CHAPTER I

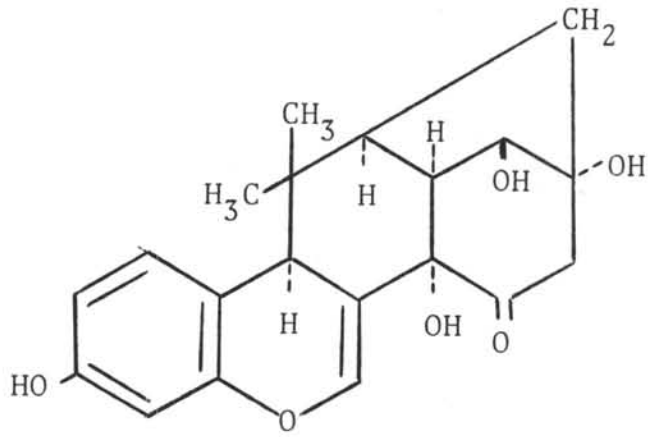
INTRODUCTION

For a long time, a tuberous root, locally known in northern part of Thailand as "Hua Guao Krüa" has been believed to produce a rejuvenating effect on old ages. It is sold by drug vendors in the preparation of local medicine not only in Thailand, but also in Burma, where it is called "pau-kse" and is recommended to old folks that one who consumed the drug, was believed to have a life span of 280 years[1].

There were many reports about the use of this drug and its effect, after compounding with honey, it was said to be used as tonic, to cure diseases and give long life[2]. In Luang Anusarn's pamphlet, different ways of compounding the drug were prescribed and many miraculous virtues of it was proclaimed, such as enlarging the mammary glands, inducing menstruation (in women of 60 - 80 years of age), regrowth of hair on bald head, producing juvenitity or improved complexion, reducing the swelling of goitre and improved impotent old men[3].

Name Guao Krüa is commonly applied to more than ten different plants, at least three kinds are sold in the market (Guao Khao, Guao Daeng, and Guao Dam). Butea superba Roxb.

is one kind of Guao Krüa which in the former was believed widely as Guao used in medicine. The vogue in its use as rejuvenator was interested to several parts of the world. Until in 1940 Schoellar, Dohrn, and Hohlweg had announced that highly potent oestrogenic substance was isolated from "Hua Guao Krüa", which arose to find the identity of the plant yielding the oestrogenic substance[4]. It was soon realised that the tuberous roots which yield oestrogenic substance was not Butea superba Roxb. but another woody climbing plant, superficially looks similar to Butea superba Roxb. having the same habitat and it was identified as a new species of Pueraria and named Pueraria mirifica Airy Shaw et Suvatabandhu[1,4]. The oestrogenic substance was isolated by Schoellar et al. and the tests on mice and rats were taken. Its effect was as twice potent as human oestrogen and they named the substance "Miroestrol". Up to 15 mg of the hormone Miroestrol content was extracted from 1 kilogram of tuber. Taylor et al. showed that the chemical composition is a monohydric phenol possessing one carbonyl and at least two alcoholic hydroxyl group, the formula is $C_{20}H_{22}O_6$ and the structure is as shown below[1,5].



MIROESTROL

Bishop had organised a carefully controlled trial of Miroestrol from Pueraria mirifica tuber to hospital patients in London. The result was unsatisfied because of the patients' complaints about headaches, queasiness, enlarged breasts and vomiting, although the dosage was lowered but the undesirable side effect still further[1]. So Miroestrol itself would not be acceptable for use in medicine, because it was impossible to minimize undesirable side-effects without lowering it potency, but on a clinical trial by Dr. Dhara Sukhavachana with crude extract of Hua Guao Krüa, the result was satisfied. The hormone was prepared by extracting powdered drug tuber with 95% ethyl alcohol, evaporated almost to dryness and the residue mixed with syrup and made up to required volume with distilled water and taken orally. One castrated patient took altogether 480 grams of the drug powder within 15 days

without ill-effects; two days after the course ended, it was found that the womb was 2 cm longer than in the previous condition and the tissue was thickened and proliferated attaining a stage corresponding to the middle of 28 days' cycle[6].

