



Antimicrobial Potential Of Berberine And Its Derivatives

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ABSTACT

Berberis aristata DC. (Berberidaceae) is one of the herbs mentioned in all ancient scriptures of Ayurveda, Charaka and Susruta have mentioned it's different properties along with various used for the treatment of numerous illnesses. It is used in various crude drug formulations and in different ayurvedic and homeopathic medicines since ancient times. The whole plant is also a good source of dye and tannin which is used for dyeing clothes and for tanning leather. Berberine is main constitute having various pharmacological actions. It is a benzylisoquinoline alkaloid, occurs as an active constituent in numerous medicinal plants and has an array of pharmacological properties. It has been used in Ayurvedic and Chinese medicine for its antimicrobial, antiprotozoal, antidiarrheal and antitrichoma. Isolated plant materials have great potential as antimicrobial agent against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. In present study the aim was to do synthetic modification of isolated berberine to check its antimicrobial activity. Antimicrobial activity were studied for zones of inhibition against the test organisms. The agar well diffusion method was used for this study. The activity was predominately observed in berberine than its modification.

KEY WORDS –Berberin, isoquinoline, Antimicrobial activity.



INTRODUCTION

Use of herbal medicines in developed countries has expanded sharply in the latter half of the twentieth century. The medicinal value of plants lies in some chemical substances that produce a definite physiological action of the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. Antimicrobials are a substance that kills or inhibits the growth of microbes such as bacteria, fungi and viruses. Berberine is a plant alkaloid with a long history of medicinal use in both Ayurvedic and Chinese medicine. The berberine alkaloid can be found in the roots, rhizomes, and stem bark of the plants. Berberine extracts and decoctions have demonstrated significant antimicrobial activity against a variety of organisms including bacteria, viruses, fungi, protozoans, helminths, and chlamydia. Moreover, several clinical and preclinical studies demonstrate ameliorative effect of berberine against several disorders including metabolic, neurological and cardiological problems. In search of new antimicrobials, in present study attempts has been made to isolate berberine from *Berberis aristata* and carry out its synthetic modification to explore its potential as new antimicrobial substances.¹⁻²⁵

MATERIAL AND METHODS

Chemicals & Reagents were obtained from Loba Chemie,, Rankem, Himedia, and UV, IR, NMR was carried on Perkin Elmer, Shimadzu, Bruker Daltonics esquire 400.

COLLECTION OF PLANT MATERIAL:-

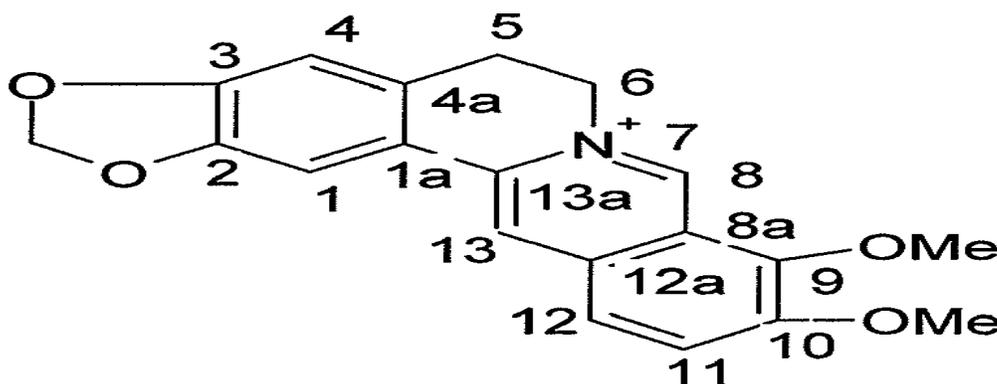
Fresh roots of plant *Berberis aristata* was collected adjoining area of Almora district of Uttarakhand in month of Jan-February. The roots are dried in shade for 12 to 15 days and then kept in oven at 35-40^o C for 2-3 hrs. The roots was crushed in small pieces and weighed it (250 gm) . The root was weighed before and after drying in order to estimate the moisture content. Than extraction of plant roots takes place with ethanol as a solvent.

ISOLATION OF BERBERINE:

Firstly the roots were defatted with pet ether for 14- 15 hrs followed by extraction with ethanol for 6 hours. The ethanolic extract is concentrated on hot water bath till syrupy mass obtained. Dissolved syrpy mass in 25ml of hot water and filtered. Added 5ml hot water to the residual syrupy mass, again filtered . To the combined filtrate added slowly while shaking 15ml of strong HCL (36.5% w/v). Cooled in ice bath and placed in refrigerator for overnight to obtain yellow crystals of Berberine hydrochloride 2gms .

PURIFICATION OF ISOLATED BERBERINE HYDROCHLORIDE:-

The salts of Berberine are dissolved in hot water and the solution was made alkaline with few drops of 10% NaOH. Thereafter a small quantity of acetone is added and solution is diluted with equal amount of water. The precipitate is allowed to settle overnight and washed with ice-cold water. The isolated berberine is was identified on the basic of TLC using silica-G as a stationary phase and chloroform methanol (95:5) and Ethyl acetate:acetone:formic acid:water(100:11:11:27) as mobile phase and visualized by spraying with the 5% sulphuric acid. The Rf value of the berberine was calculated and compared with the standard value of the same. Berberine was characterized on the basis of spectral studies and comparison with the authentic sample (table 1).



