

DETERMINATION OF BIOACTIVE PEPTIDE (4.3 KDA) AS AN APHRODISIAC MARKER IN SIX MALAYSIAN PLANTS

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Aphrodisiac is defined as a substance that increases sexual activity and libido and/or improves sexual performance. Plants are claimed to possess aphrodisiac property by many traditional medicine systems including Ayurveda, Chinese, Malay and Unani medicines. Malaysian plants such as *Eurycoma longifolia*, *Polyalthia bullata*, *Smilax myosotiflora*, *Terminalia catappa*, *Labisia pumila* and *Rafflesia* sp. are claimed to have aphrodisiac property and have been used in Malay traditional medicine. The aphrodisiac property of the plants is only a claim and has not been scientifically proven except for *E. longifolia* (Ang *et al.* 2000) and *T. catappa* (Ratnasooriya & Dharmasiri 2000). The bioactive peptide (4.3 kDa) (patented: PI 20003988, MAL; 10/362697, USA; 01920972.5, EUROPE and 2002-522919, JAPAN) isolated from *E. longifolia* (tongkat ali) is a potential phytoandrogen, which has been reported to increase the testosterone level in rat leydig cells (Sambandan *et al.* 2004). The patent describes the methods for isolating the bioactive peptide from an aqueous extract of *E. longifolia*, identifying the peptide in the aqueous extract and use of the bioactive peptide in increasing testosterone synthesis. Methods for treating male infertility and sexual dysfunction using purified bioactive peptide are also described. In the present study, we used the Surface Enhance Laser Desorption Ionization Mass Spectrometer (SELDI-MS) to detect the presence of the 4.3 kDa peptide in the studied plants as an aphrodisiac marker.

Eurycoma longifolia or locally known as tongkat ali from the family Simaroubaceae is a tree about 7–10 m in height and is indigenous to South-East Asia. It is reported that different fractions of *E. longifolia* (chloroform, methanol, water and butanol) enhanced the libido in sexually experienced male rats (Ang & Sim 1997) and also the initiation of sexual performance of inexperienced male rats (Ang *et al.* 2000). *Eurycoma longifolia* is also reported to possess antiplasmodial activity due to its quassinoids content (Chan *et al.* 2004).

Smilax myosotiflora is a climber from the family Smilacaceae and is found throughout the tropics and in the northern warm temperature regions. It is locally known as ubi jaga and has a reputation as an aphrodisiac among the Malays and aborigines. The rhizome is chewed with betel (Burkill 1966).

Polyalthia bullata, popularly known as tongkat ali hitam, is a small tree found in Peninsular Malaysia and Borneo from the family Annonaceae. According to Burkill (1966), there is no precise information of it as a medicine but the name tongkat ali suggests that it has aphrodisiac property.

Terminalia catappa is a tall tree, reaching 30.5 m in height and is native to Malaysia and the Pacific. It is locally known as ketapang from the family Combretaceae. Seeds of *T. catappa* possess potent aphrodisiac property (Ratnasooriya & Dharmasiri 2000). Other parts of the plant that are used medically include fruit and tannin from the bark (for dysentery), leaves (for rheumatic joints and dysentery) and young leaves (headache and colic) [Burkill 1966]. The kernel of the fruit mixed with beeswax can stop putrid exudation and bloody faeces (Perry & Metzger 1980).

Rafflesia sp. is a small genus of parasite from the family Rafflesiaceae whereby the flower and fruit are the only parts that come outside the tissues of the host plant (Burkill 1966). Locally known as bunga pakma and patma (Java), it is found in Sumatra and Peninsular Malaysia (hills of Perak and northern Pahang). The aborigines Sakai believe that it can expedite delivery in childbirth (Burkill & Haniff 1930) and in Java, women used it as an aphrodisiac (Burkill 1966).

Labisia pumila from family Myrsinaceae is a slightly woody plant found in western Malaysia. Commonly known as kacic fatimah or selusuh fatimah, it is used by the Malays in childbirth. A decoction of the plant is given to expedite labour and after childbirth. Although kacic fatimah is normally used by women, it has also been reported that men from several ethnic groups in Sarawak consume it to maintain and increase stamina (Runi 2001). It is also used for flatulence, dysentery, dysmenorrhoeal and gonorrhoea (Burkill 1966).

Roots (*E. longifolia* and *P. bullata*), tubers (*S. myosotiflora*), seeds (*T. catappa*), flower buds (*Rafflesia* sp.) and whole plants (*L. pumila*) were cut into small pieces, ground and individually subjected to micro extraction protocol in which they were soaked in 50% ethanol for one hour at 60 °C. The extracts were concentrated by oven drying at 40 °C and gave the following yields: 4.6, 5.1, 5.8, 5.0, 32.0 and 12.0%

respectively. A total of 0.5 mg ml⁻¹ extracts were then subjected to SELDI-MS analysis. SELDI analysis was performed using hydrophobic (H4) ProteinChip array provided by Ciphergen Biosystems (Fremont, CA, USA) and α -cyano-4-hydroxycinnamic acid (CHCA) as energy absorbing molecules (EAM), following the method provided by Ciphergen Biosystems. The ProteinChip arrays were analysed in the Protein Biological System II ProteinChip Reader and the data were analysed using ProteinChip Biomarker software version 3.0 (Ciphergen Biosystems Inc.). All data were normalized by total ion current normalization function.

