# Nutritional attributes of hemiparasitic mistletoe *Scurrula ferruginea* in Brunei Darussalam

*Tang Yuan Pin[1*], Linda B. L. Lim[1] and Kushan U. Tennakoon[2]*

*[1]Department of Chemical Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE1410, Brunei Darussalam*
*[2]Department of Biological Sciences, Faculty of Science, Universiti Brunei Darussalam, Jalan Tungku Link, Gadong, BE1410, Brunei Darussalam*

**corresponding author email: yuan_pin86@hotmail.com*

**Abstract**

Three different associations of *Scurrula ferruginea* parasites on three different hosts, namely *Tabebuia pallida*, *Acacia holosericea* and *Acacia auriculiformis* were collected from the Brunei-Muara District, Brunei Darussalam. Moisture content and chemical analyses (ash content, total carbohydrate content, crude protein, proline and mineral content composition) were determined to explain the host-parasite physiological biochemistry. *Scurrula ferruginea* contained relatively higher moisture content (47-65%) and ash content (2.1-2.5%, dry basis) than the hosts (0.7-1.4%, dry basis). High nutrient and moisture contents in *Scurrula ferruginea* make it more preferred food source than its hosts for generalist herbivores in a given community. The mistletoe exhibited differential storage profile of total carbohydrate (1.9-6.4%, dry basis) and total nitrogen (1.2-3.0%, dry-basis) when compared to hosts (total carbohydrate 2.3 - 3.0 % dry basis; total nitrogen 1.6-2.1%). Meanwhile the proline content was found to be in the range of 24.9-56.0 mg/kg, dry basis, in *Scurrula ferruginea*. Among all the minerals analysed, potassium was the most abundant mineral present in all mistletoe-host associations. Data indicated that certain host desired solutes are preferentially absorbed and stored in mistletoe.

*Index Terms:* mistletoe-host associations, parasitic plants, solute processing, plant nutrients

## 1. Introduction

Mistletoes are angiosperm obligate stem hemiparasites that inhabit branches (shoots) of completely independently growing woody trees or shrubs. Mistletoes do not produce functional roots and their photosynthetic efficiency is usually much inferior to other angiosperms. Hence, they are adapted to withdraw almost all water requirements and at least part of their nutritional requirements (by means of organic and inorganic solutes) from the hosts they parasitize.[1,2]

Studies conducted on water relations and nutrition of a range of temperate and Mediterranean mistletoe-host associations have shown that the potassium and proline contents of mistletoes were the highest among other major nutrients and osmotically active solutes, thus playing a vital role in osmotica of the parasite.[1,3-9] However, the phytochemistry of tropical mistletoes remains unclear due to paucity of studies except for a few studies conducted on *Scurrula ferruginea*[10,11], *Scurrula oortiana*[12], *Dendrophthoe falcata*[13] *Tapinanthus lugardii*, *Erianthenum ngamicum*, *Viscum rotundifolium*, and *Viscum verrucosum.*[14]

*Scurrula ferruginea* Danser [syn.: *Loranthus ferruginea* Roxb. (Loranthaceae)] attaches itself to a host by highly modified root known as the haustorium. Water and solutes are transported to the mistletoe thorough direct xylem-xylem or parenchyma cells connection present in the haustorium. Many countries including Malaysia, Indonesia, Australia and New Zealand have reported its distribution.[15] This species has been widely used in traditional medicine for the

treatment of hypertension and gastrointestinal complaints, gerontological effect and other therapeutic uses such as for treatment of ulcers and cancer.[16]

Huaxing and Michael[17] reported that the recorded hosts for *Scurrula ferruginea* include *Citrus grandis*, *Ficus hispida*, *Phyllanthus emblica* and *Prunus salicina*. Here we attempt to identify the solute partitioning and flux interaction between this tropical mistletoe and its common hosts. By comparing these variations among mistletoe-host partnerships with a single host we hope to reveal the selective manner in which nutrients are absorbed, transported and partitioned in tropical mistletoes where results are scarce.

