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Antimicrobial and antitumor activity of the fractionated extracts of Kalimusli (*Curculigo orchioides*)

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The roots of Kalimusli (*Curculigo orchioides*) were fractionated with different solvents and screened for their antimicrobial and antitumor activity. Antifungal activity was screened using agar plate method, and antibacterial activity of the extracts was determined by disk diffusion method. Antitumor activity was screened against a human breast cancer cell line (MCF-7). Methanolic extract showed maximum activity due to the saponins present.

Key words: Amaryllidaceae, antimicrobial and antitumor activity, *Curculigo orchioides*, Kalimusli, saponins

INTRODUCTION

Curculigo is a small herb found in India in the subtropical Himalayas from Kumaon eastwards and in the Western Ghats from Kinkan southwards. It belongs to the family Amaryllidaceae, commonly known as 'Kalimusli' (in Hindi) and used in various Ayurvedic medicines, especially as ingredients of aphrodisiac preparations. Pharmacognostic features of the plant have been reported by Kirtikar and Basu (1935). Tiwari and Misra (1976) have identified a new glycoside 5,7-dimethoxy myricetin 3-0 and -L-xylopyranosyl 4-0 3-D glucopyranoside from the rhizome of *Curculigo orchioides*. The tubers of the plant contain free sugars - 7.56%; mucilage - 8.12%; hemicelluloses - 12-15% and other polysachharides - 17.01%. Its tuberous roots are slightly bitter and are considered tonic, alternative demulcent, diuretic and restorative (Wealth of India, 1950). It is usually administered in combination with aromatics and bitters in piles, diarrhoea, jaundice, asthma and is used as poultice for itch and skin diseases (Wealth of India, 1950). In Chinese traditional medicine, it is used as tonic for the treatment of decline in physical strength (Gudzikiewicz *et al*., 1979). The drug is greyish brown to dull brown in colour with a distinct odour (Bisht and Nayer, 1960). Survey of literature indicates that not much work have been carried out in India. The present study was carried out to find the antimicrobial and antitumor activity of various fractionated extracts of Kalimusli.

MATERIALS AND METHODS

The plant material was purchased from the local market and identified. A herbarium specimen was kept in the Department of Botany, Agra College, Agra. The roots of Kalimusli were cleaned, dried and pulverized. The powdered drug was extracted successively by hot percolation with hexane, chloroform, acetonitrile and methanol using Soxhlet apparatus. These individual extracts were concentrated under reduced pressure to obtain dry viscous mass. The concentrate of each extract was dissolved in acetone to perform the antimicrobial activity.

These various extracts were screened for their antifungal activity using agar plate method, in which their concentration was 10 µg/ml. One millilitre of each was poured into Petri dishes having 20-25 ml of the molten potato dextrose agar medium. When the medium solidified, the Petri dishes were incubated separately with the fungal isolates and kept at 27°C for 96 h. The percentage inhibition was calculated using the following formula:

$$\text{Percentage } (\%) = \frac{C - T}{C} \times 100$$

where C is the diameter of fungus in control and T is the diameter of fungus in the test extracts.

Antibacterial activity of the extracts was determined by disk diffusion method (Verma and Imam, 1973), in which the sterile filter paper (Whatman No. 1) discs of 5-mm

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diameter, impregnated with various extracts (10 µg/ml in ethanol), were placed on the nutrient agar plate at 37°C for 24 h. The inhibition zones around the dried impregnated discs were measured after 24 h.

Antitumor activity was screened against a human breast cancer cell line (MCF-7). They were incubated with different extracts of Kalimusli at 1 µg/ml doses for 96 h and the cell growth count was measured by MTT assay, where 17-β-estradiol was used as positive control and the culture medium as negative control.

MTT Assay

Cell proliferation activity of various extracts of Kalimusli was carried out by MTT assay, which estimated the effect of the various extracts on the growth of cells *in vitro*. Measurement of cell in viability and proliferation forms is used as basis for this *in vitro* assay. The reduction of tetrazolium salt is now widely accepted to examine cell proliferation. The yellow coloured tetrazolium, MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5,diphenyltetrazolium bromide], reduced metabolically active cells in part by the action of dehydrogenase enzymes to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple colour zones was solubilized and quantified by spectrophotometric method. When metabolic event leads to necrosis or apoptosis in cells, the MTT method measures the cell viability. The assay gives low background absorbance values in the absence of necrosis of the cells.

