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A study on Seed germination of *Cassia alata* Linn an antiallergenic plant

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Abstract
Cassia alata known as candle bush or Ringworm tree belongs to Cesalpinaceae family, which was found in diverse habitats in the tropics. *C. alata* leaves containing some chemical substances like chrysophanic acid, is a common ingredient in soaps, shampoos and lotions. The effectiveness of this plant against skin diseases is confirmed by modern scientific studies. The seed germination was gradually decreased by increasing the age of the seeds. Seed propagation is still used as a specialized tool for breeding purposes and for the propagation of pathogen-free plant material.

Keywords: *Cassia alata*, seed germination, seed propagation, skin diseases

INTRODUCTION

Plants are not only important to the millions of the people to whom traditional medicine serves as the only opportunity for health care but also to those who use plant products for various purposes in this daily lives and also as a source for new pharmaceuticals. *C. alata* leaves have antiallergenic compounds are used to treat fungal infections like ringworm. This fresh leaf paste and vegetable oil mixed in equal amounts are rubbed/applied to the affected area 2-3 times a day [1]. Gujava like most *Cassia* plants contains a group of chemicals called anthroquinones [2]. These chemicals are well known for their laxative effect [3], while the ashycones including aloeemodinrhin emodin and chrysophenol exhibit antifungal activity and randomized controlled trials are carried out in provincial and community hospitals. The phytochemical studies of *in vitro* antifungal activity of *C. alata* crude stem bark extracts on dermatophytes which include chemical dermatophytes of the genera *Trichopyto*n, *Microsporium* and *Epidermopyton* was done by Chichioco-Hernandez and Leonido [4]. In the diet induced Lipidania in mice, *C. alata* methonal leaf extract was evaluated for their hypolipidemic activity [5] and antimicrobial activity [6]. The seed oil of *C. alata* extracted with chloroform exhibited antibacterial, thrombolytic and cytotoxic activity [7].

MATERIALS AND METHODS

Matured pods were collected during December 2010 from Mulugu Natural Forest of Warangal District, Andhra Pradesh, India. Seeds were extracted manually and air dried at normal temperature. Complete dry seeds were chosen for germination studies. The morphological traits of pod (pod length, width and no. of seeds per pod) and seed (seed length, width and thickness) were measured by Calipers (Table 1). The experiment was carried out in complete randomized design with five replicates and 100 seeds were taken per replication under both *in vitro* and *in vivo* conditions.

For germination studies, seeds were subjected to ten pre treatments viz., T_1 : Untreated seeds (Control), T_2: Cold water soaking for 24 hours, T_3: Cold water soaking for 48 hours, T_4: Cold water soaking for 72 hours, T_5: Hot water soaking overnight, T_6: Sulphuric acid (H_2SO_4) scarification for 2 minutes, T_7: Sulphuric acid scarification for 4 minutes, T_8: Sulphuric acid scarification for 6 minutes, T_9: Hydrogen peroxide for 5 minutes, T_{10}: Hydrogen peroxide for 10 minutes. Sulphuric Acid and hydrogen peroxide treated seeds were thoroughly washed under running tap water carefully. Afterwards the seeds were germinated in the pots filled with sand + vermicompost (2:2 ratio) mixture and irrigated regularly.

Seeds required *in vitro* studies were washed thoroughly under running tap water and soaked for 48hrs in water. After wards, seeds were sterilized with tween - 20 solution, pretreated with by $HgCl_2$ and then rinsed with sterile distilled water 3-5 times. Seeds were inoculated on Basal MS (Murashige and Skoog) medium without supplementation of any nutrients. The cultures were incubated at 16/8-hr light and dark photoperiod provided by cool white fluorescent lamps of 2000 lux and temperature of 25 $\pm 2^0$C. Germination was counted daily till 10 days and 8 days in the case of *in vivo* and *in vitro* conditions respectively (till the end of germination). Cumulative germination percentage was calculated. Germination percentage, calculated using following formula.

Germination percentage

n/N X 100 % where n was the number of normally germinated seeds and N was the number of seed kept for germination.

The data were analyzed using ANOVA in the frame of the General Linear model procedure (Gomez and Gomez 1984). Treatment means were separated using Duncan's test (Klockars and Sox 1986; Matis 1989) at significance level P<=0.005. All statistical analysis was carried using SPSS 17.0 (SPSS, INC., USA).

RESULTS AND DISCUSSION

Seed germination is the easiest and cheapest method for propagation. Germination is the growth of an embryogenic plant

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continued within a seed which results in the formation of the seedling that emerges from a seed and begins the growth. Seed grown in pots filled with sand + vermicompost (2:2) produced healthy seedlings. Germination percentage was very low due to the less viability of the seeds. Data on seed viability in both *in vivo* and *in vitro* germination is presented in Table-2 and Fig 1. Seed viability was found drastically reduced with increasing age in both *in vitro* and *in vivo* studies.