## 2. Experimental approach

*Samples*

This study was conducted on three *S. ferrruginea*-host associations, viz: *S. ferruginea-Tabebuia pallida*, (Lindl.Miers, Bignoniaceae), *S. ferruginea- Acacia holosericea* (A. Cunn. ex G. Don, Mimosoideae) and *S. ferruginea- Acacia auriculiformis* (A. Cunn. ex Benth, Mimosoideae). These associations were grown in edges of heath forest patches (N 04˚55, E 114˚56) in Brunei Darussalam. The samples were collected where mistletoes have successfully established on host trees. From the leaves of the parasite, and leaves from parasitized hosts and non-parasitized hosts were collected and stored in the freezer for further analyses.

*Chemicals*

Phenol (Sigma-Aldrich), glucose (Fluka), concentrated sulfuric acid (Merck), sodium hydroxide pellets (Merck), boric acid (BDH), Concentrated hydrochloric acid (Merck), ninhydrin (Merck), 2-propanol (Fluka), proline (Sigma), concentrated nitric acid (Ajax), formic acid (Fluka), ethylene glycol monomethyl ether (Riedel-de Haën).

*Instruments*

VIRTIS Specimen Freeze Dryer, Memmert Laboratory Oven, GALLENKAMP muffle furnace, Shidmadzu UV-1601PC UV spectrophotometer, GERHARDT automated digestion system and distillation system and Shidmadzu AA-6701F atomic absorption spectrometer.

*Sample preparation*

Two types of drying methods were used: oven-drying and freeze-drying. For oven-drying, leaf samples were dried at 45 °C until a constant weight was attained. While for freeze-drying, samples were freeze-dried using VIRTIS Specimen Freeze Dryer. Each analysis was carried out in duplicates. Dried samples were separately weighed and the change in mass of each samples were used to determine the moisture content.

*Sample extraction*

The leaf samples collected were cleaned to remove any dirt on the leaf surface. The slightly modified AOAC Official method 920.149[18] was used. Fresh leaf samples (about 10.00 g) from the parasite, parasitizing host and non-parasitizing part of each tree were blended with doubly distilled water (100 mL) at room temperature and the mixture was gravity filtered. The filtrate was then kept in the freezer prior to analysis. These sample extracts in duplicates were used for total carbohydrate and proline contents.

*Determination of ash content*

Fresh samples (about 1.00 to 2.00 g) were placed in the GALLENKAMP muffle furnace and ignited to 600 ˚C and maintained at this temperature for about 4 to 6 hours until samples turned white due to complete combustion. The change in mass was used to determine the ash content of the samples. The ash was subsequently used for the determination of mineral nutrient analyses. Each sample was carried out in duplicates.

*Determination of total carbohydrate*

The phenol-sulfuric acid method (AOAC Method 44.1.30), as stated in the Food Analysis Laboratory Manual[19] with slight modifications, was used for the determination of total carbohydrate content in leaf samples. A series of 6 calibration standard glucose solutions was prepared from the glucose stock solution with the concentrations ranging from 0 µg/2 mL to 100

µg/2 mL. The absorbance of each of the solutions was then measured using a Shidmadzu UV-1601PC UV spectrophotometer at wavelengths between 450-550 nm. The standard solution with 0 µg/2mL was used as reference.

The absorbance for each of the standard and sample solutions was recorded at 490 nm to plot a graph of absorbance against different concentrations of glucose. Each sample was carried out in duplicates.

*Determination of total nitrogen*

Total nitrogen was determined by using the standard block digestion Kjeldahl method.[18,20-21] Protein content in both freeze-dried and oven-dried leaf samples was determined using GERHARDT automated digestion and distillation systems. Each sample was carried out in duplicates.

