

A REVIEW PAPER ON *ALOCASIA MACRORRHIZA* TRADITIONAL INDIAN MEDICINAL PLANT**Santosh Kumar Singh^{1*}, Dr. Jay Ram Patel², Arvind Dangi³, Deepak Bachle⁴ and Rahul Kumar Katariya⁵**¹Ph.D. Scholar, Pacific College of Pharmacy, Pacific University, P.B. – 12 Pacific Hills, Airport Road, Pratap Nagar Extension, Debari, Udaipur – 313024, Rajasthan, India.²Oriental College of Pharmacy, NH-12, Hoshangabad Road, Misrod, Bhopal- 462047, Madhya Pradesh, India.³IES College of Pharmacy, Kalkheda, Ratibadh Bhopal- 462044, Madhya Pradesh, India.⁴Radharaman Institutes of Pharmaceutical Sciences, Ratibardh, Bhadbhada Road, Bhopal - 462046, Madhya Pradesh, India.⁵Mittal Institutes of Pharmacy, Opp. Bhopal Memorial Hospital and Research Centre, Bypass Road, Nabibagh, Bhopal – 462038, Madhya Pradesh, India.***Corresponding Author: Dr. Santosh Kumar Singh**

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ABSTRACT

Alocasia macrorrhiza also known as giant taro is a member of *Araceae* family. *Alocasia macrorrhiza* is commonly used as a household decorative plant. This plant has also some pharmacological activity like antifungal, weak hemagglutinating activity, antidiuretic, laxative, antitubercular and reduces the activity of human immunodeficiency virus reductase and also has antioxidant properties. This plant contains flavonoids, Oxalic acid, cyanogenic glycosides, alocasin, cholesterol, amino acids, gallic acid, mallic acid, ascorbic acid, succinic acid, glucose, fructose, sucrose and betalectins. This article aims to evaluate the biological activities, pharmacological applications and clinical studies of *Alocasia macrorrhiza* in an attempt to provide a direction for further research.

KEYWORDS: *Alocasia*, *Macrorrhiza*, Antioxidant, Antidiuretic.**INTRODUCTION**

The leaves of *Alocasia macrorrhiza* are nutritionally found to contain varying amounts of proteins, Ash, crude fibre, carbohydrate, starch, Ascorbic acid, oxalates, proteases, Nitrate and Tannin. It is a cultivated edible aroid in India. *Alocasia macrorrhiza* occurs naturally in tropical forests in Sri Lanka, India, Bangladesh, China and South Africa. It is mainly cultivated in India and Bangladesh.^[1] The giant taro, *Alocasia macrorrhiza* (L.) G. Don. is a member of the family *Araceae* and is closely related to taro (*Colocasia*). However, unlike taro, most of the edible part of the stem is produced above ground which allows for easier harvesting. The edible stem grows up from the planting material so is never deeper in the ground than about 6 to 8 inches. *Alocasia* is commonly grown in upland areas, high islands, or drier areas of atolls. Like the dryland taro it does not require flooding. *Alocasia* grows year around and can be harvested at any time when it is needed. However, larger plants do flower in the summer and this tends to slow growth. *Alocasia* is thought to have originated in Sri Lanka or India.^[2] It is presently cultivated in Asia in the countries of India, Sri Lanka, and Bangladesh where crop time is from 6 to 11 Months. The variety grown in Sri Lanka (Desai ala) appears to be a small variety with stems averaging 4 to 6 pounds when spaced 2 X 4 feet.^[3]

In the Pacific region, *Alocasia* is grown in mixed plantings with taro, yams and banana principally in Wallis, Futuna, Tonga and Niue. In these areas the flavor is considered superior to taro. Crop time is 18 to 24 months and corms are reported to be 3 to 4 feet long, 6 to 8 inches in diameter and weigh about 40 pounds.^[4,5,6] The stems are boiled in water or with added coconut milk, lightly salted and served. An alternate method is to bake the tubers whole alone or with ti tubers. The fructose sugar from the ti tubers during baking runs down over the *Alocasia* making them very sweet. In Hawaii, *Alocasia* was planted in upland valleys and one cultivar apparently grows rapidly and produces large edible stems. However, the Hawaiian preference is for taro and because of this *Alocasia* has not been grown commercially in Hawaii to any great extent. *Alocasia macrorrhiza*: *A. macrorrhiza* is recorded among cultivated medicinal as well as vegetable plants by the folklore of south Asia.^[7] Efforts continued to identify the potential medicinal uses of the *Alocasia macrorrhiza*, especially on liver (El-Deen et al., 2008). An anti-fungal protein designated alocasin is isolated from the plant which can reduce the activity of HIV-1 reverse transcriptase.^[8] One of the species of *Alocasia*, *A. indica* has been worked out for its efficacy to protect the liver and found to possess significant potential as

