

**Research Article****AN EXPERIMENTAL STUDY OF VRIKSHAYURVEDA SEED TREATMENTS ON GERMINATION RATE AND ACTIVE INGREDIENT OF BAKUCHI (*PSORALEA CORYLIFOLIA* LINN.) BY HPLC METHOD****Gangadhar T. Aralelimath^{1*}, Shraddha U. Nayak², Gireesh M. Ankad³, Harsha V. Hegde³, S S Shirurmath¹**¹Associate Professor, Dept. of Dravyaguna, Shri Jagadguru Gavisiddheshwara Ayurveda Medical College, Hospital & P G Studies, Koppal, Karnataka, India.²Associate Professor, Dept. of Dravyaguna, D Y Patil School of Ayurveda, Navi Mumbai, Maharashtra, India.³Herbal Medicine Division, Regional Medical Research Centre, ICMR, (NITM), Belgaavi, Karnataka, India.**ABSTRACT**

Bakuchi (*Psoralea corylifolia* Linn.) is one of the important endangered medicinal plants used in Ayurveda and other Traditional systems. Its cultivation and propagation is difficult due to its low germination rate (5-7%) and prolonged seed dormancy. *Bakuchi* seeds made into 5 groups, the experiment was conducted in a complete randomized block design with 5 treatments and 5 replications totally 500 seeds in each group) and observed for 50 days. Control Group 1 no- seed treatment, Group 2- Standard treated with 1% concentrated H₂SO₄, Group 3 *Vrikshayurvedic* treatment done by soaking in milk subsequently fumigation of *Vidanga* and ghee, Group 4- treated with paste of *Brihati*, *Tila*, *Kamalanaala*, Ghee and Group 5 treated by soaking in milk subsequently Cow dung, *Vidanga* and honey applied. Number of seeds germinated, germination percentage, emergence index and relative seed germination parameters were observed. HPLC studies carried out of post harvested *Bakuchi* seeds of all 5 groups to know the effect of seed treatments on Psoralen content quantitatively. Overall results indicates that Group 4 (8.000 ± 0.8367) seeds soaked in 12hrs milk followed by application of *Brihati*, *Tila*, *Kamalanaala* and Ghee paste for 12hrs treatment is statistically significant (P value>0.05) in comparison with group 2 (4.600 ± 0.6782) Sulphuric acid treatment and Group 3 (4.200± 0.9165) fumigation with Honey and *Vidanga*. Rest of the groups shown insignificant changes on germination parameters. HPLC results found that generally seed treatments may reduce the content of Psoralen as in control (Group 1) maximum percentage (0.04%w/w) of Psoralen is noticed. Among treatment groups Group 4 contains maximum (0.027%w/w) Psoralen next to control (0.039%w/w). Psoralen content is very less in standard Group 2 (0.022%w/w), Group 3 (0.023%w/w) & Group 4 (0.024%w/w). Maximum germination percentage was observed in Group 4 in comparison with the Group 2 conventional method of treating with sulphuric acid. Estimation of Psoralen contents in the seeds from the plants grown by various treated seeds revealed that Group 4 is qualitatively better than standard, but inferior to the control, standard and other *Vrikshayurveda* seed treatment techniques used in the current experiment.

KEYWORDS: *Vrikshayurveda*, Germination, *Bakuchi*, *Psoralea Corylifolia* Linn., Seed treatments, Psoralen, HPLC.**INTRODUCTION**

Medicinal plants play inherent and prominent roles in the general health service. Due to long-term exploitation of wild medicinal herbs, many important medicinal plants are becoming rare and endangered. In order to conserve the medicinal plant resources and to meet the increasing demand for plant-based drug and herbal remedies, the most popular medicinal plants should be cultivated under the supervision of government or grown

spontaneously by farmers. Due to increase in population, decrease in forest cover and over exploitation of natural resources has made many medicinal plants endangered. According to the Red list of Threatened Plants, 19 species are already extinct and 1236 species are facing various degrees of threat across different biogeography regions in the country (Ramprasad Naik, 2012). Hence there is essential of alternative supply sources of such species

like cultivation. Cultivation of plant species using organic manure dates back to 1000AD in India. It is dealt in *Vrikshayurveda*, which forms a part of Ayurvedic history and is treated as a separate subject owing to its importance and extensive nature. It is an age old agro practice which is of great relevance even today in sectors like agriculture and horticulture. It is interesting to know that ancient India not only had Ayurveda for the humans but also for plants called *Vrikshayurveda* written by *Surapaala* a 10th century treatise. It is the first available full-fledged text on the science of arbori-horticulture not only deals with pest and disease management of plants but also encompasses study areas like storage of seeds, sowing, germination, plant propagation, manuring etc. [1]

