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Effect of leaves of *Cassia alata* Linnaeus (Caesalpiniaceae) on the growth of *Staphylococcus aureus* Rosenbach

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Abstract

Effect of leaves of *Cassia alata* Linnaeus on growth of *Staphylococcus aureus* Rosenbach was studied. Results showed that leaves of *Cassia alata* could inhibit growth of *Staphylococcus aureus.* Seasonal variations in this effect were also studied. It was found out that leaves of *Cassia alata* during the months of July – August had maximum anti growth property against *Staphylococcus aureus.*

Key words: *Cassia alata, Staphylococcus aureus,* Anti growth property

INTRODUCTION

Resistance to antibiotics is becoming an increasingly difficult problem in the management of bacterial infections (Coates *et al* 2002). The situation is particularly critical for *Staphylococcus aureus* where methicillin-resistant (MRSA) and vancomycin intermediate resistant (VISA) strains have emerged; those are also frequently resistant to multiple classes of antibiotics (Chopra 2003). Recent reports of high level vancomycin resistance in MRSA as a result of acquisition of the vanA determinant from *enterococci* (RCDCP 2002a, b) and the emergence of MRSA resistant to linezolid (Tsiodras *et al* 2001) are further disturbing trends in the evolution of antimicrobial resistance in *Staphylococcus aureus*. Thus, Al–Bari *et al* (2006) asserted that a large number of antibacterial agents have been discovered but pathogenic bacteria are constantly developing resistance to these agents. Due to this, Rahman *et al* (2001), stated that the life threatening bacterial infection has been increased worldwide and is becoming an important cause of morbidity and mortality. Under the circumstances, stress is given to search for new antibacterial agents (Projan & Youngman 2002). Researches are on progress in this direction and is extended even in the field of medicinal plants to develop safer antibacterial drugs (Dahanar *et al* 2000).

Medicinal uses of numerous plants have been recognized by ethnic people round the world. And, many of such traditional knowledge led to the discovery/development of numerous important drugs. India is a megadiversity country with its extremely rich flora. At the same time, ethnic diversity and the knowledge acquired by them is also quite rich. Such ethnic knowledge shares so much of ethnomedicobotanical information. There are innumerable publications on Indian medicinal plants and recorded ethnobotanical knowledge (Goel 2007; Pal 2007). While scanning through the antimicrobial properties of ethnomedicinally useful plants, the recorded properties of *Cassia alata* Linnaeus (Caesalpiniaceae) (Das *et al* 2010; Sharma & Sharma 2010) prompted us to test its antimicrobial efficacy. However, some works on the medicinal properties of Cassia alata has been taken up by different workers in different regions using plants occurring outside the Himalayas (Malanichamy & Nagarajan 1990; Durainpandiyan *et al* 2006; Somchit *et al* 2003).

We, in our laboratory, screened medicinal plants from the Himalayan region for their antibacterial property. In this communication we report the results of effect of leaves of *Cassia alata* Linnaeus (Caesalpiniaceae) on the growth of *Staphylococcus aureus* as well as seasonal variations, if any, in this effect.

METHODOLOGY

Collection and authentication

Leaves of *Cassia alata* Linnaeus were collected from the foot-hill region of the Darjeeling part of the Eastern Himalaya where the species found growing upto 100 m altitude. The plant was identified at NBU Herbarium by matching. A voucher specimen was kept in the department for future reference.

Leaves were collected randomly as well as during the months of January - February, March - April, May - June, July - August, September - October and November - December.

Test drug

The collected leaves of *Cassia alata* were sundried and powdered separately. 50 g of this powder was extracted with 500 ml of 10 % acetone - ethanol mixture for 1h on a rotary shaker and then centrifuged. Supernatant was evaporated to dryness. Brown mass thus obtained was kept as test drug for anti growth study.

Growth study of *Staphylococcus aureus*

Materials

Staphylococcus aureus (strain no. ATCC 259233) used in this study was collected from the department of Microbiology, North Bengal Medical College and Hospital and maintained on nutrient agar slants.

Preparation of inoculums

Inoculums of *Staphylococcus aureus* were prepared in sterile nutrient broth containing glucose 10 %, peptone 0.5 % and beef extract 0.3 %. The pH of the medium was adjusted to 7.0. Sterile nutrient broth was prepared by autoclaving the broth at 15 pound pressure at 120 degree centigrade for 20 minutes.

Synthetic medium

The chemically defined synthetic medium selected for studying the growth of Staphylococcus *aureus* consisted of the followings. Vitamin free casein hydrolysate - 4 g, glucose – 3 g, potassium hydrogen phosphate – 5 g, cysteine hydrochloride - 20 mg, nicotinic acid – 20 mg, thiamine hydrochloride – 15 mg,

Water - 1000 ml (pH of the medium was adjusted to 7.0). Synthetic medium was made sterile by autoclaving the medium at 15 pound pressure at 120 degree centigrade for 20 minutes.

