 1ml of different concentrations of all the extracts was transferred separately to each set of tubes. The tubes were incubated at 37^0C for 24 hours. Nutrient broth tubes with extracts and without extracts were used as controls. The absorbance of each tube was measured at 600 nm by using colorimeter (Equiptronics EQ-652, India). Increase or decrease in turbidity of the tubes was considered for determining the MIC. The lowest concentration with less absorbance was taken as MIC of that extract.

2.7 Antitubercular Activity

The antitubercular activity of crude extracts was assessed against *M.tuberculosis* using Microplate Alamar Blue Assay [28] (MABA). This methodology is nontoxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Sterile deionised water (200µl) is added to all outer perimeter wells of sterile 96 well plates to minimum evaporation of medium in the test wells during incubation. The 96 well plates received 100µl of Middle brook 7H9 broth and serial dilution of compounds was made directly on plate. The final extracts concentration tested were 100 to 0.2µg/ml. Plates were covered and sealed with parafilm and incubated at 37^0C for five days. After this time, 25µl of freshly prepared 1:1 mixture of alamar bue reagent and 10% tween 80 was added to the plate and incubated for 24 hours. A blue color in the well was interpreted as no bacterial growth and pink color was scored as growth. The Minimum Inhibitory Concentration (MIC) was defined as the lowest drug concentration, which prevented the color change from blue to pink.

3. Results and Discussion

3.1 Phytochemical Screening

Priliminary phytochemical screening of crude extracts of *G. glauca* revealed the presence of various phytochemical constituents. The analysis showed the presence of alkaloids, steroids, triterpenes in pet-ether extract, flavonoids, steroids, triterpenes, tannins and phenolics in chloroform extract, while flavonoids, steroids, tannins, phenolics, carbohydrates and glycosides in ethanol extract. The detailed phytochemical investigation has been discussed in our previous publication [29].

3.2 Antibacterial Activity

The results of antibacterial activity of pet-ether, chloroform, ethanol extracts of *G. glauca* and the standard antibiotics are as shown in table 1. In the present study, a variety of Gram-positive and Gram-negative bacteria were selected for screening antibacterial effect of the extracts. The pet-ether extract was very much effective against Gram-positive bacteria *S.aureus*, *S.pyogenes*, *S.epidermidis*, *B.subtilis* and Gram-negative bacteria *P.mirabilis*, *E.aerogenes*, *S.flexneri* and *V.cholerae*. The pet-ether extract showed promising activity against *B.cereus* and *K.pneumoniae*.

The chloroform extract was potent against Gram-positive bacteria *S. aureus*, *S. pyogenes*, *B. subtilis*, Gram-negative bacteria *P. mirabilis*, *E. aerogenes*, *S. flexneri* and *V. cholerae*. The extract was also effective against *B. cereus*, *S. epidermidis* and *K. pneumoniae*.

The ethanol extract showed a considerable growth inhibitory activity against *B.subtilis* and a moderate growth inhibitory activity against all other tested organisms when compared to pet-ether and chloroform extracts. The solvent DMSO did not show any activity against the tested bacteria.

Table 1: Antibacterial activity of *G. glauca* root extracts

Sl. No	Type of Bacteria	Zone of inhibition in mm						
		Pet-ether Extract	Chloroform Extract	Ethanol Extract	Penicillin	Methicillin	Streptomycin	Tetracycline
1	*S. aureus*	26.0±1.53	29.3±1.45	10.6±0.33	00	12.6±0.67	-	-
2	*B. cereus*	21.3±.088	22.3±0.33	11.0±1.0	10.6±0.67	00	-	-
3	*S. pyogenes*	28.3±2.40	30.6±1.20	16.3±0.33	12.6±1.20	14.3±0.33	-	-
4	*S. epidermidis*	26.0±1.0	24.6±1.20	13.6±0.67	00	28.0±1.53	-	-
5	*B. subtilis*	27.3±1.20	32.0±1.15	23.0±1.15	17.3±0.88	00	-	-
6	*P. mirabilis*	25.3±1.45	26.3±0.88	14.3±0.67	-	-	00	25.0±1.0
7	*K.pneumoniae*	22.3±0.88	21.6±0.58	11.3±0.58	-	-	16.0±1.0	00
8	*E. aerogenes*	26.6±0.67	26.0±1.0	09.0±0.58	-	-	15.3±0.33	12.6±0.67
9	*S. flexneri*	28.3±1.45	26.6±1.20	08.3±0.0	-	-	16.6±0.67	00
10	*V. cholerae*	28.6±1.76	29.3±1.45	14.6±0.88	-	-	23.0±0.58	00

Zone of inhibitions are mean ± standard deviation of triplicates. The diameter of well plates was 8mm
'-': Not tested

3.3 Minimum Inhibitory Concentration (MIC)

The MIC of all the extracts was assessed by broth dilution test with the concentration range 0.781 to 25 mg/ml. Only those organisms which were highly susceptible to the extracts were selected for determining the MIC. Therefore, the MIC of the extracts on bacterial strains *S. pyogenes*, *B. subtilis*, *V. cholerae* was determined. The MIC values of all the three extracts are given in table 2.