hepatoprotective agent.^[9] The leaves of *Alocasia macrorrhiza* are nutritionally found to contain varying amounts of proteins, Ash, crude fibre, carbohydrate, starch, Ascorbic acid, oxalates, proteases, Nitrate, and Tannin. It is a cultivated edible aroid in India.^[10] Massive pachycaul with the stem decumbent or erect, to 4 m tall; petioles to 1.3 m long, sheathing in lower 1/3–1/2, blades ovato-sagittate, bluntly triangular in general outline, held more or less erect, with the margin entire to very slightly; anterior lobe ca. 70 cm to over 1 m long, ca. 60–90 cm at base, with ca. 9 rather distant primary lateral veins on each side of the anterior costa diverging ca. 60°; glands in axils of primary veins on abaxial side distinct; secondary venation flush with the lamina or but slightly raised abaxially, not forming interprimary collective veins or these poorly defined; posterior lobes ca. 1.3–1/2 the length of the anterior; inflorescences paired among the leaf bases, subtended by membranous cataphylls; peduncle barely exceeding the cataphylls at anthesis; spathe rather variable in length, ca. 13–35 cm long, constricted about 1/6th of the way from the base; lower part green, ovoid; limb broadly oblong-lanceolate, membranous, pale yellow; spadix slightly shorter than the spathe, shortly stipitate; female zone 1–2 cm long, ca. 1.5 cm diam.; ovaries pale green, ca. 3 mm diam.; stigma sessile, 3–5 lobed, the lobes conic, yellow; sterile interstice slightly shorter than to equaling the female zone, whitish, very slightly narrowed corresponding to the spathe constriction, composed of rhombo-hexagonal synandria ca. 2.5 mm diam., the lower ones paler, incompletely connate or with the central hole, the upper ones resembling synandria; male zone cylindrical ca. 3–7 cm long, ca. 2 cm diam., whitish; synandria rhombohexagonal, ca. 2 mm diam.; appendix yellowish, slightly thicker than the male zone at the base; fruiting spathe ca. 8 cm long; fruit berries, scarlet.^[11]



Figure 1: *Alocasia macrorrhiza* growing in mountainous areas of Northern Vietnam

Leaf anatomy and Morphology

To characterize the heterogeneity of structure and function within *A. macrorrhiza* leaves, we chose six mature and healthy individuals similar in leaf size, leaf age and environmental conditions and selected one leaf from each individual that was fully exposed to sunlight. The *A. macrorrhiza* leaves were excised with a razor blade and brought to the laboratory. Leaf discs of 4 cm² were sampled along transects in two directions: from the basal to apical lamina regions and from the central to outer lamina regions (Fig. 2). Abaxial epidermal impressions were made with clear nail polish and the guard cell length and stomatal density were determined. To measure vein density (i.e., vein length per leaf area), the epidermis was removed with a sharp razor blade and the remaining leaf samples were cleared in bleach (LIBY Enterprise Group Co. Ltd., Guangdong, China). The leaf samples were stained with toluidine blue to resolve and quantify vein length per area using image.^[12]

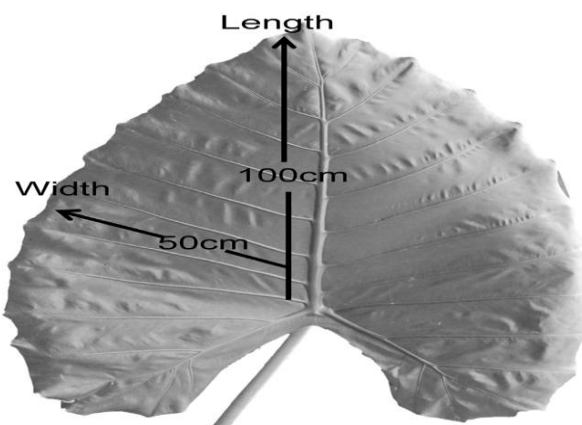


Figure 2: leaflets of *Alocasia Macrorrhiza*

PHARMACOLOGICAL ACTIVITIES

Anticancer

The IC₅₀ of the ethyl acetate extract from *Alocasia macrorrhiza* to A549, B16, BGC-823 were 94.6, 541.9, 629.5 µg·ml⁻¹ respectively and those of the

acetone extract from *Alocasia macrorrhiza* to A549 and B16 40.9,438.0 $\mu\text{g}\cdot\text{ml}^{-1}$ respectively. But there were quite large dosages of the IC₅₀ of the extracts of cyclohexane, benzene, ethyl alcohol and water from *Alocasia macrorrhiza* to all kinds of cell lines. The obvious acute toxicities were not observed for the ethyl acetate and acetone extracts of *Alocasia macrorrhiza* under the treatment doses of 60g·kg⁻¹ and 36.5 g·kg⁻¹ respectively.^[13]

Antidiarrheal activities

In vitro antidiarrheal activity of aqueous and ethanolic extracts of *Alocasia indica* was evaluated against *Escherichia coli*, *Salmonella typhimurium* and *Shigella flexneri* and *Staphylococcus aureus* by agar well diffusion method. In vivo antidiarrheal activity of the extracts was studied against ricinolic acid induced diarrhea and magnesium sulfate induced diarrhea. The aqueous and ethanol extract exhibited significant in vitro antidiarrheal activity compared to the standard drug Ciprofloxacin (10 $\mu\text{g}/\text{ml}$). The plant extracts showed significant ($P < 0.05$) and dose-dependent antidiarrheal activity comparable to that of the reference drug, loperamide (10 mg/kg).^[14]