Bacterial cell suspension

One loop full of the organism from the nutrient agar slants (2 - 3 weeks old) was inoculated to sterile synthetic medium and incubated at 37º C for 20 hours. It was then centrifuged and washed repeatedly with sterile saline to remove adhering materials in aseptic condition. A uniform cell suspension was then prepared by suspending the cells in 5 ml of sterile saline.

Preparation of test drug for experiment

0.5 mg of the mass, as obtained from leaves of *Cassia alata*, was extracted with 10 ml distilled water for 10 minutes on a rotary shaker. Mass dissolved. Solution thus obtained was made sterile by passing through the Jena glass bacteria filter (porosity G 5) which was previously sterilized by autoclaving at 15 pound pressure for 20 minutes. This sterile solution was kept for anti growth studies against *Staphylococcus aureus*.

Determination of the growth of *Staphylococcus aureus*

5 ml of sterile double strength synthetic medium was taken in Erlenmyer flask (100 ml) and was inoculated with 0.1 ml of thoroughly washed bacterial cell suspension. Total volume of the medium was made up to 10 ml with sterile distilled water. The flask was then incubated at 37 degree centigrade for 20 h and the growth of *Staphylococcus aureus* was measured turbidometrically with a colorimeter using 660 nm after stopping the growth by steaming for 5 minutes (Sarkar *et al* 1976).

Effect of the mass obtained from leaves of *Cassia alata* Linnaeus on growth of *Staphylococcus aureus*

Same as above. However, in this case four sets were prepared. Test drug was added in graded concentrations like 0.05mg (1 ml), 0.1mg (2 ml), 0.15mg (3 ml), 0.2mg (4 ml) in different flask. Total volume of the flask was made up to 10 ml with sterile distilled water. The flask was then incubated at 37 degree centigrade for 20 h and the growth of *Staphyolococcus aureus* was noted by the method described above.

Statistical analysis

The values were expressed as mean ± SEM and was analyzed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) 10th versions. Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.

RESULTS

Effect of leaves of *Cassia alata* Linnaeus on the growth of *Staphylococcus aureus* was given in Table – 1. These leaves were collected randomly. It was found out that leaves of *Cassia alata* could inhibit growth of *Staphylococcus aureus*. Inhibition was dose dependant. Out of four sets we have done, maximum inhibition (27.37%) was recorded at 0.20 mg of the test drug. This was statistically significant ($p < 0.05$) in comparison to the inhibitions made by other doses of the test drug.

Seasonal variation in the effect of leaves of *Cassia alata* on growth of *Staphylococcus aureus*. was given in Table – 2. Results showed that leaves of *Cassia alata* Linn. of all seasons had anti growth effect against *Staphylococcus aureus*. Anti growth effect was dose dependent. Out of four doses we have used, maximum inhibition of the growth of *Staphylococcus aureus* was recorded at 0.20 mg of the test drug in all cases. Results were statistically significant ($p< 0.05$ to $p<0.001$).

Results related to comparison in maximum effect of anti growth property of leaves of *Cassia alata* of different seasons against *Staphyococcus aureu* were given in Table – 3. It was found out that leaves of *Cassia alata* during the months of July – August had maximum anti growth property against *Staphylococcus aureus.* Results were statistically significant to the level of $p<0.001$ when compared with the anti growth property of the leaves of other seasons.

Table 1. Effect of leaves of *Cassia alata* on growth of *Staphylococcus aureus*

***Cassia alata* (mg)**	**Growth of *Staphylococus aureus* (O.D.)**
0.00	0.95 ± 0.05
0.05	0.89 ± 0.05
0.10	0.80 ± 0.04
0.15	0.78 ± 0.04
0.20	0.69 ± 0.04*

Results are mean ± SEM, In each case ten sets of experiments were done, * $p < 0.05$

Table 2. Seasonal variations in the effect of leaves of *Cassia alata* Linn. on growth of *Staphylococcus aureus*

Cassia alata Linn.	January-February	March-April	May-June	July-August	September-October	November-December
0.00 mg	0.94±0.06	0.95±0.05	0.94±0.06	0.94±0.05	0.96±0.06	0.95±0.06
0.05 mg	0.90±0.06	0.86±0.04	0.80±0.05	0.69±0.05	0.79±0.06	0.78±0.05
0.10 mg	0.81±0.05	0.79±0.05	0.72±0.05	0.60±0.04*	0.75±0.05	0.75±0.04
0.15 mg	0.77±0.05	0.77±0.04	0.68±0.06	0.57±0.04*	0.72±0.04	0.71±0.05
0.20 mg	0.68±0.01*	0.65±0.02*	0.60±0.02*	0.40±0.01**	0.62±0.03*	0.65±0.04