*Determination of proline*

The method was based on the original method of Ough.[22] The blended sample solutions, as described in Section 4.3, were used for the determination. The absorbances were measured using the Shidmadzu UV-1601PC UV/VIS spectrophotometer at wavelength between 400 and 800 nm. The absorbance at 510 nm was recorded for each of the tubes. The proline content was determined from the ratio of the sample solution and the proline standard solution at the wavelength of 510 nm. Each sample was carried out in duplicates.

*Determination of minerals*

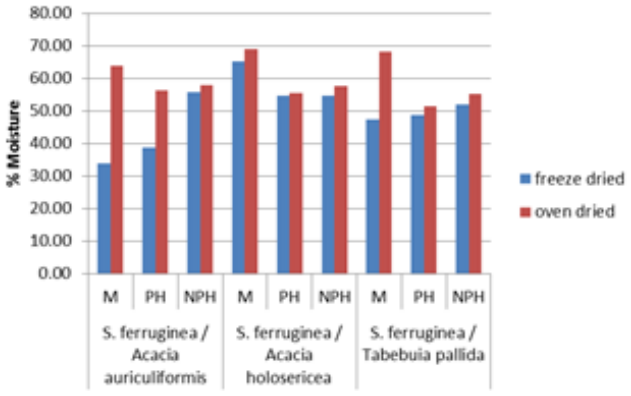
Mineral determinations were carried out in duplicate. The ash obtained was dissolved in concentrated hydrochloric acid (2.5 mL) followed by concentrated nitric acid (2.5 mL). The mixture was then left at room temperature until all the ash had dissolved. Each replicate mixture was diluted to 50 mL using ultrapure water respectively. The solution was kept in the refrigerator until further analysis using Shidmadzu AA-6701F atomic absorption spectrometer. Each sample was carried out in duplicates.

*Statistical Analyses*

All data presented are means of two determinations. Statistical analysis was done using ANOVA single factor, Microsoft Excel 2010 Data analysis Program. The level of statistical significant was set as $p < 0.05$.

## 3. Results and Discussion

There is a significant difference in the moisture content obtained using both freeze drying method and oven drying method ($p<0.05$). Moisture content data obtained in duplicates by freeze-drying method showed lower values than oven drying method (***Figure 1***). This might be probably due to the presence of volatile compounds in the leaves that vaporize when temperature was increased beyond 45 °C. Volatile compounds such as esters, terpenes and alcohols are commonly present in plant matter and can be responsible in making the cell sap of mistletoe more concentrated than host thus allowing the mistletoe to absorb host derived water and dissolved solutes along a favorable water potential gradient. General pattern of high moisture content shown in mistletoe indicates that they are more palatable for generalist herbivores than the host leaves.[23]




Key: M – Mistletoe, PH – Parasitized host, NPH – Non-parasitized host

***Figure 1.*** Percentage moisture content in *Scurrula ferruginea* and its hosts

In order to ensure uniformity, subsequent comparisons will be based on oven-dried method. Ash content reflects the total mineral content. The mean ash content in *S. ferruginea* parasitizing on the three different hosts do not show an