Another trial was carried out in a case of vegetative ovarian insufficiency with secondary amenorrhoea for 16 months. Crude extract of 200 grams of powder was administered in 10 successive days, four days after the course ended, the uterus was increased in length and endometrium was thickened and also transformed into a secreting membrane. Only five days afterwards, the patient bled and the flow ceased spontaneously for four days. It was interested that 20 days later the subject also menstruated once again without repeating treatment. No toxic effects observed but both subjects felt breasts congestion few days after receiving the drug to the close of their induced periods[6].

From these trials, it indicated that Pueraria mirifica tuber can be used as the source of sex hormone, but now it was subsided because of the rumour that it caused death, which could be regarded by two reasons, over dose or wrong plant taken[5].

Many plants look similar to Pueraria mirifica, some kind is very toxic, some may be useless. In order to prevent the fatal accident caused by using wrong plant, the identity of Pueraria mirifica Airy Shaw et Suvatabandhu may

play an important role in aiding the collector as well as the consumer to obtain the exact plant which really yields miracle effects.

PURPOSE AND SCOPE OF INVESTIGATION

Hua Guao Krüa was believed to have a rejuvenating effect long time ago, and it has been proved to have a worthy female sex hormone. A consideration of correct identity of the plant to differentiate it from the similar plants would be of great value.

Scopes of investigation are as follows :

1. To show the morphology and the histology of the plant.
2. To show foreign organic matters in the plant in order to afford the purity control of powdered drug.
3. To determine the palisade ratio, the stomatal index, stomatal number, vein-islet number, veinlet termination number, and to make use of such factors as diagnostic aids for identifying the specimen.
4. To illustrate the pattern of chemical constituents as a means of identification of drug by using chromatographic facility.

Definition of terms used

Palisade Ratio is the average number of palisade cells beneath one epidermal cell using four contiguous epidermal cells for the count[7].

Stomatal Number is the average number of stomata per square millimeter of epidermis[8].

Stomatal Index is the percentage which the number of stomata form of the total number of epidermal cells, per unit area, each stoma being count as one cell[7].

$$\text{Stomatal Index} = \frac{S}{S+E} \times 100$$

Where S = the number of stomata in unit area

and E = the number of epidermal cells in the same area.

Vein-islet Number is the average number of vein-islet per square millimeter of a leaf surface[7].

Veinlet Termination Number is the number of veinlet termination per square millimeter of leaf surface[9].

Survey of Literatures

Palisade Ratio

Zorning and Weiss introduced the use of average number of palisade cells lying under one cell of the upper epidermis as a method of identifying leaves of members of Compositae. Although the number of palisade cells per unit area increased successively from the base of the leaf to the apex, but there was a corresponding diminution in the area of the epidermal cells and the rate remained constant[10]. Silverman and Dunn found that there is no variation of a single leaf whether done on the tip, midrib or base[11].

Wallis and Forsdike also found that palisade ratio did not change with the age of leaf, habitat of the plant from year to year within either of species[12]. There were many investigators after Zornig and Weiss, who confirmed this work. Until in 1933, Wallis and Dewar, who extended to pharmacognostical examinations of leaf, introduced the term "Palisade Ratio". Wallis indicated that the palisade ratio can be used for powdered drug in which portions containing four adjacent epidermal cells are easily found[13]. This was confirmed by Hassan, and it was also stated that the statistically treated results have proved to be of diagnostic value in identification of closely related plants[14]. Markwell and Cross made use of palisade ratio as a means of detecting Ailanthus, when mixed as an adulterant of rubbed Spearmint[15].

Stomatal Index and Stomatal Number

Timmerman investigated the frequency of the occurrence of stomata on leaves, and suggested that the number of stomata per unit area of epidermis would be a useful method of distinguishing between different leaves; but the values for the stomatal number extend over wide limits, vary under different environments, and different conditions, so it was useless[16].

In 1927, Salisbury showed that there was a high corre-

lation coefficient between the number of stomata and the number of epidermal cells per unit area of leaf surface of given species. So he proposed the definition formula for calculation of Stomatal Index. He defined the stomatal index as the percentage which the number of stomata form of the total number of epidermal cells[17]. Rowson and Forsdike have shown that Stomatal Index value may be used to distinguished between leaves of cogeneric species[18,19].