Results are mean ± SEM, in each case ten sets of experiments were done, * $p< 0.05$, ** $p< 0.001$

Table 3. Comparison in maximum effect of anti growth property of leaves of *Cassia alata* of different seasons against *Staphyococcus aureus*

Months	**Maximum effect with 0.20 mg**
January – February	0.68±0.01
March - April	0.65±0.02
May - June	0.60±0.02
July - August	0.40±0.01**
September - October	0.62±0.03
November - December	0.65±0.04

Results are mean ± SEM, In each case ten sets of experiments were done, ** $p< 0.001$

DISCUSSION

Several plants have shown anti-microbial activity. Few of those are *Azadirachta indica* (Venugopal & Venugopal 1994), *Clausena anisata* (Chakraborty *et al* 1995), *Anona glabra* (Padmaja *et al* 1995), *Semecarpus anacardium* (Nair & Bhide 1996), *Garcinia mangostana* (Gopalakrishnan *et al* 1997), *Aegle marmelos* (Rana *et al* 1997), *Santolina chamaecyparissus* (Suresh *et al* 1997), *Terminalia belerica* (Valsaraj *et al* 1997), etc. *Cassia alata* Linnaeus (Caesalpiniaceae) is an erect tropical shrub, uoto 5 m tall, with leathery compound leaves and large yellow flowers. It grows throughout West Bengal

extending upto 600 m in foot hills. It is quite common in Terai and Duars. It has different names like ringworm weed in English, dadmari in Hindi and cakramard in Sanskrit. Its therapeutic values as mentioned in different literature (Gurung 2002; Das *et al* 2010) include: Leaves are anti-parasitic, used in eczema, bronchitis, asthma, ringworm and in poisonous insect bites. Bark is used to treat skin diseases. Extract of aerial parts is CNS depressant, diuretic and anti-inflammatory.

In 1998 Sakharkar & Patil found antimicrobial activity of *Cassia alata*. In the present study we also noted that leaves of *Cassia alata* could inhibit growth of *Staphylococcus aureus*. Dry mass obtained after evaporation of acetone – alcohol extract of its leaves had anti growth effect against *Staphylococcus aureus*. Effect was dose dependant.

Among the four doses we have used, maximum inhibition (27.37 %) was recorded with 0.20 mg dose of the test drug. Result was statistically significant to the level of $p<0.05$.

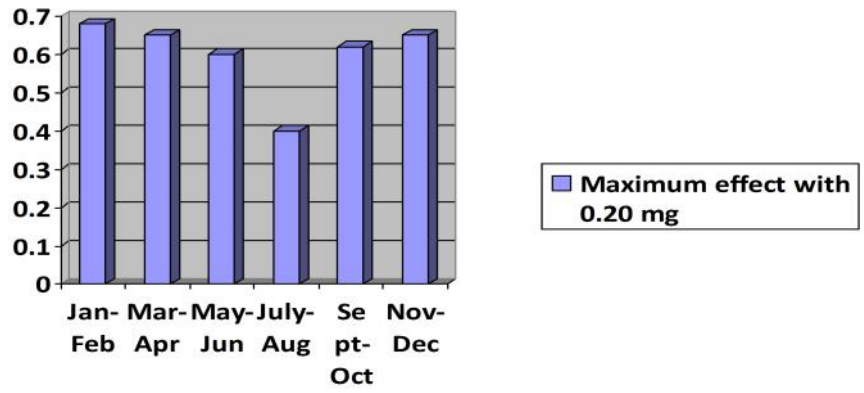

Fig. 1. Comparison in maximum effect of anti-growtrh property of the leaves of *Cassia alata* in different seasons against *Staphylococcus aureus*

We further intended to know the seasonal variations, if any, in the anti-growth effect of leaves of *Cassia alata* against *Staphylococcus aureus.* Results showed that leaves of *Cassia alata* in all seasons had anti-growth property against *Staphylococcus aureus*. Results were dose dependant and statistically significant ($p<0.05$ to $p<0.001$). But, during the months of July and August the anti-growth property against *Staphylococcus aureus* is recoded as maximum. Results were statistically significant to the level of $p<0.001$ when compared with the anti-growth properties in other seasons of the year. Growth inhibition recorded for the months of July – August was 57.45 % while the same were 27.55 %, 31.58 %, 36.17 %, 35.42 % and 31.58 % respectively for the months of January – February, March – April, May – June, September – October and November – December.

Anti-growth property of the leaves of *Cassia alata* against *Staphylococcus aureus* is due to its active ingredient(s) which need isolation and characterization. Works in this direction is now in progress.

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