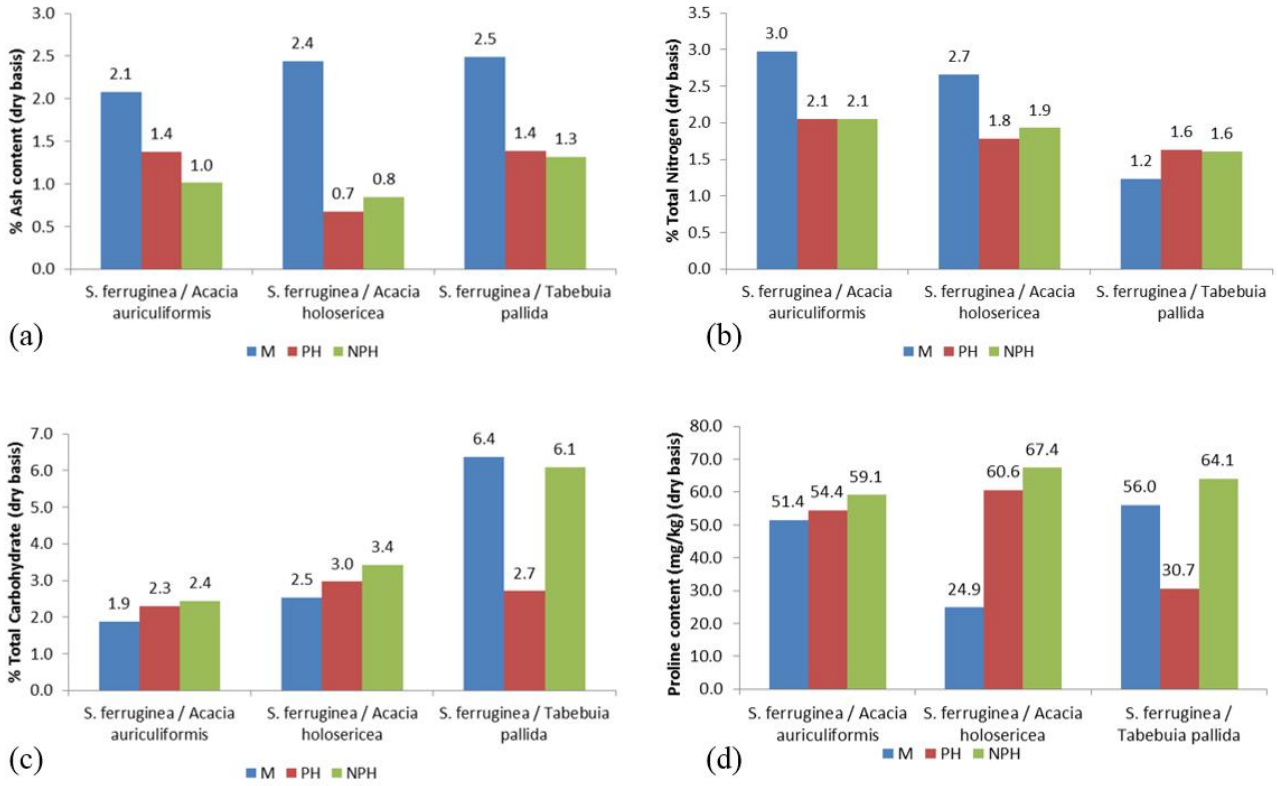


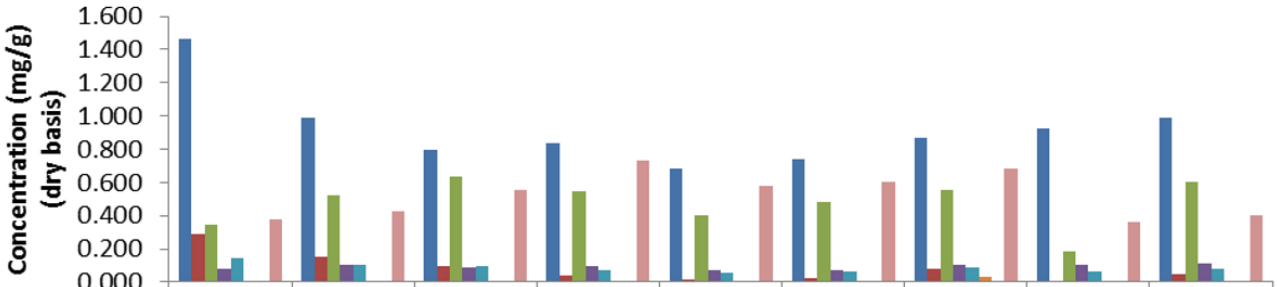

***Figure 2.*** Percentage ash content (a), total carbohydrate (b), total nitrogen content (c) and proline content (d) of *Scurrula ferruginea* parasitizing on *Tabebuia pallida, Acacia holosericea* and *Acacia auriculiformis* respectively. Each sample was carried out in duplicates.