“*Vrikshayurveda*” means ‘*Vrikshasya ayurveda*’ it deals with *Hitha* and *Ahitha* of *Vriksha*. The term *Vriksha* means ‘*Sthavara yoni visesha*’ which denotes the entire flora. It can be assumed that knowledge of *ayu* can be applied in *Vriksha* also. *Vrikshayurveda*, an ancient science of plant life deals with healthy growth of plants and their productivity.[2]

Psoralea corylifolia Linn (Indian bread root) is an endangered and medicinally important plant herbaceous plant species belonging to Fabaceae family which is distributed throughout tropical and subtropical regions of the world. Powders obtained from its seeds have been used for treatment of inflammatory diseases of skin including leucoderma, leprosy and psoriasis.[3] Psoralen is an isomer of furanocoumarin obtained from the fruits of *Bakuchi*. Seed setting commences around April-May and seed germinate immediately after shading. *Psoralea corylifolia* is propagated by seed germination, However seed germination percentage is very low (5-7%) because of hard seed coat of this useful plant. Hence there is an urgent need for cultivation of this endangered and medicinally important plant species. An observation at the post-germination growth stage revealed that *Psoralea corylifolia* is a slow-growing species. Low germination percentage and viability of the seeds, long gestation periods and delicate field-handling are some of the factors which discourage commercial cultivation of the plant.[5] It is a photosensitizing agent. *Psoralea corylifolia* (*Bakuchi*) contains furanocoumarins like psoralen. Psoralen stimulates skin to produce melanin pigment when exposed to sunlight.[4]

In practice, dormancy not only affects the number of seeds which germinate (high dormancy), but also their rate of germination (low dormancy) especially under sub-optimal conditions.[6] One-seeded pods of *Psoralea corylifolia* L., a dominant

weed of cultivated ground, are dormant due to an impermeable seed coat (Mall & Shukla, 1965).[7]

Different methods are used to break the dormancy, called as pre treatments. Based on the species and kinds of dormancy, these include mechanical scarification, chemical scarification (H₂SO₄), cold/wet, hot water, electro sonic waves and stratification. Some researches indicated the positive effect by pre sowing seed treatment with concentrated Sulphuric acid in *Bakuchi* seeds but some got insignificant results and slight increase in treatment duration resulted in hazardous effects on embryo and which also require special equipment, personal protective gear and proper disposal mechanisms.[8]

Verses 52-62 of *Vrikshayurveda* explain details about pre-sowing seed treatment techniques with organic materials which reflects the knowledge of seed dormancy existence even then. As this vast knowledge is not yet properly understood, effort is made facing the rapidly growing demands for medicinal plants, domestic cultivation is a viable and long-term way of conserving red listed medicinal plants by exploring the Vedic methods of plant rearing, cultivation and conservation.

It is the need of the day to know about *Vrikshayurveda* techniques to identify safe, alternative methods to break seed dormancy in *Bakuchi* as in today's world, global environmental issues relating to sustainable development have emerged as topics of major concern. The various seed priming processes have been carefully designed in *Vrikshayurveda* to allow early germination, to obtain good quality of seedlings like soaking in milk, rubbing seed with cow dung, applying some organic medicines to seeds.

Although there is no direct reference of seed treatments for *Bakuchi* in *Vrikshayurveda* randomly three procedures/techniques were selected among nine as these techniques were explained generally for all seeds. Hence an attempt is made in this study to know the effect of different seed treatments on germination of *Bakuchi* by comparing modern techniques with techniques explained in *Vrikshayurveda*. The aim of present work was an attempt to study the effect of different seed treatments mentioned in *Vrikshayurveda* on germination rate and active ingredient (Psoralen) of *Bakuchi* in comparison with modern techniques.

MATERIAL AND METHODS

Mature *Bakuchi* (*Psoralea corylifolia* Linn.) seeds were collected from the horticultural farm Arabhavi during October 2012. They were authenticated at K.L.EU'S Shri.B.M.K Ayurvedic College Central Research Facility (Ayush Approved

Drug testing Laboratory for ASU Drugs). (Annexure no-1) Voucher specimens were prepared and deposited in the department for reference. All other samples like *Brihati panchanga*, *Vidanga (Embelia ribes)* seeds, *Tila*, *Kamala Naala* which were needed for seed treatment were purchased from Khajarekar pharmacy, Belagavi and were authenticated in K.L.E.U'S Shri.B.M.K Ayurvedic college, Central Research Facility (Ayush Approved Drug testing Laboratory for ASU Drugs). (Annexure no- 2, 3, 4, 5) *Madhu* (Honey) Milk and cow dung was collected from locally available source.

