

Research Article

Preliminary Phytochemical Screening and Antimicrobial Activity of Methanol Extract of Crotalaria Burhia

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ABSTRACT

Methanol, chloroform, petroleum ether and aqueous extract of Crotalaria burhia (whole plant) were investigated for their antibacterial activity against Staphylococcus aureus using cup plate agar diffusion method. The 4 extracts tested, the methanol extract presented the best results. Various investigations like physicochemical standards, preliminary phytochemical screening of the plant was carried out, and the salient qualitative parameters were reported. The present paper deals with the investigation of various photochemical present in various extract and in vitro antibacterial activity. It is concluded that the plant studied may be a source of antibacterial agent.

Keywords: Crotalaria burhia, Fabaceae, Eczema, Extraction, Methanol Extract, Antibacterial activity

INTRODUCTION

The Crotalaria burhia Buch.-Ham. (Fabaceae), commonly known as *jhamo* and *khip*, is an undershurb, fibrous plant, found all over the desert extensively growing on sand dunes, common in the arid parts of West Pakistan, India (Punjab, Rajasthan and Gujarat) and Afghanistan.^[1-3] Crotalaria Flowering and fruiting are from March–August. It is known as Shinio in Rajasthan, its Hindi name is Khip^[4], in Punjab as Bhata, in Gujarat as Ghughato, in Marathi as Ghagri and in Bengal called as Ban sutra.^[3] Its leaves, branches and roots are used as a cooling medicine.^[3-4] Fresh plant juice is used in eczema and it is also very useful in gout, hydrophobia, pain and swellings. ^[5-6] Studies have shown that the plant possesses anticancer property and roots are good coolant. It is a good soil binder and has medicinal value. It is used to make ropes and sheds for animals in the desert and also used to made *jhumpa* (desert huts). It is a food for goats [7]

Phytochemical studies have revealed the presence of pyrrolizidine like alkaloids crotalarine^[8], crosemperine^[6], corburhine^[7], monocrotaline ^[7] as main compounds in this plant. In addition, flavonoids like quercetin^[9], steroids like β-sitosterol have also been isolated Antimicrobial^[10] this plant. and from antibacterial^[6] activities of *C. burhia* have been reported by previous authors. In the present study, C. burhia was selected because it is one of the medicinal plants commonly used in remedies to treat pain, swelling and fever in Indian traditional medicine and other countries in Asia.

In this work, we evaluated antibacterial activity of this whole plant using four different extracts. The reason to use methanol extract in this investigation is that methanol is more nonpolar than water; therefore, several substances including alkaloids and flavonoids, the major chemical constituents of *C. burhia*, would be

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expected to be more soluble in methanol extract than in water extract.

MATERIALS AND METHODS

Plant material

The whole plant of *Crotalaria burhia* (Fabaceae) was collected from campus area of JPC, Rajasthan. The plant was authenticated by Botanical Survey of India, Rajasthan, India.

Extraction

Freshly collected plant of *Crotalaria burhia* was shade dried and then powdered using a mechanical stirrer. 5 gms of plant powder was taken in 4 different iodine flasks, 100 ml of each solvent was added to each of these. Flasks were closed tightly and kept for 7 days. During this period, these were occasionally shaken. After 7 days, it was filtered and marc was pressed. Extractive yield was calculated and extract was converted into dry mass to use it for further evaluation. The yield of different extracts is depicted in Table **1**.

Physical evaluation

Drug was evaluated for following physical parameters using referred methods and values obtained are depicted in Tables **2** & **3**.

- 1. Moisture content: 14 (% loss on drying)
- 2. Total ash values
- 3. Acid insoluble ash value
- 4. Water soluble ash value
- 5. Sulphated ash value
- 6. Extractive value

Phytochemical screening

Preliminary phytochemical screening was performed to identify phytochemicals in each extract of *Crotalaria burhia*. The phytochemicals were detected by colour tests as depicted in Table **4**.

Antimicrobial activity

Microorganism

The antibacterial activity of the extracts was tested using gram positive, *S. aureus.* The bacterial strain was maintained on nutrient agar at 4°C and sub-cultured into nutrient broth in our laboratory.

