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Investigation of Anti-Acne Potential of Leaves of *Cassia fistula* and *Abrus precatorius*

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Abstract: Leaves of *Cassia fistula* and *Abrus precatorius* have been reported to possess anti-bacterial and anti-fungal activity. This indicates that they may be effective in acne vulgaris, which is one of the most prevalent skin diseases, and which is mainly caused by the bacteria *Propionibacterium acnes*. However, leaves of these plants have not been investigated for their anti-acne activity. The objective of the present work is to investigate anti-acne activity of leaves of *C. fistula* and *A. precatorius*. Successive extraction of the leaves of the two plants was performed separately using Petroleum Ether, Chloroform, Methanol and Distilled Water. Anti-acne activity was evaluated against *Propionibacterium acnes* by measuring Zone of Inhibition of all four extracts of leaves of each plant using agar disc diffusion method. This was followed by calculation of Minimum Inhibitory Concentration of the most active extract using serial tube dilution method. Methanolic extracts of leaves of both *C. fistula* and *A. precatorius* were found to be most effective against *P. acnes* with a Minimum Inhibitory Concentration of 10µg/ml. The present work provides proof of anti-acne potential of leaves of *C. fistula* and *A. precatorius*. Also, its activity at a much lower concentration can help in formulation development at much lower doses, and indicates that development of resistance of *P. acnes* against the methanolic extracts will be minimal. The methanolic extracts can be further investigated for their phytochemical composition and for isolating anti-acne phytoconstituents.

Keywords: Abrus precatorius, Acne, Cassia fistula, Minimum Inhibitory Concentration, Propionibacterium acnes.

Introduction

Figure 1. Cassia fistula

Figure 2. Abrus precatorius

Cassia fistula (Caesalpiniaceae; Fig. 1) is also known as Aaragvadha (Sanskrit), Amaltaas (Hindi), Purging Cassia and Golden Shower (English)[1]. Its fruits have Sennosides, but they are present maximum in new

leaves. The leaves are antioxidant and also used in inflammation, skin eruptions and eczema[2, 3]. Juice of leaves is used in ringworm infection dressing and various skin diseases[3]. *Abrus precatorius* (Fabaceae; Fig. 2) is also known as Gunja (Sanskrit) and Indian Wild Liquorice (English)[4]. Its leaves are used in leucoderma, skin disease, itching, in painful swellings and as a germicidal in wounds. The leaves contain triterpenoids Glycyrrhizin and Abrusosides[5]. The leaves are also anti-inflammatory, anti-allergic and larvicidal[6, 7]. Leaves of both these plants have been proven to possess anti-bacterial and anti-fungal activity[2, 3, 5-8]. This indicates that they may be effective in acne, which is one of the biggest skin problem nowadays, mainly caused by the bacteria *Propionibacterium acnes*. The objective of the present work is to investigate anti-acne activity of leaves of *C. fistula* and *A. precatorius* against *P.acnes.*

Experimental

Collection of plant material

Leaves of *C. fistula* and *A. precatorius* were collected from the medicinal garden of School of Pharmacy, RK University in August, 2014. Herbarium was prepared and authenticated by botanist of School of Science, RK University.

Successive extraction

Leaves of *C. fistula* and *A. precatorius* were dried completely in hot air oven at 50°C. 500g leaves each of *C. fistula* and *A. precatorius* were successively extracted separately in round bottom flask using 1l each of Petroleum Ether, Chloroform, Methanol and Distilled Water at 50°C for 3h.

Anti-Microbial screening

Agar disc diffusion method was employed. Nutrient Broth was prepared by dissolving 13g of nutrient medium in 1l distilled water and boiled to dissolve the medium completely. Fresh culture of the *Propionibacterium acnes* (Gram positive anaerobic bacteria, MTCC 1951) isolates was inoculated in Nutrient Broth and incubated for 24h at 37°C. Nutrient Agar medium was prepared by dissolving 33.9g of agar in 1l distilled water. Prepared bacterial culture (40µl) was added in 20ml soft agar media. This media was added in the Nutrient Agar petriplate. This media was solidified and cavities were made using cup borer. The extracts (Petroleum ether, Chloroform, Methanol and Water) were added in the cavities in 50% and 100% concentration. Dimethylsulfoxide (DMSO) was used as control and Erythromycin was used as standard. The plates were then incubated at 37°C for 24h. Anti-acne activity was assayed by measuring the diameter of the Zone of Inhibition (ZOI) in mm.

Minimum Inhibitory Concentration (MIC)

It was measured by serial tube dilution technique. Extract of *C. fistula* and *A. precatorius* showing the largest ZOI was taken (1mg/ml) and serial dilution of the extract (0 to 100µg/ml) with nutrient broth for *P. acnes* inoculums was used. The test tubes were incubated for 24h at 37°C. The lowest concentration without visible growth was defined as MIC.

