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The Study of Pharmacological Efficacy in Dry Mulberry Burirum 60 in Mild Stage of Alzheimer's Disease

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Abstract

Alzheimer's Disease is a condition in which occurs in the area concerning the deteriation of brain function. This condition of disease is not completely curative treatment by medicines and always happens in the eldery with ages over 55 years old. However it can delay and maintain the condition of the stages of deteriation of brain function. In this experiment. We used *Morus alba* Linn. Burirum 60 leaves collected from Mahasarakham University Research Plantation Field, the Division of Research Innovation and were prepared as a powder in capsule form to control the incidence and maintain brain function condition in Mild stage of Alzheimer's disease patients. *Morus alba* Linn. contains many minerals and vitamin A, vitamin B, vitamin B3, vitamin B12, vitamin C, essential amino acids, Zinc, Magnesium, Sodium, deoxynojirimycin, GABA, Phytosterol, Calcium, Potassium, folic acid etc.. The samples of this research were collected from silk weaving women, ages of 55-70 years old in Silk Innovation Weaving Village, supported by Mahasarakham University. The samples were divided into 4 groups as follows: group no.1 of feeding silkworms weaving women and intake *Morus alba* Burirum 60 capsule, group no.2 of feeding silkworms weaving women and intake placebo, group no.3 of non feeding silkworm weaving women and intake *Morus alba* Burirum 60 capsule, group no.4 of non feeding silkworms weaving women and intake the placebo. All samples were tested with Cognitive Testing Scale and the selected samples were Mild cognitive memory function group. The dosage *of Morus alba* capsule would have 2 capsules after meals, once daily for 3 months and all the samples were tested for cognitive memory function using the scales of Sage1, Mini-Mental state Examination (MMSE), Functional Assessment Stage tool (Fast) for evaluation and confirmation of the efficacy of Mulberry (*Morus alba* Burirum 60) effecting the brain function. The data were tested with Wilcoxon Signed Rank Test and Kruskal Wallis statistical analysis. The result revealed that each 4 groups of Group no.1, no.2, no.3, and no.4 were 3.00, 1.50, 3.00 and 2.00 respectively. It showed that in each group had indicated mean scores with statistical significant difference at *p-value 0.05*. It showed the development of changing scores tested by SAGE1 scale within the group. Then we compared the mean scores difference among or in between groups by using the Kruskal Wallis statistical method. The result found that the mean rank score among 4 groups before the experiment were 14.10, 12.70, 7.30 and 7.90 respectively and the mean rank score among 4 groups after the experiment were 17.70, 8.70, 11.00 and 4.60 respectively. The higher rank of each groups indicated the higher development in obtaining the scores of SAGE1 tested by Kruskal Wallis after the experiment by oral intake of *Morus alba* Linn. Burirum-60 capsules with the dosage of 200 mg daily for 3 months. It showed the development of cognitive memory function by increasing the ranking scores of group no.1, group no.3 and group no.2 respectively. The suggestion of this study for the future research is to increase the size of samples and clinical confirmed with CT-SCAN or NMR in order to measure the comparison of improvement in the density and brain image before and after the experiment and also the duration of administering or intake Morus alba Linn. Burirum 60 should be recommended to extend longer period of time and may be increase in the daily dosage.

Key words: Mild cognitive memory impairment; MMSE; SAGE1; FAST; Alzheimer disease

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1. RESEARCH METHODOLOGY

The Screening Stage of 625 Silk Weaving Village

The population was 625 silk village women in Ban Thamuang, Amphor Selaphum, Roet Province, Thailand. The 125 samples were selected purposively by screening and testing with MMSE Scale (Mini-Mental State Examination) in order to quantify cognitive function cognitive loss. The samples used in this experiment were the persons who were determined as Mild Cognitive Memory Impairment. The samples were female, of age range between 50-70 years old, without administered of any medication, no other complication of diseases especially brain-function diseases. MMSE was the scale to test the individual orientation, attention, calculations, recall, language and motor skills. Each section of the test involved a related series of questions or commands. The individual received one point for each correct answer. The result of screening by MMSE were obtained and the groups of samples who had been received the scores between 20-25 would be considered as Mild Cognitive Impairment for experimental and control groups. The samples of this experiment were randomized control trial selected for 20 women and divided into 4 groups as follows: (1) Silk Worm Feeding Weaving Women treated with *Morus alba* Linn. (Mulberry Burirum 60); (2) Silk Worm Feeding Weaving Women treated with Placebo; (3) Non Silk Worm Feeding Weaving Women treated with *Morus alba* Linn. (Mulberry Burirum 60); (4) Non Silk Worm Feeding Weaving Women treated with Placebo. After all samples were selected, then they also were reconfirmed with FAST Scale (Functional Assessment Staging of Alzheimer's disease Test) to indicate that all the samples showed Mild Cognitive Impairment Patients.