appreciable difference ranging from 2.1 to 2.5 % dry basis (***Figure 2a***). The values of the mistletoes showed a generally higher value than the comparable hosts[24], thus we expect generally low ash content in mistletoe. However, it can be argued that mistletoes have absorbed and retained a large content of nutrients along the host derived xylem flux *via* the haustoria. Hence, the phenomenon of the presence of high ash content is generally in agreement with the physiology of mistletoes.

Hull and Leonard[25] stated that it is important to maintain sufficient carbohydrate reservoir in mistletoes for the survival and production of new foliage during the unfavorable periods. There was a significant difference among all the total carbohydrate contents analysed ($p<0.05$). The tropical mistletoe *S. ferruginea* shows generally lower carbohydrate contents (1.9-6.4% dry basis) than the corresponding non-parasitized (2.4-6.1 % dry basis), (***Figure 2b***). This can be attributed to generally favorable environmental conditions prevailing in tropical Brunei in contrast to results reported for arid and temperate species.

There is significant difference among the three associations of *S. ferruginea* ($p<0.05$). The nitrogen content (***Figure 2c***) indicates that *S. ferruginea* parasitizing the nitrogen fixing *A. holosericea* and *A. auriculiformis* have higher nitrogen content than those parasitizing on non-nitrogen fixing *T. pallida.* The nitrogen concentrations in mistletoe leaves are dependent on the type of host it parasitizes.[24,26-27] Results of nitrogen partitioning clearly are in agreement with the literature that storage of some nutrients in mistletoe is influenced by host species. Furthermore, nitrogen profile on *S. ferruginea* reflecting those of hosts may be interpreted as little solute processing related to nitrogenous compounds taking place in the haustorium.

Ehleringer *et al.*[28] have stated that proline often serves as an essential role in osmotic relations of




| | M | PH | NPH | M | PH | NPH | M | PH | NPH |
|---|---|---|---|---|---|---|---|---|---|
| | S. ferruginea / Acacia auriculiformis | | | S. ferruginea / Acacia holosericea | | | S. ferruginea / Tabebuia pallida | | |
| K | 1.461 | 0.991 | 0.793 | 0.840 | 0.683 | 0.737 | 0.865 | 0.923 | 0.992 |
| Na | 0.293 | 0.155 | 0.099 | 0.042 | 0.016 | 0.021 | 0.081 | 0.011 | 0.048 |
| Ca | 0.346 | 0.525 | 0.638 | 0.543 | 0.401 | 0.486 | 0.553 | 0.188 | 0.600 |
| Mg | 0.083 | 0.102 | 0.085 | 0.094 | 0.070 | 0.074 | 0.102 | 0.105 | 0.110 |
| Fe | 0.142 | 0.107 | 0.093 | 0.073 | 0.060 | 0.063 | 0.091 | 0.064 | 0.078 |
| Zn | 0.010 | 0.010 | 0.011 | 0.007 | 0.007 | 0.007 | 0.032 | 0.008 | 0.009 |
| Mn | 0.002 | 0.010 | 0.011 | 0.003 | 0.004 | 0.005 | 0.007 | 0.004 | 0.003 |
| Cu | 0.374 | 0.428 | 0.559 | 0.729 | 0.581 | 0.605 | 0.687 | 0.361 | 0.402 |

Key: PL – Parasite leaves, PHL - parasitized host leaves, NPHL - non-parasitized host leaves

***Figure 3.*** Concentration of potassium, calcium, sodium, magnesium, copper, zinc, manganese, iron of *S. ferruginea* and its hosts. Each sample was carried out in duplicates.

parasitic plants and it is expected that mistletoes would have higher concentrations of proline than the hosts. However, a reverse effect was observed as where the non-parasitized host showed the highest proline content in our investigation. Even though the total nitrogen content of *S. ferruginea* is always higher than the hosts, the proline content of the mistletoe *S. ferruginea* was always lower than the non-parasitizing host partner (***Figure 2d***). Leaf nitrogen can be attributed to the storage of polyphenol[29]. Hence, this implies that proline is not the major component of nitrogen in the leaf of *S. ferruginea.* However consistent with the literature the mistletoe had higher proline content than the parasitized host in the *S. ferrugina-T. pallida* association, interestingly this was the only host that was not a N fixer.