The seeds of *C. alata* responded differently to each treatment. The normal water soaked seeds for 24hr resulted in better germination percentage of around 76.80% than 72hr treatment (68.6%). Similarly 48hr treatment exhibited highest percentage (80.2%) of germination. The data showed that treatment of normal water was higher than sequenced time, decreased the germination percentage and resulted in showing significant difference than normal water treatment.

The germination percentage of seeds treated with concentrated H_2SO_4 was significant. Highest percentage (51.20%) germination was observed at 2 min treatment followed by 4 min (47.40%) and 6 min (41.80%) respectively, the germination percentage in H_2SO_4 scarification was dropped while increasing the period treatment time (i.e. 2 min to 6 min). Similarly, H_2O_2 treatment also showed acid scarification after 5min and exhibited 64.8% germination which was higher than 61.40% of H_2O_2 treatment of 10 min.

The germination percentage of *C. alata* seeds treated with normal water, concentrated sulfuric acid and hydrogen peroxide exhibited significant differences compared to normal water treatments. Germination percentages of all treatments viz., T_2 to T_{10} were higher than the control group.

The percentage of seed germination was higher *in vitro* level, which was around 86.40% and was comparatively much higher and significant than all other samples treated in normal water, hot water, sulfuric acid and hydrogen peroxide for various durations. *In vitro* germination percentage was not only noted higher but was also observed two days earlier than treated samples.

In *Phytolacea acinosa*, sulfuric acid treated seeds exhibited germination percentage significantly higher than that of other treatments [8, 9] and conc. H_2SO_4 treatment showed better germination percentage. The present investigation reported that normal water treatment showed higher germination percentage than sulfuric acid, hydrogen peroxide treatments or control treatments. It might have been because the seed coat of *C. alata* is highly thin which could have easily reacted and imbibed the acid and H_2O_2 leading to embryo damage and influencing the germination percentage.

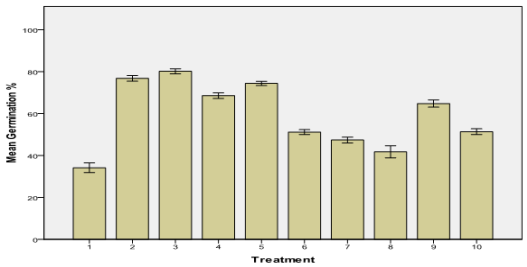
The germination of seeds immediately after collection exhibited 99% germination without any treatments. Hence, it could be concluded that the increasing age of the seed results in reduced viability and percentage of germination.

Table 1. Morphological characteristics of pod and seed of *Cassia alata*

Morphological Character	Mean
Pod length (cm)	16.84
Pod width (cm)	1.68
Number of seed per pod	53.6
Seed length (mm)	4.9
Seed Width (mm)	4.18
Seed thickness (mm)	1.54
Weight of 1000 seed (g)	36.54
Seed Collection period	December-February

Table 2. Effect of pretreatment on *in vivo* seed germination of *Cassia alata*

Treatment	*In vivo* Germination	
	Germination (%)	S. Deviation ± S. Error
T_1 Untreated seeds (Control)	34.20	2.58 ± 1.15
T_2 Cold water soaking for 24 hours	76.80	1.48 ± 0.66
T_3 Cold water soaking for 48 hours	80.20	1.30 ± 0.58
T_4 Cold water soaking for 72 hours	68.60	1.51 ± 0.67
T_5 Hot water soaking overnight	74.40	1.14 ± 0.51
T_6 Sulphuric acid (H_2SO_4) scarification for 2 minutes	51.20	1.30 ± 0.58
T_7 Sulphuric acid (H_2SO_4) scarification for 4 minutes	47.40	1.51 ± 0.67
T_8 Sulphuric acid (H_2SO_4) scarification for 6 minutes	41.80	3.19 ± 1.42
T_9 Hydrogen peroxide (H_2O_2) for 5 minutes	64.80	1.92 ± 0.86
T_{10} Hydrogen peroxide (H_2O_2) for 10 minutes	51.40	1.12 ± 0.67
In vitro germination	86.40	0.548 ± 0.245



T_1: Untreated seeds (Control), T_2: Cold water soaking for 24 hours, T_3: Cold water soaking for 48 hours, T_4: Cold water soaking for 72 hours, T_5: Hot water soaking overnight, T_6: Sulphuric acid (H_2SO_4) scarification for 2 minutes, T_7: Sulphuric acid (H_2SO_4) scarification for 4 minutes, T_8: Sulphuric acid (H_2SO_4) scarification for 6 minutes, T_9: Hydrogen peroxide (H_2O_2) for 5 minutes, T_{10}: Hydrogen peroxide (H_2O_2) for 10 minutes.

Fig 1. Effect of pretreatment on *in vitro* seed germination of *Cassia alata*

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