2. PREPARATION OF MULBERRY BURIRUM 60

2.1 The mulberry leaves (Burirum 60) was selected from Silk Innovation Center, Division of Research Dissemination and facilitation, Mahasarakham University, Thailand in August 2011.

2.2 The leaves of mulberry were collected from the top of the mulberry stem to the leaf at level of 5th position from the top in order to contain the high concentration of chemical constituents.

2.3 Mulberry leaves were cleaned with purified water to remove the risks of contamination such as contamination of possible insecticides, pesticides, heavy metals, fertilizers, bacteria or other foreign bodies. Then they were washed twice with the purified water.

2.4 The cleaned mulberry Burirum 60 leaves were dried in a vacuum oven at 50^{o}C for 48 hours prior to extraction of flavonoid and carotinoid antioxidant and also other vitamin contents in *Morus alba* Linn. Burirum-60. After the specific drying time with spray drying technique, then dried mulberry leaves were comminuted to fine powder. Mulberry fine powder was transferred to be encapsulated in aseptic capsules as mulberry capsule for clinical study. The capsules were very convenience for administration orally.

2.5 All capsules also were standardized to obtain the required quality of herbal medicine from Thai FDA, Ministry of Health and lastly were passed Gamma ray to assure aseptic condition.

3. CLINICAL STUDY

All 4 sample groups of 20 Mild Cognitive Memory Impairment function Silk village women which were (1) Silk Worm Feeding Weaving Women treated with *Morus alba* Linn. (Mulberry Burirum 60); (2) Silk Worm Feeding Weaving Women treated with Placebo; (3) Non Silk Worm Feeding Weaving Women treated with *Morus alba* Linn. (Mulberry Burirum 60); (4) Non Silk Worm Feeding Weaving Women treated with placebo capsules. Dosage of *Morus alba* Linn. Burirum 60 was 200 mg daily, after meal. The duration was period of 3 months.

3.1 Before introducing of *Morus alba* capsules for group samples no. (1) and no. (3) then also introducing of the placebo capsules in each groups of samples no. (2) and no. (4) as mentioned above, the subjects were educated with the health behavioral patterns in the frame of dietary, free from other vitamins, minerals and other medications by the group of researchers and following up with the Health Volunteers at Saelaphum Health Station during experiment for 3 months. All samples were tested for Cognitive Brian Function Scale with SAGE1 in order to collect the scores of each groups of total 4 groups before the experiment and after the experiment of introducing *Morus alba* Burirum 60 and Placebo for period of 3 months by using SAGE1 Scale.

3.2 The scores of SAGE 1 were collected and has been interpreted in each of samples group before and after the experiment followed the instruction of SAGE1 Scale. After using SAGE1 Scales for evaluating the stage of cognitive function before and after the experiment, we also used FAST Scale (Functional Assessment Staging of Alzheimer's disease Test) for confirmation of the test.