As a consequence to the absence of retranslocation of solutes between the mistletoe and host due to lack of phloem links in the haustorium that connects these two separate plants, mistletoes rely exclusively on host xylem for their water and mineral requirements.[1] The major and minor mineral nutrients profiles are in ***Figure 3***. Potassium was the major mineral found in the all samples investigated. As reported by Glatzel,[1] potassium enrichment in mistletoe is directly linked to the absence of retranslocation between mistletoe and host because of the lack of phloem links. Mistletoes have the ability to divert a steady stream of potassium from the xylem-phloem cycle of the host. The second most abundant mineral found in the samples was calcium followed by sodium. The distribution of other minerals between the mistletoe and the parasitized host were not consistent.

Mistletoes lack a usual plant-root system capable of active uptake. They rely upon a host connection through the haustorium for nitrogen and mineral nutrients. Both the host leaves and mistletoe leaves draw from the same xylem sap[30]. Nevertheless, whether or not the mistletoe should have higher mineral content than the host may be dependent on as of yet unresolved factors as Lamont[4] reported the calcium levels were lower in the mistletoe than in the host, while McDowell[3]

reported that the mistletoes have higher concentrations of nitrogen, phosphorus, potassium and magnesium than the host.

Furthermore, Singh and Carew[6] did not detect meaningful differences in macronutrient or micronutrient concentrations for any plant parts analyzed in a range of mistletoe-host association. Such variations might be due to the age of the plant analysed, the growing environment of these tropical mistletoe-host association, the type of parasite and the specific host and the time chosen for sample collection.[4,7] In addition, loss of nutrients can also occur to the older part of the host plant and mistletoe independently by the phloem-mediated cycling or remobilization from senescing leaves.[1]

Some evidence suggest that the complex nature of solute partitioning between mistletoes and hosts due to selective (active) transportation of solutes across the haustoria. The haustorium cells at the interface have enhanced concentrations of mitochondria and show signs of being able to mobilize energy. The main function of these cells is to pump unwanted materials back to the host. However, the mechanisms behind these processes are only partly understood.[31]

## 4. Conclusion

Results of this study revealed that solute partitioning of the mistletoes is likely to be complex, but interacts in a highly ordered fashion in relation to the composition of host derived solutes. The compositional changes shown across the mistletoes, parasitized and non-parasitized host branches occur in favor of the successful continuation of mistletoe-host association partnership. Further studies on nutritional partitioning of tropical mistletoes at molecular level must take into account the haustorial anatomy, co-evolutionary strategies adopted by both host and mistletoe that ensures successful co-habitation, and the confounding tropical environmental conditions that many influence physiological biochemistry of mistletoes.

**Acknowledgements**

The authors would like to thank Aywen Wang H. Chak for field assistance and the Universiti Brunei Darussalam for logistic support. Financial assistance for this study was provided by Universiti Brunei Darussalam, Science and Technology Grant No. 8.