MTT was dissolved in PBS at a concentration of 5 mg/ml. Then, 50 µg of the MTT solution was added to each well of the 96-well culture plate, containing the 100-µl medium, and incubated at 37°C for 4 h. This medium was then removed carefully without disturbing the purple coloured formazan crystals, 50 ml of dimethyl sulphoxide (DMSO) was added to each well and mixed thoroughly to dissolve the crystals of the formazan. These plates were then seen on a microplate reader at a wavelength of 670 nm. The readings were presented as optical density (OD). The growth inhibition of the cells by the extracts are identified.

The cell proliferation activity was qualified on MCF-7 cell line, by using positive and negative controls (positive control - 17-β-estradiol; negative control - culture medium only).

Antimicrobial and Antitumor Activity

Antifungal activity of four different extracts, i.e. methanol, acetonitrile, chloroform and hexane, is shown in Table 1. They were tested against *Aspergillus flavus* and *Aspergillus niger* for their antifungal activity. All the four extracts showed significant activity against *A. flavus*; however, maximum activity was observed for methanolic extract followed by acetonitrile and chloroform extracts, which have similar activity. Minimum activity was observed for hexane extract. On the one hand, only methanolic extract showed significant activity against *A. niger*, while, on the other hand, three extracts, i.e. acetonitrile, chloroform and hexane extract, showed moderate activity.

The antibacterial activity of different extracts was carried out against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Klebsiella pneumonia*. Out of different extracts tested, methanolic extract of Kalimusli was found to be the most active against *Pseudomonas aeruginosa* followed by *S. aureus* and *K. pneumonia* (Hutchinson, 1968), which showed moderate activity (Table 2). Maximum antitumor activity was again observed by the methanolic extract, which was

Table 1: Antifungal activity of the different extracts of Kalimusli

S. no.	Sample	*Aspergillus flavus*		*Aspergillus niger*	
		Col. dia (mm)	% inhibition	Col. dia (mm)	% inhibition
1	K.M. (MeOH)	0.4	86.7	0.4	80.0
2	K.M. (Acetonitrile)	0.5	83.3	0.8	60.0
3	K.M. ($CHCl_3$)	0.5	83.3	0.8	60.0
4	K.M. (Hexane)	0.6	80.0	0.5	75.0
5	Control	3.0	-	2.0	-

K.M. - Kalimusli

Table 2: Antifungal activity of the different extracts of safed musli

S. no.	Sample	Control	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Klebsiella pneumonia*
1	K.M. (MeOH)	-	+++	++	++
2	K.M.(Acetonitrile)	-	+	-	-
3	K.M.($CHCl_3$)	-	-	+	-
4	K.M. (Hexane)	-	+	+	+

K.M. - Kalimusli, + = 6-10 mm (slightly active); ++ = 10-15 mm (moderate active); +++ = >15 mm (highly active); - = inactive

Table 3: Antitumor activity of the different extracts of Kalimusli

S. No.	Sample	Cell count x 10^4	Activity
1	K. M. (MeOH)	9.35 ± 0.61	+
2	K. M. (Acetonitrile)	12.34 ± 1.09	-
3	K. M. ($CHCl_3$)	9.21 ± 0.82	+
4	K. M. (Hexane)	11.89 ± 1.05	-

K. M. - Kali Musli, Positive control: (+) Inactive: (-)

followed by the chloroform extract that showed a little less activity. No activity was observed in hexane and acetonitrile extracts (Table 3).

RESULTS AND DISCUSSION

The antimicrobial and antitumor activity of different fractioned extracts of Kalimusli is due to presence of saponins, which are the glycosides (Varshney and Sharma, 1996) present in plant. These plant glycosides are the polar compounds, therefore the methanolic extracts is showing the maximum activity.

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