4. STATISTICAL ANALYSIS METHOD

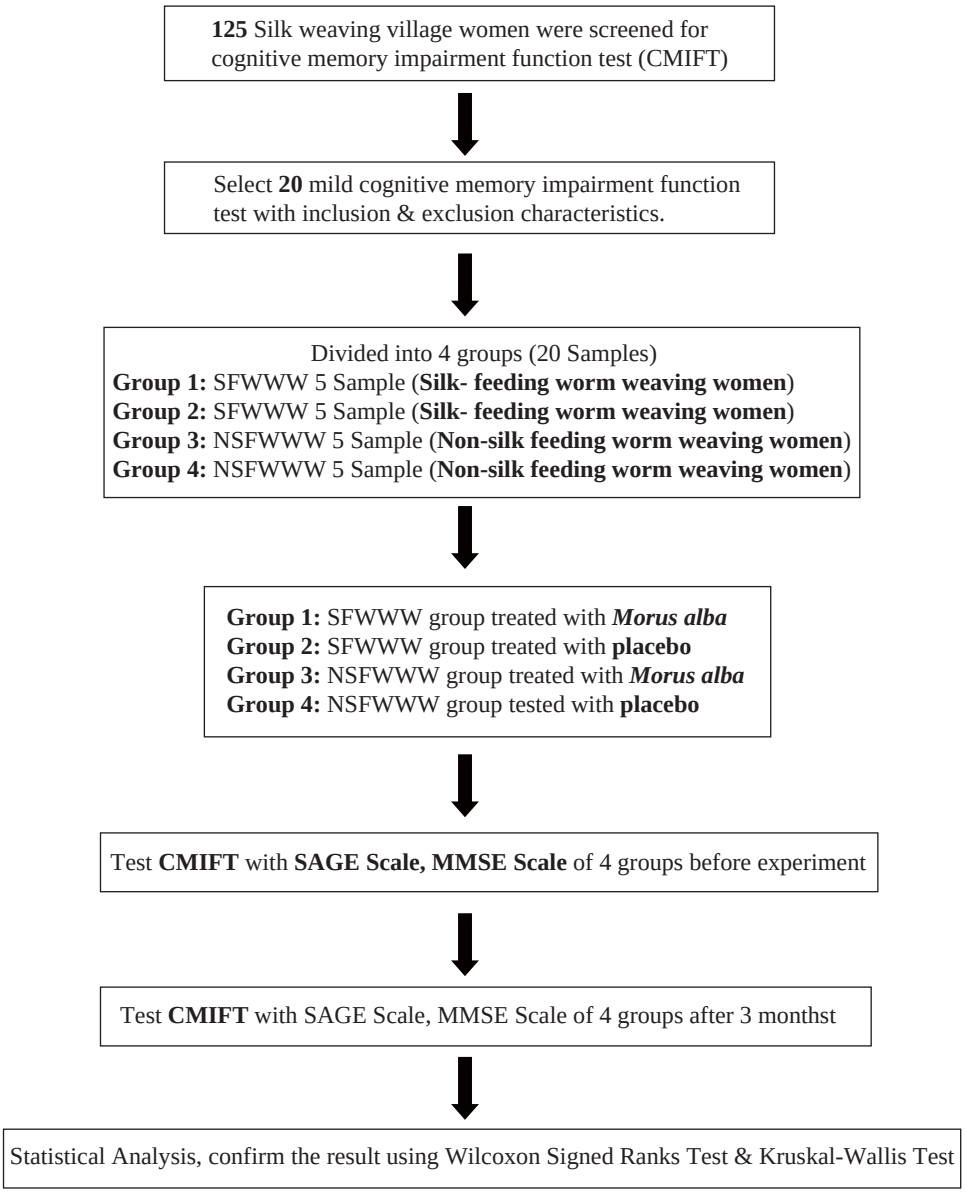
In this experiment, the statistical analysis used were Wilcoxon Signed Ranks test and Kruskal Wallis method for

quantitative comparison of each mean scores from each 4 groups and also to identify the hypothesis of 1. Mild cognitive impairment memory patient will or will not have the better condition or maintaining the condition of cognitive memory after administering *Morus alba* Linn. Burirum 60 orally for 3 months period with dosage of once a day, 200 mg after meal; 2. Silk-worm feeding Weaving women will have the tendency of not having Alzheimer disease more than normal people. Normally the weaving women who feed the silkworm with mulberry leaves. All conclusions of scores would follow the instructions and criteria of SAGE1, MMSE Scale, and FAST Scale. Therefore the result of this study would reveal the comparative analysis in efficacy of *Morus alba* Linn. Burirum 60 in maintaining, preventing, or improving the condition of Mild Cognitive Memory Impairment of the patients among the 4 groups of samples in this experiment.

5. RESEARCH FRAMEWORK

Research Methodology

Research Methodology



6. RESULTS AND CONCLUSIONS

The Screening Stage of 125 Silk Weaving Village Women

All questionnaires were translated to Thai language and 2 pictures were changed to Thai Cultural style of picture in which the samples could understand and interpret easily. The researchers have explained the method of answering step by step before starting the MMSE test and Fast test and followed the regulations of examination procedures strictly in order to find out mild cognitive memory function impairment samples. The result after screening showed that 90 Silk weaving village women were normal, 25 Silk weaving village a women were mild cognitive memory impairment and 10 Silk weaving village women were moderate cognitive memory impairment function and the rest were in exclusion criteria characters. Then 25 of mild cognitive memory function impairment samples were confirmed for the inclusion criteria and were finalized as 20 samples of mild cognitive memory impairment function.