Antifungal activities

An anti-fungal protein designated alocasin was isolated from the rhizomes of the *Alocasia macrorrhiza* (L.) G. Don. The isolation protocol involved in ion exchange chromatography on diethylaminoethyl (DEAE)-cellulose, ion exchange chromatography on sulfopropyl (SP) - sepharose and gel filtration on Superdex 75. Alocasin has shown some similarity miraculin like antifungal protein from *Pisum sativum* legumes. It demonstrated a molecular mass of 11 kDa in sodium dodecyl sulfate polyacrylamide gel electrophoresis and gel filtration and displayed anti-fungal activity against *Botrytis cinerea*. Alocasin reduced the activity of HIV-1 reverse transcriptase. It exhibited weak hemagglutinating activity, only at a concentration of 1 mg/ml.^[8]

Anthelmintic activity

Hydroalcoholic extract of leaves of *Alocasia indica* Linn. And its different fraction exhibited anthelmintic activity in dose-dependent manner giving shorter time of paralysis (P) and death (D) with 50 mg/ml concentration. At 10 mg/ml concentration hydroalcoholic extract caused paralysis of 4.27 min and time of death of 10.58 min. While petroleum ether (40-60 °C) and ethyl acetate fractions caused paralysis of 6.28 and 5.63 min and time of death of 21.59 and 11.92 min. The reference drug Piperazine citrate showed the same at 19.26 and 63.25 minutes respectively at concentration of 10 mg/ml. The effect of reference drug was compared with each crude extract (10, 25 and 50 mg/ml), which was found to produce grade 3 paralysis within 90 minutes.^[15]

Antihyperglycemic activity

Alocasia macrorrhizos (L.) a coarse and erect plant that is widely cultivated to be eaten as a vegetable throughout

Bangladesh has been reported to possess a number of medicinal properties. The antihyperglycemic, antioxidant and cytotoxic effects of the methanol extract of *A. macrorrhizos* rhizomes were investigated. The extract was given at a single dose (250 and 500 mg/kg) in alloxan-induced hyperglycemic mice. The extract at 500 mg/kg produced a significant ($P < 0.05$) decrease in the blood glucose level (55.49%) at 8 h of treatment when compared with the control and was comparable with the reference drug, metformin. The extract was also subjected to antioxidant potentiality and brine shrimp lethality bioassays. The IC₅₀ value of the extract was 693 $\mu\text{g}/\text{ml}$ and the LC₅₀ was 188.14 $\mu\text{g}/\text{ml}$.^[16]

Anti-inflammatory activity

In this experiment gels containing *Alocasia indica* and marketed formulation of Diclofenac sodium produced dose-dependent inhibition of carrageenan and formalin induced paw edema as compared to the control ($P < 0.05$). In xylene-induced mouse ear edema assay gels containing *Alocasia indica* and marketed formulation of Diclofenac sodium significantly inhibited arachidonic acid and xylene-induced ear edema in dose dependent as compared to the control ($P < 0.05$)²⁵ (Mulla *et al.*, 2010). The researcher has reported the carrageenan induced rat paw edema model of inflammatory activity, the ethanolic extract of dried rhizome of *Alocasia indica* Schott showed a significant inhibitory effect on the edema formation from the first hour to fifth hour. The highest inhibitory effect was found during the third hour where the inhibition was 25.43% ($P < 0.001$) and 41.05% ($P < 0.001$) at the dose of 300 and 600 mg/kg of body weight respectively. These findings were comparable to standard drug aspirin where the inhibition was 51.38% ($P < 0.001$) at the dose of 150 mg/kg of the body weight.^[16]

Antimicrobial activity

Antimicrobial activity *Alocasia indica* was evaluated by using 5 and 10 mg/ml concentration against Gram-positive, Gram-negative bacterial and fungal strains under test. Result is comparable with standards. The MIC of different extracts was evaluated across a concentration range of 5 mg/ml to 20 mg/ml. The lowest MIC values were observed for ethanol extract in the range of 10.23 to 13.18 mg/ml. MIC values of chloroform extract 11.47 to 15.46 mg/ml, acetone extract 12.14 to 14.32 mg/ml, petroleum ether extract 11.45 to 14.16 mg/ml were observed against the microorganism under test. The result reveals that various extracts of leaves of *Alocasia indica* were effective against both Gram-positive, Gram-negative bacteria and fungi which are associated with microbial disorders. The ethanolic extract of leaves of *Alocasia indica* also found to be effective in inhibiting growth of *Staphylococcus aureus* and *Klebsiella pneumoniae* profoundly in range of 2-8 hours. The *Alocasia indica* leaf extract also inhibited growth of *Aspergillus Niger*, *Candida albicans* and *Saccharomyces cerevisiae*. Ethanolic extract of leaves of *Alocasia indica* inhibited growth of *Aspergillus Niger* and

saccharomyces cerevisiae till the end of the 4 hours incubation period but growth was observed after 4h incubation period.^[14]