Agar diffusion method

In vitro antibacterial activity of the crude extracts was studied against gram positive, S. aureus by the cup-plate agar diffusion method. Nutrient agar was used as the bacteriological medium. 10 mg of each extract was dissolved separately in 10 ml of aqueous DMF to get concentration of 1000 μ g/ml. From these stock solutions, further dilutions were made to get concentration of 100 µg/ml and 150 µg/ml of each extract. Pure DMF was used as a negative control and amoxicillin as the positive control. 100 µl of inoculum was aseptically introduced on to the surface of sterile agar plates and sterilized I-shaped glass rod was used for even distribution of the inoculums. Wells were prepared in the agar plates using a sterile cork borer of 8 mm diameter. 100 µl of test and control compound was introduced in the well. The plates were incubated at 37°C for 24 hrs. The diameter of zone of inhibition produced by each extract was measured and compared with those produced by the antibiotic amoxicillin. Antibacterial activity of different extracts is depicted in Table 5.

RESULTS AND DISCUSSION

The yield of extracts in petroleum ether, chloroform, methanol and water was found to be 3.4, 3.6, 6.9 and 16.1 respectively. Ash parameters, extractive values were evaluated. The phytochemical analysis of all extract showed the presence of carbohydrate. Petroleum extract, methanol extract and aqueous extract showed the presence of glycoside. Chloroform and aqueous extract showed the presence of saponin. All other secondary metabolites were not detected in any of these extracts. Methanol extract showed



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the presence of alkaloids and flavonoids. Antibacterial screening data showed that methanol extract has soluble constituents hence exhibit antibacterial activity. Whereas the remaining extracts i.e., Petroleum extract, chloroform extract and aqueous extract does not have the constituents responsible for antibacterial activity.

S.No. **Different parameters** Values (%) 1. Total ash values 4.8 2. Acid insoluble ash 4.5 value Water soluble ash 3. 3.66 value 4. Sulphated ash value 3.33

Table 1: Yield of extracts

S.No.	Extract	% dry weight
1	Petroleum ether	3.4
2	Chloroform	3.6
3	Methanol	6.9
4	Water	16.1

Table 2: Ash parameters

Table 3: Extractive value

S.No.	Extract	% Extractive	
		value	
1.	Petroleum ether	2.8	
2.	Chloroform	3.2	
3.	Methanol	6.3	
4.	Water	15.5	

S.No	Const.	Pet.Ext.	Chl.Ext.	Met.Ext.	W.Ext.
1.	Alkaloids	-	-	+	-
2.	Flavonoids	-	-	+	-
3.	Saponins	-	+	-	+
4.	Carbohydrate	+	+	+	+
5.	Proteins	-	-	-	-
6.	Volatile oil	-	-	-	-
7.	Coumarins	-	-	-	-
8.	Steroids	-	-	-	-
9.	Glycosides	+	-	+	+
10.	Tannins	-	-	-	-

Where:-

(+)-sign indicate presence and (-)-sign indicate absence of constituent

Const.: Constituents, Pet.Ext.: Petroleum extract, Chl.Ext.: Chloroform extract, Met.Ext.: Methanol extract, W.Ext.: Water extract

Table 5: Antibacterial activity of extracts of Crotalaria burhia

S.No.	Sample	Conc. (µg/ml)	Zone of inhibition (mm)
1	Pet. ether extract	100	-
1.		150	-
2	Chloroform extract	100	-
Ζ.		150	-
3.	Methanol extract	100	-
		150	6.7



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4.	Aqueous extract	100	-	
		150	-	
5.	Amoxicillin	100	5.5	
		150	7.5	

Where, (-)-sign indicate no inhibition

CONCLUSION

Methanol extract showed the presence of carbohydrate, glycoside and saponin. The major chemical constituents of *C. burhia*, would be expected to be more soluble in methanol extract than in other extract.

From the antibacterial screening, it was found that methanol extract showed good antibacterial activity in the concentration of 150 μ g/ml whereas remaining extracts were found inactive. The methanol extract showed the zone of inhibition of 6.7 mm. This is an indication that methanol extract possess substances that can inhibit the growth of microorganism. However, the zone of inhibition diameter produced by methanol extract was less than those produced by amoxicillin. Hence the antibacterial activity of methanol extract was found slightly less than those of amoxicillin.

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