Table 2: Minimum Inhibitory Concentration (MIC) of *G. glauca* root extracts

Extracts	MIC (mg/ml)		
	S. pyogenes	***B. subtilis***	***V. cholerae***
Pet-ether	3.125	1.562	1.562
Chloroform	6.25	3.125	3.125
Ethanol	6.25	3.125	6.25

The MIC values of pet-ether extract indicated that *B. subtilis* and *V. cholerae* were most sensitive organisms with lowest MIC of 1.562 mg/ml. *S. pyogenes* was other sensitive one to pet-ether extract with MIC 3.125 mg/ml. The chloroform extract was highly susceptible to *B. subtilis* and *V. cholerae* with MIC of 3.125 mg/ml. *S. pyogenes* was moderately sensitive towards chloroform extract with MIC of 6.25 mg/ml.

The ethanol extract was highly sensitive towards only *B. subtilis* MIC of 3.125 mg/ml. *S. pyogenes* and *V. cholerae* have MIC 6.25 mg/ml indicated that they were moderately sensitive to ethanol extract.

3.4 Antitubercular Acitvity

All the extracts were tested for their *in-vitro* antitubercular activity against *M. tuberculosis* by MABA with the use of Middle brook 7H9 broth and the MIC was determined. The MIC was defined as the lowest drug concentration, which prevented the color change from blue to pink. The MIC values of various extracts are presented in Table 3. The MIC of pet-ether, chloroform and ethanol extract was 25 µg/ml, 50 µg/ml and 100 µg/ml and those of standard drugs pyrazinamide and streptomycin were 3.125 µg/ml and 6.25 µg/ml respectively. It was found that among the tested samples, pet ether extract was more active and chloroform extract was moderately active whereas, ethanol extract was less active towards *M. tuberculosis* when compared to standard drugs.

Table 3: MIC of *G. glauca* root extracts against *M. tuberculosis*

Extracts	MIC (µg/ml)
Pet-ether	25
Chloroform	50
Ethanol	100

3. Conclusion

The results of this study showed that, the pet-ether and chloroform extracts of the plant *G. glauca* were highly effective towards most of the Gram-positive bacteria and Gram-negative bacteria. But the ethanol extract was highly active against *B. subtilis* and moderately active towards all other organisms tested. When the MIC values of

the extracts are compared, the pet-ether extract has showed lower MIC values than chloroform and ethanol extracts on tested bacteria and on *M. tuberculosis*. Pet-ether extract had MIC of 1.562 mg/ml on *B. subtilis*, *V. cholerae*, 3.125 mg/ml on *S. pyogenes* and 25 µg/ml on *M. tuberculosis*. The demonstration of antibacterial activity against both Gram-positive bacteria, Gram-negative bacteria and on *M. tuberculosis* may be an indicative of the presence of broad spectrum antibiotic compounds in the extracts. This investigation has opened up the possibility of the use of this plant in drug development for human consumption. However, further studies are needed to be conducted to isolate the active principles responsible for observed activity to understand the exact mechanism of action.

References

[1] Chandra M. Antimicrobial activity of medicinal plants against human pathogenic bacteria. *International Journal of Biotechnology and Bioengineering Research* 2013; 4: 653-658.

[2] Laila F. Nimri; M. M. Meqdam; A. Alkofahi. Antibacterial Activity of Jordanian Medicinal Plants. *Pharmaceutical Biology* 1999; 37: 196-201.

[3] Ahmad I, Aqil F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. *Microbiol Res* 2007; 162: 264-275.

[4] Barbour EK, Al Sharif M, Sagherian VK, Habre AN, Talhouk RS, Talhouk SN. Screening of selected indigenous plants of Lebanon for antimicrobial activity. *J Ethnopharmacol* 2004; 93: 1-7.

[5] Iwu MW, Duncan AR, Okunji CO. New antimicrobials of plant origin: In Perspectives on new Crops and new Uses, eds. J. Janick, ASHS Press, Alexandria, VA 1999; p. 457- 462.

[6] Gonzalez C E, Venzon D, Lee S, Mueller B U, Pizzo P. A, Walsh T. J. Risk factors for fungemia in children infected with human immunodeficiency virus: a case-control study. *Clin Infect Dis* 1996; 23: 515-521.

[7] Rajesh Dabur, Amita Gupta, T K Mandal, Desh Deepak Singh, Vivek Bajpai, A M Gurav, G S Lavekar. Antimicrobial activity of some Indian medicinal plants. *Afr. J. Trad. CAM* 2007; 4: 313-318.