Antinociceptive activity

The Antinociceptive activity is determined by using the oral administration of the ethanolic extract of *Alocasia indica* (200 and 400 mg/kg) significantly ($P < 0.05$) reduced the number of writhing induced by acetic acid in rat. Acetyl salicylic acid (200mg/kg, p.o.) used as a reference drug and the test activity was comparable with reference drug. Moreover, the extract induced protection in rat in tail immersion test that is comparable with the standard drug Pentazocine (mg/kg, p.o). The result of hot plate test presented showed that the I.P administration of ethanolic extract of *Alocasia indica* at the doses of 200-400mg/kg significantly raised the pain threshold at observation time of 45 min in comparison with control ($P < 0.001$).^[17]

Antioxidant activity

In this experiment five concentrations ranging from 200 to 1000 $\mu\text{g/ml}$ of the ethanolic extract of *Alocasia indica* were tested for their antioxidant potential using different in vitro models. It was observed that free radicals were scavenged by test compound at different concentrations. The maximum inhibitory concentration (IC₅₀) in all models viz., DPPH, nitric oxide radical, superoxide, hydroxyl radical scavenging activity, were found to be 7.30, 10.97, 9.8 and 7.86 $\mu\text{g/ml}$, respectively (Mulla *et al.*, 2010). In this experiment seven extract of *Alocasia macrorrhiza* (L.) G. Don (rhizome, roots and leaves) and two standards tested for antioxidant activity using DPPH method; the diethyl ether extract of roots and rhizome showed the maximum antioxidant activity with IC₅₀ value 34.51} 2.72 and 48.01} 6.68 $\mu\text{g/ml}$, respectively. The diethyl ether and aqueous extracts of leaves also exhibited good quantum of antioxidant activity with lesser IC₅₀ value 103.39} 7.12 and 58.37} 6.21 $\mu\text{g/ml}$, respectively. However, hexane, benzene, toluene, chloroform and diethyl acetate extract of rhizome and roots have lower antioxidant activity with higher IC₅₀ value. The known antioxidants ascorbic acid and quercetin exhibited IC₅₀ value of 78.17} 4.05 and 53.60} 1.79 $\mu\text{g/ml}$, respectively.^[11]

Antiprotozoal activities

In vitro antiprotozoal activity of aqueous and ethanolic extract of *Alocasia indica* was studied against *Entamoeba histolytica* and *Giardia intestinalis*. The plant extracts significant in vitro antiprotozoal activity and the result was compared to the standard amebicidal and giardicidal drugs, metronidazole and emetine.^[18]

Antitumour activity

Antitumour activity of water extract of *Alocasia macrorrhiza* was evaluated by using transplanted tumour in mice and human cancer enograft in nude mice models. Results showed that the inhibitory rate against S180 in mice was 29.38%, and the inhibitory rate against

transplantable human gastroadenitis in nude mice was 51.72%. No antitumour effect was shown against ECA in mice. The amino acid sequences of the trypsin inhibitors from taro *Colocasia esculenta* var. *esculenta* and giant swamp taro *Cyrtosperma chamissonis* have been determined and are compared with the protein sequence of the trypsin/chymotrypsin inhibitor from giant taro *Alocasia macrorrhiza*. Both inhibitors display polymorphism and there is evidence of two components in the giant swamp taro. The positional identity between the proteins is highest at 73-75% for the comparison of the giant taro (GT) with the polymorphic forms of the taro (T) inhibitors and lowest at 56-58% for the pairs of taro and giant swamp taro (GST) proteins. The comparisons show that the inhibitors from T and GT are more related to each other than to GST, which supports their taxonomic classification into different tribes. Location of the P1 site for the trypsin inhibitors of aroids is different from that of other Kunitz-type inhibitors and could be at Leu.^[19]

Diuretic activity

Alocasia macrorrhizos (AM) belongs to the family Araceae, different parts of this plant have shown hepatoprotective, anti-oxidant and anti-inflammatory action. Though it is traditionally used as a diuretic it needs substantial experimental evidence to support this. Hence we aimed to evaluate the diuretic activity of hydroalcoholic extract of leaves of AM in wistar rats. Acute diuretic activity of hydroalcoholic (50%) extract of leaves of AM (250 mg/kg and 500 mg/kg body weight orally) was studied in saline primed wistar albino rats ($n=6$). Furosemide (10 mg/kg) orally was used as the standard. Total 24 hours urine volume was measured using metabolic cages. The concentration of Na⁺, K⁺ in the urine at the end of 24 hours was estimated. Data was analyzed by One-way ANOVA followed by Dunnett test. Hydroalcoholic extract of leaves of AM showed a significant ($P < 0.05$) dose dependent increase in urine volume ($8.1 \pm 0.97 \text{ ml/100g/24hr}$ and $9.7 \pm 0.75 \text{ ml/100gm/24hr}$). At 500 mg/kg AM increased the excretion of sodium but decreased the excretion of potassium significantly compared to control. This preclinical study showed a potential diuretic activity but further studies regarding the mechanism of action is required to validate this finding. Herbal medicines derived from plant extracts are being progressively more utilized to treat a variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is increasing interest in the health and wellness benefits of herbs and botanicals. This is with good reason as they might offer a natural safeguard against the development of certain conditions and be a putative treatment for some diseases. One such area may be the lowering of blood pressure in those where it is elevated. One class of clinical medicines used to lower blood pressure are known as diuretics and work by increasing the excretion of urine from the body as well as the amount of sodium in urine. There are a growing number of studies purporting diuretic effects with