Experimental study

Seeds Treatment Techniques

Table No 1: Showing the Groups of Experimental Study and a type of seed treatment on *Bakuchi* seeds

	Groups	No. of seeds	Treatment*
01	Group 01	100x5	Control group- no treatment for seeds
02	Group 02	100x5	<i>Bakuchi</i> seeds treated with 1% H ₂ SO ₄ (Sulphuric acid) for 50 minutes and washed in running water for 5 min, a day prior to sowing ^[10]
03	Group 03	100x5	Seed sprinkled with warm milk for 12hrs and dried for 12hrs continued for 5 days, then smoked with <i>Ghrita</i> (Ghee) and <i>Vidanga (Embelia ribes burm)</i> at the distance of 3 inches for 10 minutes a day prior to sowing ^[11]
04	Group 04	100x5	Seeds soaked in milk, for 12hrs and dried for 12hrs continued for 5 days, rolled into powder of <i>Brihati</i> , <i>Sesame (Sesamum indicum)</i> and <i>Kamala Naala</i> (hallow stalk of lotus { <i>Nelumbo nucifera</i> }) mixed with <i>Ghrita</i> (Ghee) for 12hrs a day prior to sowing. ^[12]
05	Group 05	100x5	Seed sprinkled with milk, for 12hrs and dried for 12hrs continued for 5 days, rubbed with cow dung, dried and profusely smeared with Honey and <i>Vidanga (Embelia ribes burm)</i>) for 12hrs a day prior to sowing ^[13]

*The Quantity of ingredients and the duration of processing (fumigation/soaking/drying) varied according to the need/requirement. Consequently Standard Operative Procedures were developed for quantity of ingredients and the duration of processing (fumigation/soaking/drying).

Experimental Design

Experimental Design: Randomized Block Design (RBD)

Treatment for the seeds

Number of treatment groups: 5 Number of Replications: 5

Number of seeds per Replications: 100

Total number of seeds per treatment group: 500

Seeds Sowing

Bakuchi (Psoralea corylifolia) seeds- 100x5=500 were sown directly on land bed with the gap of 1 inch between each seeds and about 3 inches gap in between each rows. Same procedure was followed for all other group seeds. 5 land beds were made for 5 groups. 500 seeds were sown in each group. As this land bed was made in a greenhouse uniform temperature was maintained and recorded daily on an average temperature between 23-26° C and trays were watered with tap water according to the need. Seed germination was recorded daily.

HPLC Analysis: Quantitative analysis of Psoralen content Seeds obtained after 8 months from the plants grown by various treated seeds including control i.e., from group 1 to group 5 were done in Natural Remedies Pharmacy, Bangalore.

Results

Experimental study

Table 2: Effect of different seed treatments on Germination of *Bakuchi (Psoralea corylifolia)* (Each value is an average of five replicates)

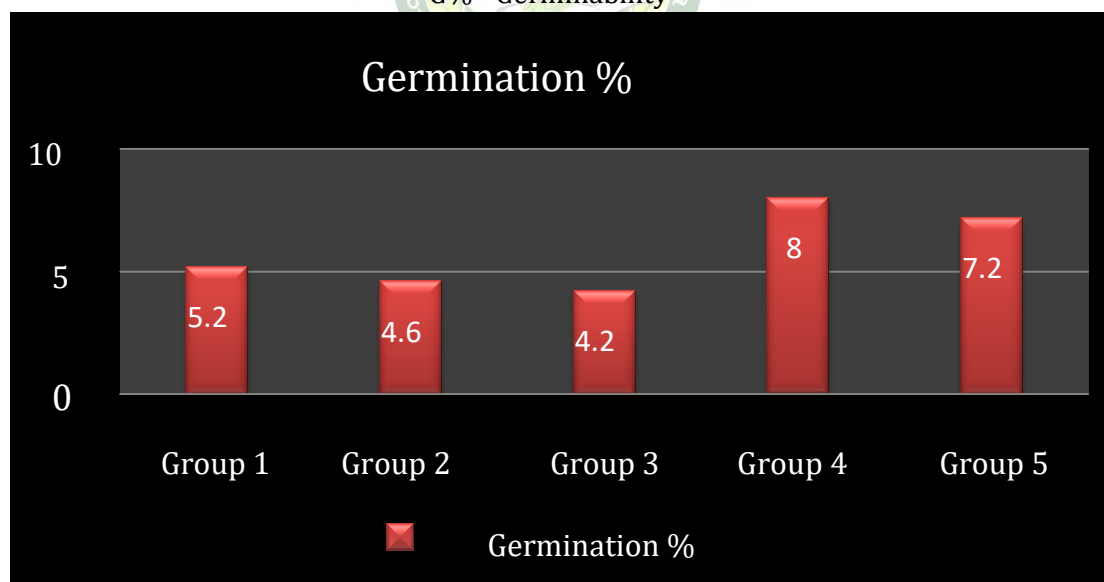
Days of germination Groups	5 th day	10 th day	15 th day	20 th day	25 th day	30 th day	35 th day	40 th day	50 th day	Total
Group 1	6	11	9	-	-	-	-	-	-	26
Group 2	7	6	5	-	5	-	-	-	-	23
Group 3	8	3	3	4	-	-	3	-	-	21
Group 4	6	14	12	2	4	-	2	-	-	40
Group 5	8	11	12	3	-	-	2	-	-	36