Mini-Mental Status Examination (MMSE)

The Mini-Mental Status Examination offers a quick and sample way to quantify cognitive function and screen for cognitive loss. It tests the individual orientation, attention, calculation, recall, language and motor skills. Each section of test involves a related series of questions or commands. The individual receives one point for each correct answer during examination of a control quiet environment, well-lit room, asking each of 4 testing sample groups to listen carefully and answering each questions as accurately as possible. The maximum score is 30 points. If the received scores from each person in each sample groups is below 25-30 the samples will be considered still questionably significant and may have clinical signs of cognitive Impairment present but mild deficits only most demanding activities of daily living. If the received scores is 20-25, the sample will be considered as Mild cognitive impairment condition. Significant effect may be required some supervision, support and assistance. If the received scores is below 10-20, the samples will be considered as Moderate degree of Cognitive Impairment. Formal assessment may be required to help to better determine pattern and extent of deficits. Significant effect may be required 24-hour supervision. If the scores are below 10-0, the patients will be marked for impairment and required 24-hour supervision and assistance with ADL.

Functional Assessment Staging of Alzheimer diseases (FAST)

Fast Scales also had been used for screening and evaluating before and after experiment along with Sage1 Scale and MMSE Scale. Fast Scale consists of 1, 2, 3, 4, 5, 6a, 6b, 6c, 6d, 6e, 7a, 7b, 7c, 7d, 7e, 7f, stages which are 16 stages categories in screening and measuring stage of Alzheimer diseases. In this experiment, we used patients in stage of 2, 3, 4, 5 which are indicated or considered as Normal Older Adult - some functional decline, Early Alzheimer's disease noticeable deficits in demanding job situations, Mild Alzheimer's assistance needed for complex tasks respectively.

7. CONCLUSIONS AND SUGGESTIONS

7.1 The result was collected from 4 Mild Cognitive Memory Impairment groups before and after the experiment (of introducing oral medicating of *Morus alba* Linn. Burirum 60) capsules and Placebo by using the SAGE1 Scale and MMSE Scale for cognitive memory testing. The data of SAGE1 scores was tested with Wilcoxon Signed Ranks Test from each 4 groups of Group no.1, no.2, no.3, and no.4 were 3.00, 1.50, 3.00 and 2.00 respectively. It showed that in each group had indicated mean scores with statistical significant difference at *p-value 0.05*. It showed the development of changing scores tested by SAGE1 scale within the group. Then we compared the mean scores difference among or in between groups by using the Kruskal Wallis statistical method. The result found that the mean rank score among 4 groups before the experiment were 14.10, 12.70, 7.30 and 7.90 respectively and the mean rank score among 4 groups after the experiment were 17.70, 8.70, 11.00 and 4.60 respectively. The higher rank of each groups indicated the higher development in obtaining the scores of SAGE1 tested by Kruskal Wallis after the experiment by oral intake of *Morus alba* Linn. Burirum 60 capsules with the dosage of 200 mg daily for 3 months. It showed the development of cognitive memory function by increasing the ranking scores of group no.1, group no.3 and group no.2 respectively. It showed that there were the increasing of cognitive memory function of the samples of Silkworm Feeding Weaving Women with intake of *Morus alba* Linn. Burirum 60, Non Silkworm Feeding Weaving Women with intake of *Morus alba* Linn. Burirum 60 and Silkworm Feeding Women with intake of Placebo.

7.2 The confirmation test of cognitive memory function was continued by using the MMSE Scale to indicate the effect of *Morus alba* Linn. Burirum 60 on the memory function of the 4 groups of Mild cognitive memory impairment function patients. The comparison within group and in between 4 groups were repeated to confirm the result by using the statistical analysis of the Wilcoxon Signed Ranks test and Kruskal Wallis test. The result of Wilcoxon Signed Ranks test showed the difference in mean sore rank of within each group of no.1, 2, 3 and 4 were 2.00, 1.00, 3.00, and 1.00 respectively. Therefore the conclusion of using MMSE Scale test could show that there were the improvement of cognitive memory function after the experiment with intake of *Morus alba* Linn. Burirum 60 to groups of samples which showed the

significant statistical difference at p-value 0.05. In addition, the result in between 4 groups were also calculated by Kruskal Wallis statistical Method which the mean ranks before the experiment of groups no.1, no.2, no.3, and no.4 were 17.40, 10.50, 6.70 and 7.40 respectively and the mean ranks after the experiment of groups no.1, no.2, no.3, and no.4 were 17.60, 10.10, 8.50 and 5.80 respectively. Therefore, it showed the increasing of MMSE score of group. no.1, group no.3 and group no.2 respectively. However, there was the repetition of confirmation in data statistical analysis by using the difference value of the NPar Kruskal-Wallis test which the highest mean rank was 14.80 from group no.3 and the second highest was 11.60 from group no.1, the third mean rank was 8.40 from group no.2, and the lowest mean rank was 7.20 from group no.4. Therefore the conclusion of this study would indicate that the development of increased the cognitive memory function which had a higher score in the group of intake of *Mourus alba* Linn. Burirum 60 in Silk Weaving Women at Ban Tha Maung Silk Village, Selaphum, Roiet, Thailand.