traditional medicines. Any substance that tends to increase the flow of urine, which causes the body to get rid of excess water, is known as diuretic drugs. Substances that induce "diuresis," or the removal of fluids from the body through urination, are considered diuretics. These agents were widely explored in Indian ancient system of medicine. Diuretics increase the rate of urine outflow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephritic syndrome and cirrhosis. The aim of this review is to abridge the work on diuretics of herbal origin.^[20]

Hepatoprotective activity

Hydroalcoholic extract of *Alocasia indica* leaves were studied using the model of hepatotoxicity induced by CCl₄ (carbon tetra chloride) and PCM (Paracetamol) in rats. CCl₄ and PCM administration induced the significant increase in the levels of serum marker enzymes ALT, AST, ALP, Total serum bilirubin (SB), Total cholesterol (CHL) and decrease in the levels of total protein (TP), Serum albumin (SA) were observed significantly indicating acute hepatocellular damage and biliary obstruction. Pretreatment with Hydroalcoholic extract of *Alocasia indica* produced a significant reduction in serum marker enzymes ALT, AST, ALP, SB, CHL and increase in the level of TP, SA similar to silymarin (100mg/kg, po) treated group which seems to offer the protection and maintain the functional integrity of hepatic cells. Pretreatment with Hydroalcoholic extract of *Alocasia indica* and silymarin significantly reduced the increase in the liver weight and liver volume seen after CCl₄ and PCM intoxication. A significant reduction in thiopentone- induced sleeping time was observed with *Alocasia indica* extract as compared to the CCl₄ and PCM treated group. Histological examination of the liver tissues from CCl₄ and PCM treated animal disclosed that CCl₄ and PCM had produced profound inflammation, severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus congestion especially in the sinusoids and occasional necrosis. Pre treatment of animal with silymarin, *Alocasia indica* (250mg/kg, po) and *Alocasia indica* (500mg/kg, po) reduced the inflammation, degenerative change and steatosis.^[21]

Hepatorenal activity

RBCs, Haemoglobin were significantly decreased after treated period. Total protein, albumin and globulin were significantly decreased, while Aspartate transaminase (AST), Alanine transaminase (ALT), gamma glutamyl transpeptidase (GGT), Lactate dehydrogenase (LDH), urea, creatinine, total lipid and cholesterol were significantly increased after treated and recovery period of 10 days. Histological changes in the treated section of the liver showed evidence of cellular degeneration and necrosis and in kidney section, tubular necrosis, glomerular shrinkage and atrophied glomerular tuft of capillaries were prominent. Mallory stained section in

liver showed increased collagen fibers around congested central vein, blood sinusoids and portal areas. While kidney sections, the extract could not induce any change in the collagen fibers in the connective tissue. The change which was observed after treatment disappeared after a recovery period of 20 days. However, the vascular congestion persisted. Histochemical studies revealed a significant decrease in PAS positive material in kidney after treated and recovery periods of 10 days.^[22]

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Laxative activity

On wistar albino rats of either sex, fasted for 12 hours before the experiment, but with water provided ad libitum. Rats were divided into five groups, each group consisting six rats. The first group of animal, received normal saline (25ml/kg, p.o.), second group of animal, received agar agar (300mg/kg, p.o.) the third, fourth and fifth group of animals received simultaneously (100, 200, 400mg/kg, p.o.) ethanolic extract of *Alocasia macrorrhiza*. After administration the animals were placed in a plastic container suitable for collection of faeces. After 8 hours of drug administration, the faeces were collected and weighed. Thereafter, food and water were given to all rats and faecal outputs were again weighed after a period of 16 hours.^[24]

Reproductive activity

This investigation was aimed at understanding the morphological variation and reproductive biology of wild populations of giant taro (*Alocasia macrorrhizos*) in Vanuatu. It is an aroid species, which grows in vigorous, relatively small and dense populations, consisting of phenotypically uniform or very similar individuals. The most variable traits observed in wild populations are number of inflorescences, number of infructescences per plant and corm length. These traits are highly variable even within genetically uniform populations and are highly influenced by age differences between plants. Flowering is frequent and most of the plants are highly

fertile. However, sexual reproduction is rare due to self-incompatibility (between plants genetically homogeneous within the same population) and the absence of efficient pollinators. Self incompatibility can be partly overcome by repeated self-pollination. Thermogenesis was studied on a large sample of inflorescences and little variation concerning the thermogenic potential was observed. Our results showed that, despite optimal flowering conditions (concerning pollen fertility, stigma receptivity and thermogenic potential), propagation of wild *Alocasia macrorrhizos* populations in Vanuatu was mainly vegetative. Giant taro plants might have been carried beyond the range of their most effective pollinators. Other factors were considered: the presence of numerous earwigs (*Labiduratruncata*) insects within inflorescences, which could have a competing effect with the usual pollinators of giant taro and the long distances between genetically homogeneous populations.^[25]