Germinability (G %) = Total No. of seeds germinated/ Total No. of seeds sown x 100

Table 3: Effect of different seed treatments on Germination Percentage parameter (Each value is an average of five replicates)

Treatment	G %
G 1	5.2
G 2	4.6
G 3	4.2
G 4	8
G 5	7.2
Mean	5.84
S. Em ±	1.11
CD (0.01)	4.47
CV %	46.0

G%= Germinability



Graph 1: Comparison of different seed treatment groups on Germination Percentage parameter

EMERGENCE INDEX (EI) is calculated by the formula of Baskin (1969)

$$EI = (n1/dn1) + (n2/dn2) + (n3/dn3) + \dots + (nx/dnx)$$

Where n=No. of seeds emerged on the day 1st, 2nd, 3rdnth day dn= No. of days from the day of sowing.

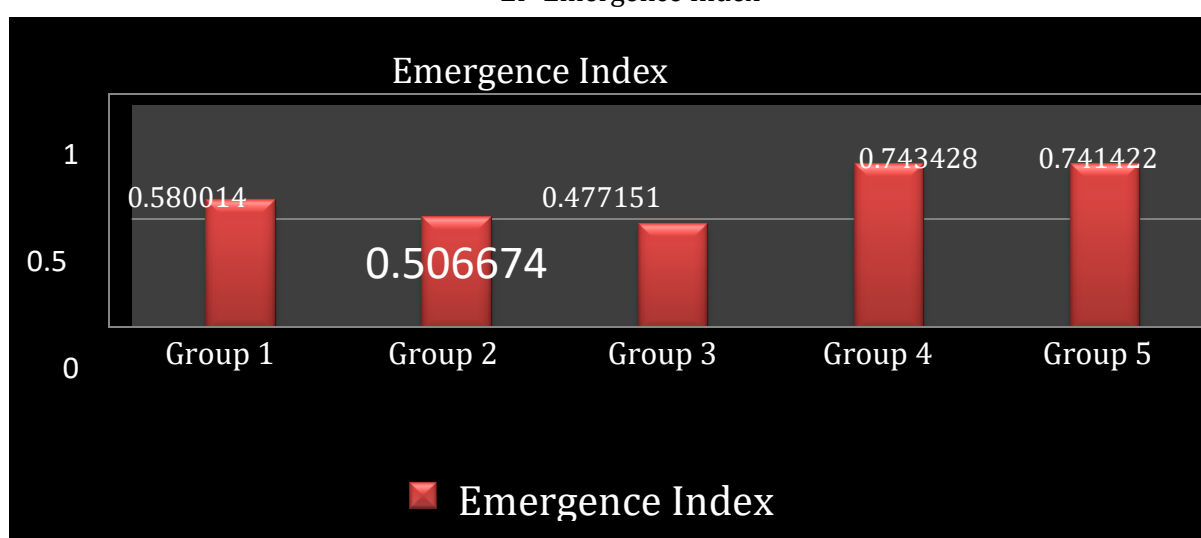
dnx= No. of days to the final count

$$EI = n1/dn5 + n2/dn10 + n3/dn15 + n4/dn20 + n5/dn25 + n6/dn30 + n7/dn35 + n8/dn40 + n9/dn45 + n10/dn50 + n11/dn55 + n12/dn60$$