**References**

[1] G. Glatzel, *Oecologia*, **1983**, 193.
[2] J. Kujit, *The Biology of Parasitic Flowering Plants*, University of California Press, Berkeley, **1969**, 45.
[3] L. C. McDowell, *Physiological relationships between dwarf mistletoe and ponderosa pine*, Oregon State University, Corvallis, OR, Oregon State University, **1964**, 68.
[4] B. Lamont, *Mineral nutrition of mistletoes*. *The biology of mistletoes*, M. Calder and P. Bernhardt, **1983**, 185.
[5] W. H. Chak, *Preliminary investigation on the mineral nutrition and water relations of tropical hemiparasitic mistletoes found in Brunei-Muara District (Brunei Darussalam)*, Universiti Brunei Darussalam. **2009.**
[6] P. Singh and G. C. Carew, *Euro. J. Forest Pathology*, **1989**, 305.
[7] K. U. Tennakoon and J. S. Pate, *Plant, Cell & Environ.*, **1996**, 517.
[8] M. Popp, R. Mensen, A. Richter, H. Buschmann and D. J. Willert, *Trees*, **1995**, 303.
[9] J. M. Hibberd and W. D. Jeschke, *J. Exp. Botany*, **2001**, 2043.
[10] D. F. Lohézic-Le, A. Bakhtiar, C. Bézivin, M. Amoros and J. Boustie, *Fitoterapia*, **2002**, 400.
[11] O. Z. Ameer, I. M. Salman, M. J. Siddiqui, M. F. Yam, R. N. Sriramaneni, A. Sadikun, Z. Ismail, A. M. Shah and M. Z. Asmawi, *The American J. Chinese Med.*, **2009**, 991.
[12] C. Kirana, *Bioactive compounds isolated from mistletoe (Scurulla oortiana (Korth).Danser) parasitizing tea plant (Camellia sinensis L.)*, University of Adelaide, South Australia. **1996**.

[13] D. N. Raut, C. P. Subodh and C. M. Subhash, *Int. J. Pharmaceutical Res. Develop*., **2009**, 6.
[14] O. R. Madibela, M. Letso, W. S. Boitumelo, M. Masedi and K. Alton, *Animal Feed Sci. Technol.*, **2002**, 159.
[15] B. A. Barlow, *Flora Malesiana Bull.*, **1991**, 335.
[16] O. Z. Ameer, I. Salman, M. Yam, A. H. Allah, M. Abdulla, A. Shah, A. Sadikun and M. Asmawi, *Int. J. Pharmacology*, **2009**, 44.
[17] Q. Huaxing Q and G. G. Michael, *Flora of China*. **2003**, 220.
[18] P. Cunniff, *Methods of Analysis of AOAC International*. AOAC International, USA. **1998**, 37.
[19] S. S. Nielsen, *Food Analysis Laboratory Manual*, Indiana, **2003**, 39.
[20] L. S. P. Madamba, *Laboratory Instruction Manual: Technical analysis I - Foods and Feeds*, The Philippines, **2000**, p 28-31.
[21] S. S. Nielsen, *Food Analysis Laboratory Manual*, Indiana, **2003**, 33.
[22] C. Ough, *J. Food Sci.*, **1969**, 228.
[23] D. V. Canyon and C. J. Hill, *Australian J. Ecology*, **1997**, 395.
[24] J. Ehleringer, E. Schulze, H. Ziegler, O. Lange, G. Farquhar and I. Cowan, *Science*, **1985**, 1479.
[25] R. J. Hull and O. A. Leonard, *Plant Physiology*, **1964**, 996.
[26] P. Bannister and G. L. Strong, *Oecologia*, **2001**, 10.
[27] E. D. Schulze, O. Lange, H. Ziegler and G. Gebauer, *Oecologia*, **1991**, 457.
[28] J. R. Ehleringer, C. Craig and L. T. Larry, *Oecologia*, **1986**, 279.
[29] J. C. Rambourg and B. Monties, *Plant Foods for Human Nutrition*, **1983**, 169.
[30] G. Glatzel and B. Geils, *Canadian J. Botany*, **2009**, 10.
[31] H. Shen, W. Ye, L. Hong, H. Huang, Z. Wang, X. Deng, Q. Yang and Z. Xu, *Plant Biol.*, **2006,** 175.