The result also showed that there should have more data to assure the different in efficacy of *Morus alba* in comparison of Feeding Silkworm Weaving Women and Non-Feeding Silkworm Weaving Women at Ban Tha Maung Silk Village, Salaphum, Roiet, Thailand.

The suggestion of this study for the future research is to increase the size of samples and clinical confirmed with CT-SCAN or NMR in order to measure the comparison of improvement in the density and brain image before and after the experiment and also the duration of administering or intake *Morus alba* Linn. Burirum 60 should be recommended to extend longer period of time and may be increasing in the daily dosage.

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The Result Scores of 4 Sample Groups Tested by SAGE I

Table 1
Before Experiment (Without Administering Rral *Morus alba* Burirum 60) SAGE 1 Score Before the Experiment

Questions of Sage I test format /(total score)	Average score of group No. 1 SWFWW with *Morus alba*	Average score of group No.2 SWFWW with (Placebo)	Average score of group No. 3 NSWFWW with *Morus alba)*	Average score of group No.4 NSWFWW with placebo
1. General/ (total score=4)	2	3	3	3
2. Name of pictures/(2)	2	2	2	2
3. Name of similarities/(2)	1	1.5	1	1
4. Calculations / (1)	1	1	1	1
5. Calculation / (1)	0	0	0	1
6. Memory questions/(12)	1	1	0	0
6.1Building 3 dimensions/(2)	0	1.5	1	0
6.2 Building clock images/(2)	1	1	1	1
6.3 Easy vocabulary/(2)	1.5	0.2	0.8	0.5
6.4 Management/(2)	1.5	1	1	0.5
6.5 Finish score/(2)	1.6	1	1	1
6.6 Memory/(2)	2	1	1	2
Total score = 0 - 22 / (min-max)	14.6	14.2	12.8	13.0

Table 1. Sage I scale before the experiment
Total score = 22 scores
Maximum score = 22 below or equivalent of 17 considered as Cognitive memory Impairment

The Result Scores of 4 Sample Groups Tested by SAGE I

Table 2
After the Experiment (with Administering Oral *Morus alba* Burirum 60)

Questions of Sage I test format /(total score)	Average score of group No. 1 SWFWW with *Morus alba*	Average score of group No.2 SWFWW with (Placebo)	Average score of group No. 3 NSWFWW with *Morus alba)*	Average score of group No.4 NSWFWW with placebo
1. General/ (total score=4)	3	3	3	3
2. Name of pictures/(2)	2	2	2	2
3. Name of similarities/(2)	1.6	1	1	1
4. Calculations / (1)	1	1	1	0
5. Calculation / (1)	1	0	1	1
6. Memory questions/(12)				
6.1 Building 3 dimension)2	1	1	1.4	0
6.2 Building clock images/(2)	1	1	1	1
6.3 Easy vocabulary/(2)	1	1	1	1
6.4 Management/(2)	2	1.8	1	1.6
11. Finish score/(2)	2	1	1	1
12. Memory/(2)	2	2	2	2
Total score = 0 - 22 / (min-max)	17.6	14.8	15.4	13.6

Table 2. Sage I Scales after the experiment
Total Score = 22 scores
Maximum score = 22 below or equivalent of 17 considered as Cognitive Memory Impairment

Result Score of 4 Sample Groups Tested by MMSE Scale

Table 3
(MMSE Score Before the Experiment)

Questions of MMSE test scale/(total score)	Test full score	Average score of group No. 1	Average score of group No. 2	Average score of group No. 3	Average score of group No.4
1. Orientation to time	5	3	3	2.5	2.5
2. Orientation to place.	5	3	2	2.5	2.5
3. Immediate recall	3	2	2	1.5	1.0
4. Attentions	5	3	2	2	2.6
5. Delay verbal recalls	3	1.8	1.8	1.5	2.0
6. Naming	2	2	2	1.4	1.0
7. Repetition	1	1	0	1	1
8. 3 Stage command	3	2	2	1	1
9. Reading	1	0	1	1	1
10. Writing	1	0	0	0	0
11. Copying	1	1	1	1	1
Total scores	30	18.8	16.8	15.4	15.6

Table 3. Total score or maximum score =30 below 20 is considered as Cognitive memory Impairment.
MMSE Scores before the experiment.