Vein-islet Number

The term vein-islet was introduced by Benedict in 1915, and he defined as the size of aggregation of photosynthetically active cells surrounding by the veinlet[20]. Eames defined as the minute area encircled by the ultimate division of the conducting strands of leaf[21]. Benedict has suggested that the average vein-islet area has more direct physiological significance[20], and Ensign has shown that it is not related to the age of the plant[22]. Levin found that it was more practical to express the results in the terms of the number of vein-islet number. He found this to be a specific character independent of the total area of leaf, and should be used for differentiation of species[23]. Forsdike has shown that the appearance of leaf venation under a hand lens, when viewed by transmitted, and by relfected light, may be used to distinguish medicinal leaves from their

common adulterants[19]. Smith et al. reported that all or almost all of the cells of a typical leaf enlarge and mature more or less simultaneously, the cells having been formed relatively early with cell divisions then ceasing[24]. These early cell divisions occur chiefly in the apex of the leaf primodium, but later divisions occur throughout until the leaf has assumed the form and shape of a mature leaf. The increase in size is due, in part, to the enlargement and maturation of cell already present and not primarily to an increase in the number of cells of the conducting tissue originally present in the immature leaf, merely increase in size during the period of leaf expansion. Few or no additional cells are developed in the central portion of the lamina, the area usually used for vein-islet determination. Levin suggested to count four square millimeters area of leaf[23]; and Wallis stated that the result calculated from four contiguous square millimeters was accurate[7]. Ensign found that vein-islet number remains constant within a given species regardless of age, and this was accepted by Levin[22,23].

Veinlet Termination Number

Hall and Melville suggested that the number of veinlet termination per square millimeter of leaf surface may be used to differentiate coarse powders of certain leaves

belonging to cogeneric species[25]. They defined the term veinlet termination as the ultimate free termination of a veinlet or branch of a veinlet, and they showed the applications for the identification of drug. They also showed that veinlet termination number is not significantly dependent on the position at which it is determined[26].

Chromatography

Attempts have been made by many investigators to use the chemical components as a means of identification or classification of living organisms. Plant taxonomists are well reversed in morphological characteristics; and found that chromatography is useful for classification of plants. The biochemists' knowledge is generally limited to the chemical nature of plant. A combination of the two approaches might conceivable further considerably the classification of any vegetable species. The principle formulated by De Candolle that only specific plant constituents (tannins, bitter principle, volatile oils, resins, etc.) are useful for classification purpose has been widely accepted (Tyler). Mez et al. have used antigen-antibody techniques in studying relationships of plants and had published a system of classification based on their results. Data from serodiagnosis, used in conjugation with other characters, no doubt are valuable for classification, but it has been pointed out

that the protein content of the juice of plant is no more an infallible index of relationship than is any other single feature of the plant[27]. Benson and Greshoff tried to classify the plants by combination of morphology and chemical constituents, he suggested that a short chemical description of the plants should be added to the genus of species[27]. Mc. Nair studied the occurrence of gums, tannins, resins, oils and fats in Bignoniaceae, Solanaceae, Scrophulariaceae and Martynaceae and pointed to the possible usefulness of these constituents for establishing taxonomic relation. He consulted all data available to him and came to conclusion that, in general, the evolution of chemical substances in plants has followed evolution of the plant themselves[27]. Mirov suggested that the most promising groups of plants should be studied with regard to chemical and botanical relationships[27]. However, rapid progress in these fields can be expected in the future because the chromatographic method have been developed and applied. Horhammer and Wagner had shown that paper chromatography could give valuable help in Pharmacognosy, particularly of materials that were difficult to distinguish in morphology and histology[27].

König suggested about the use of ultraviolet light to aid in locating the fluorescent compounds is very useful[27]. Fox had pointed out the advantages of two-dimensional chromatographic analysis over one-dimensional analysis. The

great resolution obtained, he maintained, insured better results effort to provide finger-print identification of plant species and crude drugs (Mc. Phail)[27].

Two-dimensional Chromatography

The advantage of this technique is that the area of the paper is larger and thus a complicated mixture of substances (amino acids, organic acids, nucleic acid component, etc.) may be more completely resolved. Descending method in chromatographic system is preferable for the slow moving solvent or substances that fail to migrate[28].