Table 4: Effect of different seed treatments on Emergence Index (EI) parameter (Each value is an average of five replicates)

Treatment	EI
G 1	0.580014
G 2	0.506674
G 3	0.477151
G 4	0.743428
G 5	0.741422
Mean	0.609738
S. Em ±	0.117
CD (0.01)	0.471
CV %	15.09

EI=Emergence Index



Graph 2: Comparison of different seed treatment groups on Emergence Index (EI) parameter
 Relative Seed Germination (RSG) = No. of seeds germinated in Treatment group/ No. of seeds germinated in control group) x 100

Table 5: Effect of different seed treatments on Relative seed germination parameter (Each value is an average of five replicates)

Treatment	RSG
G 1	100 (2.0)*
G 2	178.2142(2.01)
G 3	173.9284(1.92)
G 4	280.642(2.25)
G 5	230(2.20)
Mean	192.55(10.38)
S. Em ±	110.74(0.192)
CD (0.01)	445.64(0.77)
CV %	798.10(13.35)

* Values in the parentheses are transformed RSG= Relative Seed Germination

Results Showing Significant Difference between Germination Percentage of various seed treatments between the Groups by unpaired' Test (Only Groups of Significant P Value Has Been Mentioned).

Table 6: Germination Percentage of treatments between G2 and G4

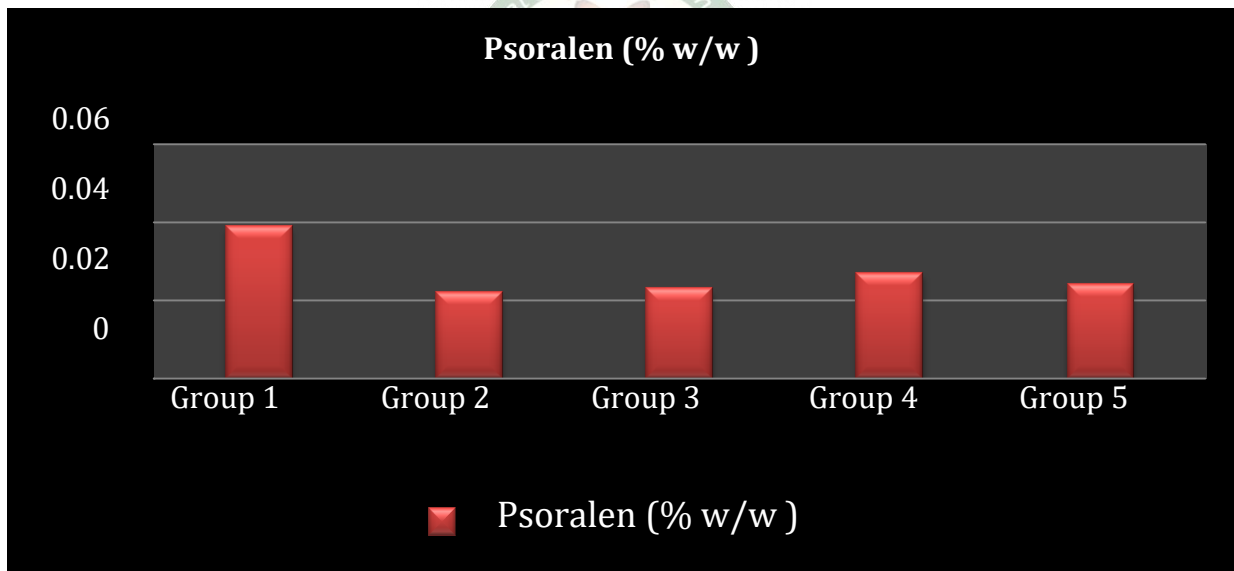
S. No	Parameters	G 2 Mean± SEM	G 4 Mean± SEM	Difference between means	P Value	P Value Summary
1	Germination Percentage	4.600 ± 0.6782	8.000 ± 0.8367	-3.400 ± 1.077	0.2946	Significant