Result Score of 4 Sample Groups Tested by MMSE Scale

Table 4
(MMSE Score After the Experiment)

Questions of MMSE test scale/(total score)	Test full score	Average score of group No. 1	Average score of group No. 2	Average score of group No. 3	Average score of group No.4
1. Orientation to time	5	4	3	2.5	2.5
2. Orientation to place.	5	4	3	3	2
3. Immediate recall	3	2	2	2	2
4. Attentions	5	2.8	3	2.5	2
5. Delay verbal recalls	3	2	2	2.1	1.5
6. Naming	2	1	2	1	1.8
7. Repetition	1	1	1	2	1
8. 3 stage command	3	2	1	1	1
9. Reading	1	1	1	1	1
10. Writing	1	0	0	1	1
11. Copying	1	1	1	1	1
Total Scores	30	20.8	19	17.6	15.8

Table 4. Total score or maximum score = 30 Below 20 is considered as Cognitive memory Impairment. (After administering *Morus alba* Linn. Burirum-60)
MMSE Scale before the experiment.

Statistical Analysis

Table 5
MMSE Scores Among 4 Groups Before the Experiment by Confirmation of Kruskal Wallis Test

Ranks		
Group	**N**	**Mean Rank**
Before 1.00	5	17.40
2.00	5	10.50
3.00	5	6.70
4.00	5	7.40
Total	20	

Test Statistics [a,b]	
	Before
Chi-Square	10.473
df	3
Asymp. Sig.	.015
a. Kruskal Wallis Test	b. Grouping Variable: Group

Table 6
MMSE Scores Among 4 Groups After the Experiment by Confirmation of Kruskal Wallis Test

Ranks		
Group	**N**	**Mean Rank**
After 1.00	5	17.60
2.00	5	10.10
3.00	5	8.50
4.00	5	5.80
Total	20	

Test Statistics [a,b]	
	After
Chi-Square	11.813
df	3
Asymp. Sig.	.008
a. Kruskal Wallis Test	b. Grouping Variable: Group

Table 7
The Result Showed the Difference of Before and After Scores,Therefore, We Confirmed with the Scores Difference for NPAR Tests for Evaluating the Mean Ranking by Kruskal Wallis Test

Ranks		
Group	**N**	**Mean Rank**
DIFF 1.00	5	11.60
2.00	5	8.40
3.00	5	14.80
4.00	5	7.20
Total	20	

Test Statistics [a,b]	
	DIFF
Chi-Square	5.996
df	3
Asymp. Sig.	.112
a. Kruskal Wallis Test	b. Grouping Variable: Group

Figure 1
***Terminalia Chebula* (Retz) (SamaoThai)**
URL http://www.mpbd.info/plants/terminalia-chebula.php

Figure 2
***Curcuma Zedoaria* (Berg) Rosco. (Kamin Aoi)**
URL http://blog.naver.com/PostView.nhn?blogId=bbe8&logNo=120111302564&redirect=Dlog&widgetTypeCall=truehttp://blog.naver.com/PostView.nhn?blogId=bbe8&logNo=120111302564&redirect=Dlog&widgetTypeCall=true http://blog.naver.com/PostView.nhn?blogId=bbe8&logNo=120111302564&redirect=Dlog&widgetTypeCall=true

Figure 3
***Croton Tiglium* Lin. (Purging croton)**
URL http://www.payer.de/amarakosa/amara206.htm http://blog.naver.com http://blog.naver.com/PostView.nhn?blogId=bbe8&logNo=120111302564&redirect=Dlog&widgetTypeCall=truehttp://blog.naver.com/PostView.nhn?blogId=bbe8&logNo=120111302564&redirect=Dlog&widgetTypeCall=truehttp://blog.naver.com/PostView.nhn?blogId=bbe8&logNo=120111302564&redirect=Dlog&widgetTypeCall=true

Figure 4
***Diospyros Mollis* (Griff.) (Ebony Tree)**
URL http://www.biogang.net/biodiversity_view.php?menu=biodiversity&uid=898&id=3524

Figure 5
Mebendazole
URL http://www.stanford.edu/class/humbio103/ParaSites2006/Enterobius/

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