The aphrodisiac activity in the plants was determined by the presence of 4.3 kDa peptide based on report by Sambandan *et al.* (2004). The bioactive peptide that is found in *E. longifolia* could increase the testosterone level in rat leydig cell. With reference to *E. longifolia* as a control, the peak of 4.3 kDa was also detected in three other species: *S. myosotiflora*, *Rafflesia* sp. and *L. pumila* using H4 ProteinChip array (Figure 1). This suggests that these three plants possess a bioactive peptide similar to that in *E. longifolia*. The 4.3 kDa bioactive peptide peak was not detected in *T. catappa* even though the plant has been reported to have aphrodisiac activity scientifically. The presence

of 4.3 kDa in *E. longifolia* and absence in *T. catappa* may contribute to certain aphrodisiac properties, hence, the dissimilar action of aphrodisiac activity. *Eurycoma longifolia* is reported to enhance the libido in sexually experienced male rats (Ang & Sim 1997), whereas *T. catappa* contributed to the prolongation of the ejaculatory latency (Ratnasooriya & Dharmasiri 2000).

The reproducibility of the 4.3 kDa protein in *E. longifolia* was reported earlier by Nurhanan *et al.* (2004). From SELDI-MS analysis, they found that the 4.3 kDa protein was present in most parts of the plant including root, bark, callus and tissue cultured plantlets.

In order to validate this bioactive peptide (4.3 kDa) as an aphrodisiac marker, pure 4.3 kDa bioactive peptide will be isolated from *S. myosotiflora*, *Rafflesia* sp. and *L. pumila*, and subjected to animal assay for aphrodisiac activity. The pure bioactive peptide will then be sequenced and compared with the peptide sequence of *E. longifolia* isolated earlier by Sambandan *et al.* (2000). This is to confirm whether the bioactive peptide in *S. myosotiflora*, *Rafflesia* sp. and *L. pumila* is the same as that in *E. longifolia*. The discovery of this biomarker will allow fast and reliable screening procedure for aphrodisiac activity in plants.

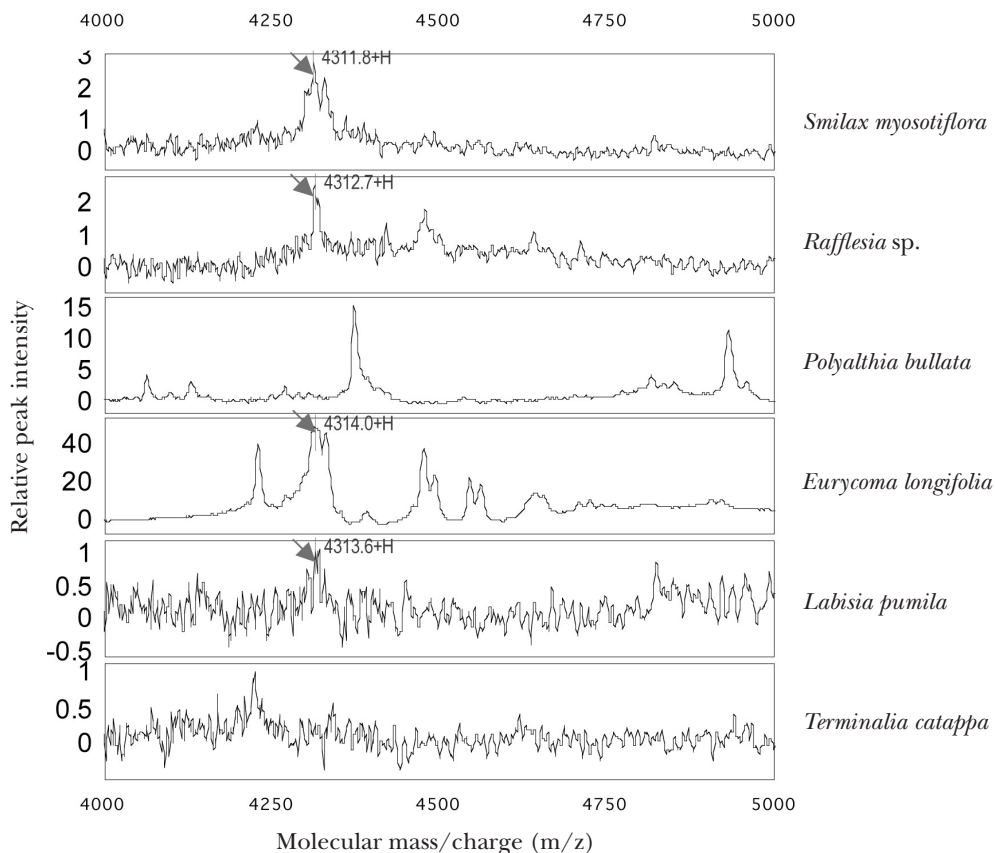


Figure 1 SELDI-MS data obtained with H4 ProteinChip array loaded with 50% ethanol extract of six plants species. Arrow indicates the 4.3 kDa bioactive peptide peak.

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