Table 7: Germination Percentage of treatments between G3 and G4

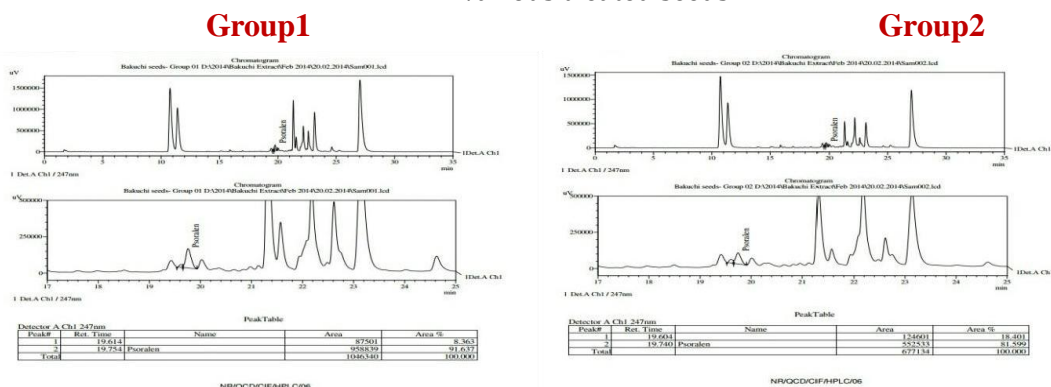
S. No	Parameters	G 3 Mean± SEM	G 4 Mean± SEM	Difference between means	P Value	P Value Summary
1	Germination Percentage	4.200± 0.9165	8.000 ± 0.8367	-3.800 ± 1.241	0.0155	Significant

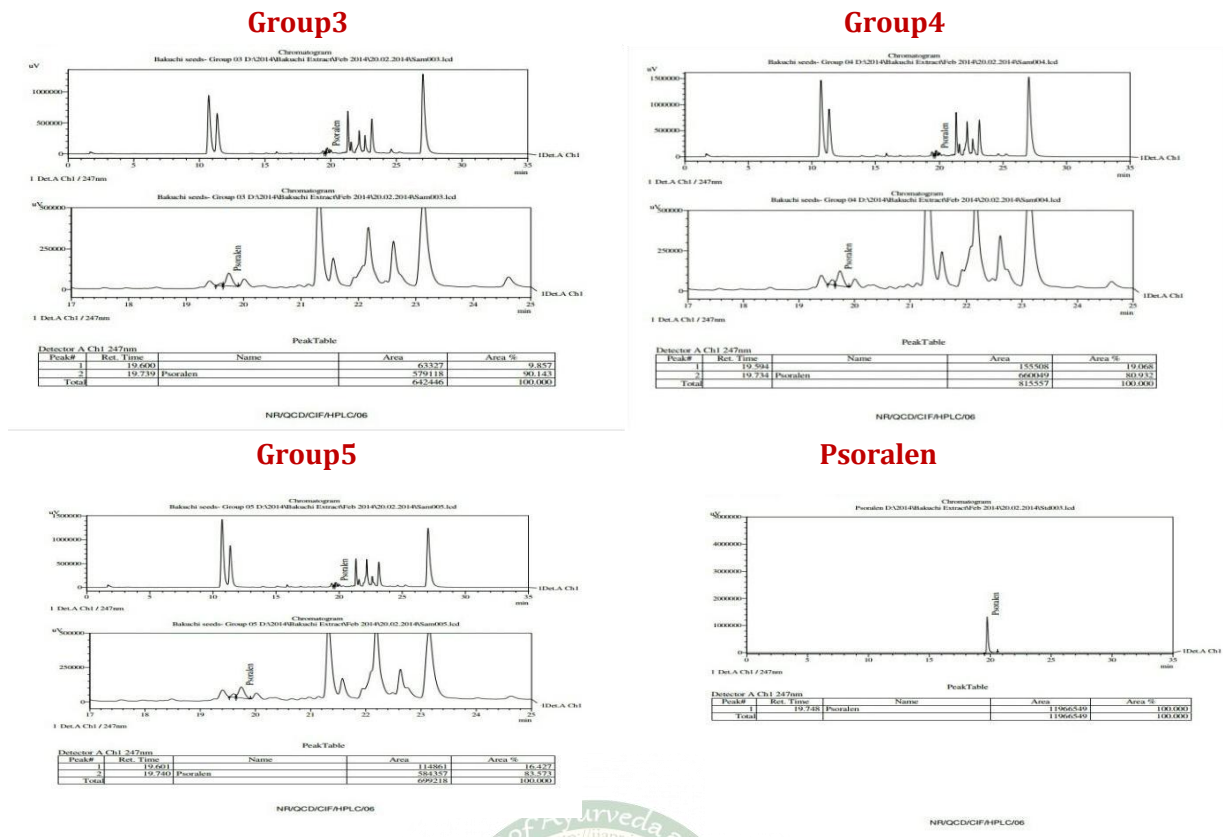
Table 8: Estimation of Psoralen in *Psoralea corylifolia*. Linn by HPLC from the plants grown by various treated seeds