Berberine



DERVATIVES OF BERBERINE

1 SYNTHESIS OF 11 CHOLOROBERBERINE

Chlorination is carried out by treating berberine with chlorine gas at about 30°. In order to remove any moisture from chlorine, the gas is passed through at least two washing bottles containing concentrated sulfuric acid. The reaction flask is placed in a water bath and is fitted with an efficient reflux condenser. The hydrogen chloride escaping from the condenser is absorbed in a round-bottomed flask containing sodium hydroxide or water. After completion the residue is cooled and extracted with ether to obtain chloro berberine as yellow solid

2 SYNTHESIS OF 11 NITRO BERBERINE

A mixture of Berberine (35 mmol, 3.75 ml), nitric acid (120 mmol, 5.04 ml), chloroacetic anhydride (120 mmol, 20.52 g), zeolite H β (Si/Al = 12.5; 2.0 g) and 1,2-dichloroethane (10 ml) was stirred in a bath set at 50 °C for 2 h yielded 11 nitro berberine as yellow coloured crystals.

ANTIMICROBIAL ACTIVITY

To test antimicrobial activity, the agar well diffusion method was used on the Muller Hinton Agar (MHA). The media was seeded with bacterial cultures. A measured quantity 25ml. of culture medium inoculated with bacterial culture was transferred to petriplates and kept for some time until the medium solidified completely. Holes were punched in nutrient Agar with a sterile cork borer, (dia. 6 mm.). 0.3 ml. of test solutions were poured in one well and 0.3 ml of control solution in another well for comparative study. After pouring, the petriplates were kept for one hour to facilitate diffusion of test solution at room temperature and incubated at 37°C for 12 hours. After completion of Incubation time Zone of inhibition was measured in mm. with the help of zone reader. Four bacterial strains were used in this study. Gram positive bacteria include Bacillus, Aureus, Salmonella typhi, Klebsiella pneumonia and Echerichia coli.

RESULTS AND DISCUSSION

All the spectral data are well in agreement with the documented structures of synthesized compound. The spectral data are summarized in table 1. Further, compounds were screened for antimicrobial activity.

Table 1 SPECTRAL DATA OF BERBERINE AND DERIVATIVES

compound	Mass EIMS (m/z)	UV (λ_{max} , nm)	IR _{KBr}	¹ H NMR (CDCl ₃): δ
Berberine	336 (m ⁺ , 20%), 322 (35%), 281 (50%), 267 (38%), 207 (76%), 147 (100%)	230, 266 348, 431	2820 cm ⁻¹ (C-H str) 1597 cm ⁻¹ (C = C, C = N str) 1354-1383 cm ⁻¹ (C-H deformation)	1.75 (m, 2H, -CH ₂ -), 2.4 (m, 2H, N ⁺ -CH ₃), 3.9 (s, 6H, 2 X OCH ₃), 6.0 (s, 2H, O-CH ₂ -O), 6.6-9.4 (m, 6H, Ar-H)
11-chloro berberine	370 (m ⁺ , 40%), 335 (25%), 281 (43%), 267 (37%), 207 (62%), 147 (100)	235, 246 352, 430	2829 cm ⁻¹ (C-H str) 1600 cm ⁻¹ (C = C, C = N str) 1354-1383 cm ⁻¹ (C-H deformation)	1.75 (m, 2H, -CH ₂ -), 2.4 (m, 2H, N ⁺ -CH ₃), 3.9 (s, 6H, 2 X OCH ₃), 6.0 (s, 2H, O-CH ₂ -O), 6.6-9.4 (m, 5H, Ar-H)
11-nitro Berberine	382 (m ⁺ , 39%), 336 (55%), 281 (43%), 267 (37%), 207 (76%), 147 (100)	255, 256 367, 445	3100, 2829 cm ⁻¹ (C-H str) 1600 cm ⁻¹ (C = C, C = N str) 1400-1383 (NO stretch)	1.75 (m, 2H, -CH ₂ -), 2.4 (m, 2H, N ⁺ -CH ₃), 3.9 (s, 6H, 2 X OCH ₃), 6.0 (s, 2H, O-CH ₂ -O), 6.6-9.4 (m, 5H, Ar-H)



ANTIMICROBIAL ACTIVITY OF BERBERINE AND DERIVATIVES

According to the literature survey and preliminary studies it has been revealed that not much work has been done on synthetic modification of berberine. It was thought worthwhile to isolate active constituent from roots of the berberis aristata. For it, roots were dried, powdered and extracted with ethanol as a solvent system with, The isolated berberine and the modifications were screened for the antibacterial activity because the facilities were available. The present work was pursued under the following headings:- Microbiological evaluation technique is employed for evaluation of antibiotic principles of plant extracts. The screening for antibacterial activities of bio-constituents is done by these techniques.

Table 5: Antimicrobial screening of Berberine And Derivatives

S.no	Test Organism	Mean inhibition zone diameter (mm)		
		Berberine	11-Choloro berberine	11-Nitro berberine
1	E.Coli	29	27	22
2	Bacillus aureus	24	25	28
3	Salmonella typhii	30	27	32
4	Klebsiella pneumonia	22.5	20	2

Antimicrobial activity were studied for zones of inhibition against the test organisms. The agar well diffusion method was used for this study. The activity was predominantly observed in 11- nitro berberine while chloro derivative has comparable zone of inhibition with respect to Berberine.



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