Self- and cross-(in)compatibility

The first step of the self- and cross-(in)compatibility study (from November 2001 to February 2002) was the observation of the incidence of fruit formation in: (1) wild populations; (2) after 20 artificial self-pollinations (female portion pollinated with pollen originating from the same spadix); (3) after 20 artificial intraclonal pollinations (pollinations among plants belonging to different individuals within the same clone); and (4) after 88 artificial pollinations among five different morphotypes. The second step (from November 2002 to February 2003) employed repeated self-pollinations. Inflorescences were emasculated in an early stage of development and pollinated 2-3 times (on 2-3 successive mornings) with pollen originating from different inflorescences of the same plant. This was made possible because the chosen plants developed five or more clusters of inflorescences. Self-pollination could be conducted despite protogyny because our preliminary studies showed that stigmas remained fully receptive at least 3 days.^[26]

Acclimation of Respiratory Properties

To clarify the way in which the light available for growth affects respiration in leaves of sun and shade plants, we examined the respiratory properties of mature leaves of *Spinacia oleracea* L., a sun species and of *Alocasia macrorrhiza* (L.) G. Don., a shade species, that had been grown at various irradiances. In leaves of *S. oleracea*, the respiratory rates, on a dry mass basis, decreased with time during the night and the higher was the growth irradiance during the day, the higher was the respiratory rate. The marked decreases in the respiratory rate during the night were accompanied by decreases in the concentration of carbohydrates in the leaves. By contrast, the respiratory rates of leaves of *A. macrorrhiza* were virtually constant throughout the night and the absolute rates were lower than those of *S. oleracea* even though the absolute value of the concentration of carbohydrates and its decrease at night resembled to those in *S.*

oleracea. The maximum activities of respiratory enzymes were also similar to those in *S. oleracea*. However, the leaves of *A. macrorrhiza* contained less soluble protein than those of *S. oleracea*. These results suggest that, in *S. oleracea*, the concentration of carbohydrates might determine the respiratory rate while such is not the case in *A. macrorrhiza*. The lower respiratory rates in *A. macrorrhiza* might be due to a lower demand for ATP.^[27]

Lead and cadmium induced alterations of cellular functions

Alocasia macrorrhiza is a fast growing and propagating herbaceous species commonly found in South China. To determine its physiological responses to Pb and Cd stresses, the biochemical, histochemical and cytochemical changes under PbAC2 and CdCl2 phytotoxicity were detected using leaf discs as an experimental model. After leaf discs were infiltrated in different concentrations of PbAC2 and CdCl2 solutions (0,50,100,150,200 mM) for 72h, the formation of reactive oxygen species (H₂O₂ and O₂⁻) in plant tissue were found to be exaggerated together with elevated dOH concentration and cell death. Changes in chlorophyll fluorescence (Fv/Fm, FPSII, qP and NPQ) imaging colours/areas of leaf discs indicated decreased photo system II functions by both heavy metal treatments and positive reactions of antioxidants under Pb²⁺ stress. Results showed that fluorescent detection of hydroxylated terephthalate using terephthalic acid as dOH trap is a simple, yet valuable and specific method for monitoring dOH generation in plant tissue under heavy metals stresses. As compared with Cd²⁺, Pb²⁺ was found to be less toxic, indicating that *A. macrorrhiza* tissue might have a potential tolerance to Pb.^[28]

In Situ Localisation of Superoxide

Leaf discs of *Alocasia macrorrhiza* were treated with various stress factors, including two photo-oxidants, methyl viologen (MV) or riboflavin (RB); three pollutants, sodium bisulphite (NaHSO₃), or the heavy metals lead or cadmium; or an osmotic medium, polyethylene glycol 6000. The in situ localisation sites for O₂⁻ generation were identified using specific dye nitro blue tetrazolium as a probe. The level of superoxide production was determined by scanning the blue-stained formazan area and was defined as the percentage of pixels from the stained portion versus the total number of pixels in the entire leaf disc area. All stress factors induced the generation of O₂⁻ in a time- or concentration-dependent pattern. Although superoxide production also was enhanced by longer time periods in untreated discs (control), the degree to which this occurred was less than that measured in leaves treated with either MV or RB. Generation sites were primarily found in the chloroplasts of stomatal guard cells and in the plasma membrane of the epidermis and mesophyll cells, indicating that they were most responsive to stress conditions. Nevertheless, the site of O₂⁻ generation varied among these stress factors.^[29]