S.No	Sample	Tests		Protocol
		Description	Psoralen (%w/w)	
1.	Group 1 (1402053E)	Dark Brown Dried Seeds	0.039	By HPLC
2.	Group 2 (1402054E)	Dark Brown Dried Seeds	0.022	
3.	Group 3 (1402055E)	Dark Brown Dried Seeds	0.023	
4.	Group 4 (1402056E)	Dark Brown Dried Seeds	0.027	
5.	Group 5 (1402057E)	Dark Brown Dried Seeds	0.024	

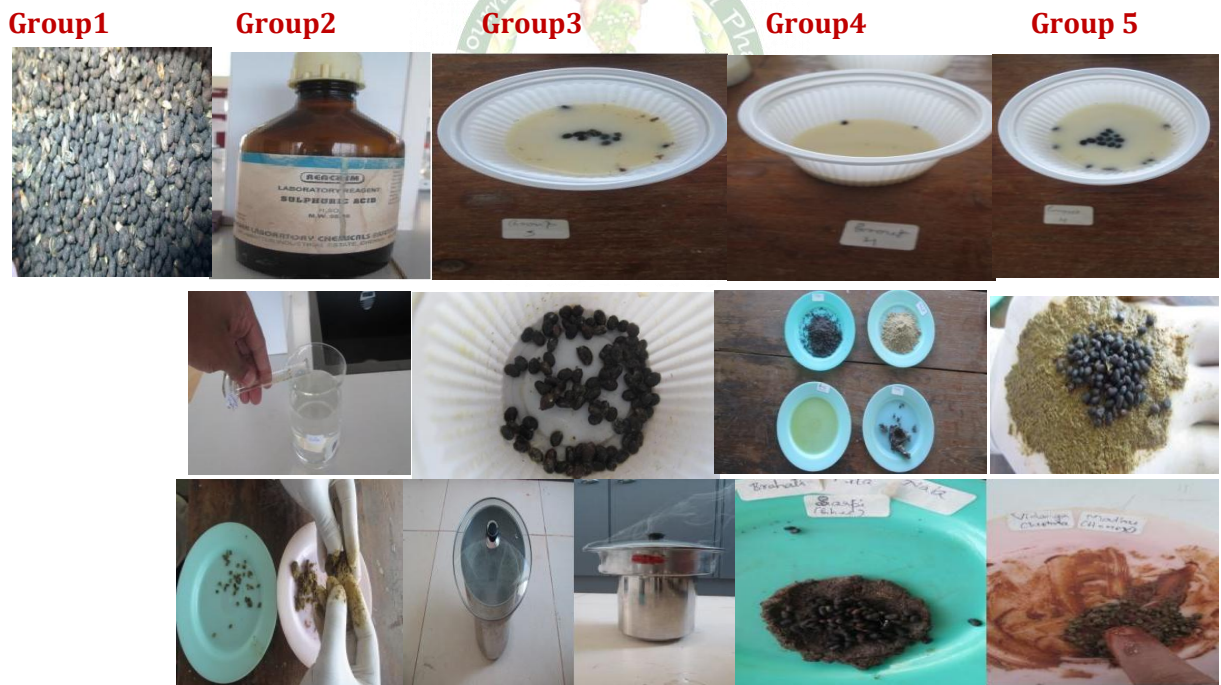


Graph 3: Comparison of estimation of Psoralen in *Psoralea corylifolia*. Linn by HPLC from the plants grown by various treated seeds





Picture Plate 2: Seed treatment techniques in different groups



Picture plate 1- Group 1 To Group 5 Cromatographs of Estimation of Psoralen in Psorali corylifolia Linn. by HPLC Method from the plants grown by various treated seeds

Picture Plate 3

Group 1

Group 2

Group 3

Group 4

Group 5

Germinated *Bakuchi* seedlings after 2



Germinated *Bakuchi* Plants after 8 months with flowers and seeds



DISCUSSION

The life cycle of plant begins with germination which in turn determines its survival. Ayurveda states that germination of seed depends on four factors *Rutu* (season), *Kshetra* (soil), *Ambu* (water), *Beeja* (seed). If all these four factors are good then the germination will be better, but if problem persists in one or more factors it may hamper the germination. Seeds are the delivery systems for agricultural biotechnology, and high levels of "field" performance (seed quality) are essential for predictable seedling establishment. High seed quality and seedling establishment can be considered as cornerstones of profitable, efficient and sustainable crop production (Finch-Savage, 1995). Dormancy (usually low) is an important component of physiological seed quality and so plants with a long history of domestication and plant breeding generally have a lower seed dormancy than wild or more recently domesticated species (Li & Foley, 1997; Copeland & McDonald, 2001; Benech- Arnold, 2004). However, dormancy can increase when germination takes place under stress (i.e. poor field conditions).