[8] Sankar Kumar Dey, Debdulal Banerjee, Sourav Chattapadhyay, Krishnendu Bikash Karmakar. Antimicrobial activities of some medicinal plants of West Bengal. *International Journal of Pharma and Bio Sciences* 2010; 1: 1-10.

[9] Monroe S, Polk R. Antimicrobial use and bacterial resistance. *Curr Opin Microbiol* 2000; 3: 496-501.

[10] Dye C, Scheele S, Dolin P, Pathania V, Raviglione MC. Global burden of tuberculosis: estimated incidence prevalence and mortality by country. *JAMA* 1999; 282: 677-686.

[11] Gupta R, Thakur B, Singh P, Singh HB, Sharma VD, Katoch VM, Chauhan SVS. Anti-tuberculosis activity of selected medicinal plants against *Mycobacterium tuberculosis*. *Ind J Med Res* 2010; 131: 809-813.

[12] Gizachew YE, Giday M, Teklehaymanot T. Antimycobacterial activity of selected Ethiopian traditional medicinal plants used for treatment of symptoms of tuberculosis. *Glo Adv Res J Med Plants* 2013; 2: 022-029.

[13] Ilango K, Arunkumar S. Synthesis, antimicrobial and antitubercular activities of some novel trihydroxy benzamido azetidin-2-one derivatives. *Trop J Pharm Res* 2011; 10: 219-229.

[14] Panda VS, Ashar HD, Sharan A. Antioxidant and hepatoprotective effects of *Garcina indica* fruit rind in antitubercular drug-induced liver injury in rats. *Botanics: Targets and therapy* 2013; 3: 29-37.

[15] Singh MM. XDR-TB-danger ahead. *Ind J Tuberc* 2007; 54: 1-2.

[16] Udwadia ZF, Amale RA, Ajbani KK, Rodrigues C. Totally drug-resistant tuberculosis in India. *Clin Infect Dis* 2012; 54: 579-581.

[17] BWRC Amarajeewa, Mudalige AP; Kumar V. Proceedings of the Peradeniya university Research sessions, Sri Lanka, Vol.12, Part-I: 2007.

[18] Kareru PG, Kenji GM, Gachanja AN, Keriko JM, Mungai G. Traditional Medicines among the Embu and Mbeere peoples of Keenya. *African Journal of Treditional, complementary and Alternative medicines* 2005; 4: 75-86.

[19] Teklehaymanot T, Giday M. Ethanobotanical studies of medicinal plants used by people in Zegie Peninsula, north-western Ethiopia. *Journal of Ethanobiology and Ethano medicine* 2007; 3: 12.

[20] Borris R P, Cordell G A. Studies of the thymelaeaceae II. Antineoplastic principles of *Gnidia kraussiana*. *Journal of Natural Products* 1984; 47: 270–278.

[21] Franke K, Porzel A, Schmidt J. Flavone-coumarin hybrids from *G. socotrana*, *Phytochemistry* 2002; 61: 873-878.

[22] Javaregowda K, Naik L K. Ovicidal properties of plant extracts against the eggs of teak defoliator, hyblaea puera cramer Karnataka. *Journal of Agricultural Science* 2007; 20: 291-293.

[23] Kupchan S M, Shizuri Y, Sumner W C, Haynes H R, Leighton A P, Sickles B R. Isolation and structural elucidation of new potent antileukemic diterpenoid esters from *G.* species. *Journal of Organic Chemistry* 1976; 41: 3850–3853.

[24] Gowrish A, Vagdevi H M, Rajashekar H. *In vitro* antioxidant and antitubercular activity of *Leucas marrubioides* Desf. root extracts. *Journal of Applied Pharmaceutical Science* 2015; 5: 137-142.

[25] Parekh, Chanda. *In-Vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plants. *Turkish J Biol*, 2007; 31: 53-58.

[26] Antara Sen, Mala Batra. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia Azedarach L*. *Int J Curr Pharm Res* 2012; 4: 67-73.

[27] Shanmugapriya Kaminidevi, Thayumanavan Thangavelu, Jinu Udayabhanu, Senthil Murugan Thangavel. Antimicrobial activity of methanolic extracts of indigenous traditional Indian folk Medicinal Plant, *Gnaphalium polycaulon*. *Int J Green Pharm* 2015; 9: 39-44.

[28] Munna S, Basha CS, Reddy RP, Pramod N, Kumar PY, Pasha MG. Antitubercular activity of *Actiniopteris radiata* linn. *Journal of Global Trends in Pharmaceutical Sciences*, 2014; 1: 1443-1445.

[29] Gowrish A, Vagdevi H M, Rajashekar H, Vijaya Kumar M L, Shobha K S. *In-Vitro* Cytotoxic and Antioxidant Activity of *Gnidia glauca* (Fresen) Gilg Root Extract. *Journal of Applicable Chemistry* 2013; 2: 1362-1369.