Molecular cloning of a lectin cDNA

The cDNA of *Alocasia macrorrhiza* lectin (aml, GenBank accession number: DQ340864) was cloned by RACE-PCR and its characteristics were predicted by various bioinformatics tools. GSPs (Gene Specific Primers) were designed according to the conserved regions of the genes encoded for lectins and similar proteins from the same family Araceae. Total RNAs were extracted from the tubers of *A. macrorrhiza* by Qiagen RNeasy mini kit. The 3'- and 5'-RACE-PCRs were performed with the isolated total RNAs by SMART(TM)RACE cDNA amplification kit from BD Biosciences Clontech Company, respectively. The purified PCR products were ligated with pMD 18-T vector, and the confirmed clones were sequenced. The full-length cDNA of aml was obtained by combination of 3'- and 5'-end sequences, and was then confirmed by full-length 3'-RACE-PCR. The aml cDNA is 1 124 bp long. The deduced amino acid length of AMLlectin is 270 aa. Its relative molecular weight is 29.7 kD. The results of homologous analysis showed a high similarity between AML and other mannose-binding lectins and similar proteins from Araceae family. Two typical B-lectin domains and three mannose-binding motifs were found in the sequence of AML. With all these taken together, it can be concluded that this newly cloned aml cDNA encodes for a mannose-binding lectin.^[30]

New ceramide

A new ceramide from *Alocasia macrorrhiza* A was isolated from the ethanolic extract of the plant *Alocasia macrorrhiza* (L.) Schott. Its chemical structure was elucidated as (2S, 3S, 4R)-2A/-(2'R)-2'-hydroxy-hexacosanoyl]-tetradecane-1,3,4-triol based on extensive 1D, 2D NMR, EI-MS, FABMS, HR-FAB-MS spectroscopic data and chemical degradation studies.^[31]

Wetting and Self-Cleaning Properties of Artificial Superhydrophobic Surfaces

The wetting and the self-cleaning properties (the latter is often called the "Lotus-Effect") of three types of superhydrophobic surfaces have been investigated: silicon wafer specimens with different regular arrays of spikes hydrophobized by chemical treatment, replicates of water-repellent leaves of plants, and commercially available metal foils which were additionally hydrophobized by means of a fluorinated agent. Water droplets rolled off easily from those silicon samples which had a microstructure consisting of rather slender spikes with narrow pitches. Such samples could be cleaned almost completely from artificial particulate contaminations by a fog consisting of water droplets (diameter range, 8-20 μm). Some metal foils and some replicates had two levels of roughening. Because of this, a complete removal of all particles was not possible using artificial fog. However, water drops with some amount of kinetic impact energy were able to clean these surfaces perfectly. A substrate where pronounced structures in the range below 5 μm were lacking could not

be cleaned by means of fog because this treatment resulted in a continuous water film on the samples.^[32]

An improved dynamic model of photosynthesis for estimation of carbon gain in sunfleck light regimes

A dynamic model of leaf photosynthesis for C3 plants has been developed for examination of the role of the dynamic properties of the photosynthetic apparatus in regulating CO₂ assimilation in variable light regimes. The model is modified from the Farquhar-von Caemmerer-Berry model by explicitly including metabolite pools and the effects of light activation and deactivation of Calvin cycle enzymes. It is coupled to a dynamic stomatal conductance model, with the assimilation rate at any time being determined by the joint effects of the dynamic biochemical model and the stomatal conductance model on the intercellular CO₂ pressure. When parametrized for each species, the model was shown to exhibit responses to step changes in photon flux density that agreed closely with the observed responses for both the understory plant *Alocasia macrorrhiza* and the crop plant *Glycine max*. Comparisons of measured and simulated photosynthesis under simulated light regimes having natural patterns of lightfleck frequencies and durations showed that the simulated total for *Alocasia* was within $\pm 4\%$ of the measured total assimilation, but that both were 12-50% less than the predictions from a steady-state solution of the model. Agreement was within $\pm 10\%$ for *Glycine max*, and only small differences were apparent between the dynamic and steady-state predictions. The model may therefore be parametrized for quite different species, and is shown to reflect more accurately the dynamics of photosynthesis than earlier dynamic models.^[33]

Acclimation of Respiratory Properties

To clarify the way in which the light available for growth affects respiration in leaves of sun and shade plants, we examined the respiratory properties of mature leaves of *Spinacia oleracea* L., a sun species and of *Alocasia macrorrhiza* (L.) G. Don., a shade species, that had been grown at various irradiances. In leaves of *S. oleracea*, the respiratory rates, on a dry mass basis, decreased with time during the night and the higher was the growth irradiance during the day, the higher was the respiratory rate. The marked decreases in the respiratory rate during the night were accompanied by decreases in the concentration of carbohydrates in the leaves. By contrast, the respiratory rates of leaves of *A. macrorrhiza* were virtually constant throughout the night and the absolute rates were lower than those of *S. oleracea* even though the absolute value of the concentration of carbohydrates and its decrease at night resembled to those in *S. oleracea*. The maximum activities of respiratory enzymes were also similar to those in *S. oleracea*. However, the leaves of *A. macrorrhiza* contained less soluble protein than those of *S. oleracea*. These results suggest that, in *S. oleracea*, the concentration of carbohydrates might determine the respiratory rate while such is not the case in *A. macrorrhiza*. The lower

respiratory rates in *A. macrorrhiza* might be due to a lower demand for ATP.^[27]