The conservation of this species is necessary as gene source, thus increasing seedling production. The various seed priming processes have been carefully designed in *Vrikshayurveda* to allow early germination, to obtain good quality of seedlings like soaking in milk, rubbing seed with cow dung, applying some organic medicines to seeds etc.

All seeds may not need all treatments so the quantity of ingredients and the duration of processing (fumigation/ soaking/ drying) may vary according to the need/requirement. Consequently

Standard Operative Procedures was developed for the same by doing three pilot studies before conducting actual experiment.

Hence on the basis of classical references and the previous researches it was essential to validate the *Vrikshayurveda* for safe and alternative methods to break the dormancy thus improving germination rate and to analyze the effect of *Vrikshayurveda* seed treatment techniques on active ingredient (Psoralen) of *Bakuchi* so this study was chosen.

The *Bakuchi* seeds have in built dormancy mechanisms which protect them from germinating before killing frosts or in times of drought. In wild or natural habitat, *Bakuchi* seeds lie dormant until the proper conditions for growth occur but in cultivation the successful gardener must become familiar with several simple pre-sowing seed treatment methods which will unlock the dormancy mechanism and stimulate quicker, more consistent germination.

An important use of psoralen is in PUVA treatment for skin problems such as psoriasis, eczema and vitiligo.^[14] Psoralen is found to have photocarcinogenic properties however psoralen although safe to mammals it should be used with care since many furocoumarins are extremely toxic to fish.^[15]

So Psoralen content must not be too high nor too low because if it is too low then may not exhibit its therapeutic action and if too high it may cause some adverse effects, from the experiment of present study we may conclude that *Vrikshayurveda* methods of seed treatment will balance the active ingredient content,

and Conventional methods and other two *Vrikshayurveda* seed treatment methods is not suitable for *Bakuchi* seeds as these will reduce the active ingredient of *Bakuchi*.

CONCLUSION

Overall results indicates that maximum germination percentage was observed in Group 4 (8.000 ± 0.8367) seeds soaked in 12 hrs milk followed by application of *Brihati*, *Tila*, *Kamala naala* and Ghee paste for 12 hrs treatment is statistically significant (P value > 0.05) in comparison with group 2 (4.600 ± 0.6782) Sulphuric acid treatment and group 3 (4.200 ± 0.9165) fumigation with honey and *Vidanga*.

Rests of the groups have shown insignificant changes on germination parameters. HPLC Results found that generally seed treatments may reduce the content of Psoralen as in control (Group 1) maximum percentage (0.04%w/w) of Psoralen is noticed. Among treatment groups Group 4 contains maximum (0.027%w/w) Psoralen next to control (0.039% w/w). Psoralen content is very less in standard Group 2 (0.022%w/w), Group 3 (0.023% w/w) and Group 4 (0.024%w/w).

Estimation of Psoralen contents in the seeds from the plants grown by various treated seeds revealed that, group 4 (i.e. seeds soaked in 12 hrs milk followed by application of *Brihati*, *Tila*, *Kamala naala* and ghee paste for 12 hrs) is qualitatively better than standard, but inferior to the control. However, the method has the added advantage of safety over the standard (acid treated) group. Seed treatment may reduce the Psoralen content in seeds.

Present study revalidates the germination behavior of dormant seeds of *Psoralea corylifolia* Linn. Dormancy factor is present in these seeds and must be counteracted to obtain prompt germination. From the obtained results it can be recommended to treat the seeds of *Bakuchi* by soaking in milk for 12 hrs, drying followed by application of *Brihati*, *Tila*, *Kamala naala* and ghee paste for 12 hrs for safe and better germination, in comparison with the conventional method of treating with sulphuric acid.

Group 4 is qualitatively better than standard and other *Vrikshayurveda* seed treatment techniques used in the current experiment. *Vrikshayurveda* seed treatment techniques may be thus better than the conventional seed treatment technique.

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***Address for correspondence**

Dr Gangadhar T. Aralelimath

Associate Professor,
Department of Dravyaguna,
Shri Jagadguru Gavisiddheshwara
Ayurveda Medical College, Hospital & P G
Studies, Koppal, Karnataka, India
Email: gangadharswamy@gmail.com

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