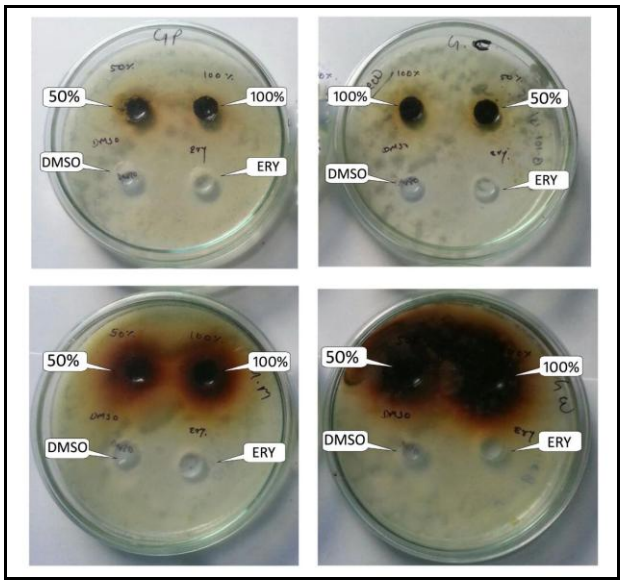
Results and Discussion

Zone of Inhibition

Table 1: Zone of Inhibition of *Cassia fistula* leaves

Cavity filled with	Pet. Ether Extract (mm)	Chloroform Extract (mm)	Methanol Extract (mm)	Water Extract (mm)
1mg/ml extract	0 mm	8 mm	11 mm	8 mm
0.5mg/ml extract	0 mm	7 mm	6 mm	6 mm
DMSO	5 mm	5 mm	5 mm	5 mm
Erythromycin (1%)	13 mm	13 mm	13 mm	13 mm

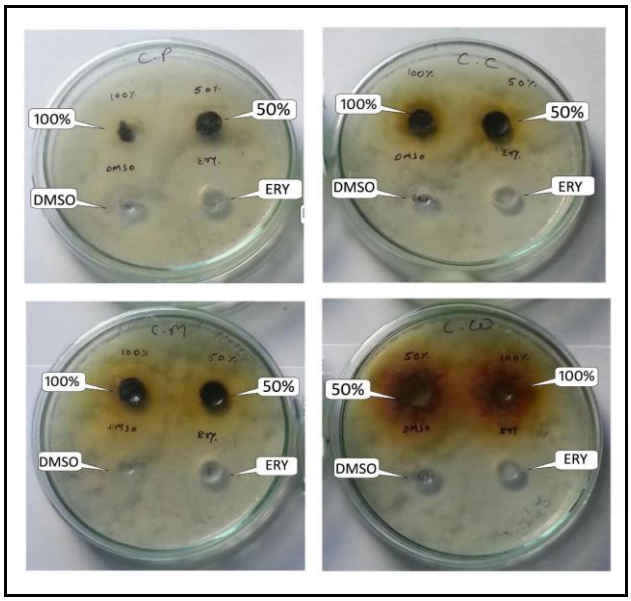
Pet. Ether extract **Chloroform extract**



Methanolic extract **Water extract**

Figure 3. Zone of Inhibition of *Cassia fistula* leaves
(ERY: Erythromycin, DMSO: Dimethylsulfoxide, 50%: 0.5mg/ml, 100%: 1mg/ml)

Petroleum ether Extract **Chloroform Extract**



Methanolic Extract **Water Extract**

Figure 4. Zone of Inhibition of *Abrus precatorius* leaves
(ERY: Erythromycin, DMSO: Dimethyl sulfoxide, 50%: 0.5mg/ml, 100%: 1mg/ml)

Table 2: Zone of Inhibition of *Abrus precatorius* leaves

Cavity filled with	Pet. Ether Extract (mm)	Chloroform Extract (mm)	Methanol Extract (mm)	Water Extract (mm)
1mg/ml extract	0 mm	5 mm	11 mm	3 mm
0.5mg/ml extract	0 mm	4 mm	8 mm	0 mm
DMSO	5 mm	5 mm	5 mm	5 mm
Erythromycin (1%)	13 mm	13 mm	13 mm	13 mm

Minimum Inhibitory Concentration

From the observation of Zone of Inhibition of all extracts, it was found that the methanol extract of leaves of *C. fistula* and *A. precatorius* has the best anti-acne activity, almost comparable to that of Erythromycin in 1mg/ml concentration (Table 1, 2 and Fig. 3, 4). Hence, the methanol extract of leaves of *C. fistula* and *A. precatorius* was chosen to find out the Minimum Inhibitory Concentration of each (Table 3).

Table 3: Minimum Inhibitory Concentration

C. fistula		***A. precatorius***	
Concentration of methanol extract (µg/ml)	***P. acnes* Growth**	**Concentration of methanol extract (µg/ml)**	***P. acnes* Growth**
0	Present	0	Present
10	Absent	10	Absent
20	Absent	20	Absent
30	Absent	30	Absent
40	Absent	40	Absent
50	Absent	50	Absent
100	Absent	100	Absent

10µg/ml is the MIC of methanol extracts of leaves of *C. fistula* and *A. precatorius* against *P. acnes*, which is the main cause of acne. Such a low MIC indicates that resistance of *P. acnes* towards the methanol extract will be very less.

This work shows the effectiveness of leaves of *C. fistula* and *A. precatorius* against the acne-causing microbe *P. acnes*. Also, its low Minimum Inhibitory Concentration can help in formulation development at lower doses. The methanol extract of leaves of both *C. fistula* and *A. precatorius* can be further explored for isolating anti-acne compounds which will be beneficial to patients in acne, which is currently one of the most prevalent skin disease.

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