Biomolecular evidence for plant domestication

The question of the introduction of domesticated plants from the Sunda plate (South-east Asia) to Sahul (New Guinea, Australia and Tasmania) has been a subject of speculation and debate for decades. This paper reviews recent phylogenetic studies conducted with biomolecular markers on bananas (*Musa* spp.), breadfruit (*Artocarpus altalis*), sugarcane (*Saccharum* spp.), taro (*Colocasia esculenta*) and the greater yam (*Dioscorea alata*). Biomolecular evidence for plant domestication in Sahul is presented and discussed. Biomolecular markers reveal that for these crops at least, domestication has occurred in New Guinea and further east in Melanesia. This domestication produced cultivated genotypes that were selected from the endemic wild gene pools. These areas of domestication still are important centres of diversity for crop species that also exist in Asia. For most crops, genetic distances are very important between the two gene pools due to the geographic isolation of the two continental plates. The implications of these findings have obvious bearings on genetic resources programme strategies and future surveys.^[34]

Stress-Induced Alteration of Chlorophyll Fluorescence Polarization and Spectrum

The value of intrinsic chlorophyll fluorescence polarization, and the intensity in emission spectrum were investigated in leaf segments of *Alocasia macrorrhiza* under several stress conditions including different temperatures (25–50°C), various concentrations of NaCl (0–250 mM), methyl viologen (MV, 0–25 µM), SDS (0–1.0%) and NaHSO₃ (0–80 µM). Fluorescence emission spectrum of leaves at wavelength regions of 500–800 nm was monitored by excitation at 436 nm. The value of fluorescence polarization (P value), as result of energy transfer and mutual orientation between chlorophyll molecules, was determined by excitation at 436 nm and emission at 685 nm. The results showed that elevated temperature and concentrations of salt (NaCl), photooxidant (MV), surfactant (SDS) and simulated SO₂ (NaHSO₃) treatments all induced a reduction of fluorescence polarization to various degrees. However, alteration of the fluorescence spectrum and emission intensity of F685 and F731 depended on the individual treatment. Increase in temperature and concentration of NaHSO₃ enhanced fluorescence intensity mainly at F685, while an increase in MV concentration led to a decrease at both F685 and F731. On the contrary, NaCl and SDS did not cause remarkable change in fluorescence spectrum. Among different treatments, the negative correlation between polarization and fluorescence intensity was found with NaHSO₃ treatments only. We concluded that P value being measured with intrinsic chlorophyll fluorescence as probe in leaves is a susceptible indicator responding to changes in environmental conditions. The alteration of P value and fluorescence intensity might not always be

shown a functional relation pattern. The possible reasons of differed response to various treatments were discussed.^[35]

The Heterogeneity and Spatial Patterning of Structure and Physiology across the Leaf Surface

Leaf physiology determines the carbon acquisition of the whole plant, but there can be considerable variation in physiology and carbon acquisition within individual leaves. *Alocasia macrorrhiza* (L.) Schott is an herbaceous species that can develop very large leaves of up to 1 m in length. However, little is known about the hydraulic and photosynthetic design of such giant leaves. Based on previous studies of smaller leaves, and on the greater surface area for trait variation in large leaves, we hypothesized that *A. macrorrhiza* leaves would exhibit significant heterogeneity in structure and function. We found evidence of reduced hydraulic supply and demand in the outer leaf regions; leaf mass per area, chlorophyll concentration and guard cell length decreased, as did stomatal conductance, net photosynthetic rate and quantum efficiency of photosystem II. This heterogeneity in physiology was opposite to that expected from a thinner boundary layer at the leaf edge, which would have led to greater rates of gas exchange. Leaf temperature was 8.8uC higher in the outer than in the central region in the afternoon, consistent with reduced stomatal conductance and transpiration caused by a hydraulic limitation to the outer lamina. The reduced stomatal conductance in the outer regions would explain the observed homogeneous distribution of leaf water potential across the leaf surface. These findings indicate substantial heterogeneity in gas exchange across the leaf surface in large leaves, greater than that reported for smaller-leaved species, though the observed structural differences across the lamina were within the range reported for smaller-leaved species. Future work will determine whether the challenge of transporting water to the outer regions can limit leaf size for plants experiencing drought and whether the heterogeneity of function across the leaf surface represents a particular disadvantage for large simple leaves that might explain their global rarity, even in resource-rich environments.^[36]

Toxic activity

Toxic effects of crude *Alocasia macrorrhiza* lectin (AML) on larvae of cabbage butterfly (*Pieris rapae* L.), asiatic corn borer (*Ostrinia furnacalis* Guenea) and tobacco cutworm (*Spodoptera litura* Fab.) were studied by feeding, mouthparts infusion, pleopoda injection and potter spraying methods, respectively. The results showed that AML had certain stomach toxic, contact toxic to the above tested lepidopteran insects. The stomach toxic effect of AML on the larvae of cabbage butterfly was the strongest, of which the corrected mortality was 62.5% and 75% after infusing by AML 5d and 6d later, respectively. The results showed that the stomach toxic was the main toxic mode of AML to lepidopteran pests.^[36]

CONCLUSION

We recommend that people should be educated about these problems so as to prevent further poisonings and decrease use of this plant for decorative household purpose. This plant also has some pharmacological activity like antibacterial, antiviral, antioxidant and some more activities.

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