EFFECTS OF *Nigella sativa*, LINOLEIC ACID AND *Eurycoma longifolia* ON RABBIT PENILE ERECTION

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EFFECTS OF *Nigella sativa*, LINOLEIC ACID AND *Eurycoma longifolia* ON RABBIT PENILE ERECTION

by

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Dedicated to my beloved parents and husband,

Sahdom Mohd Yussuf

Siti Rahmah Jamih

&

Sidi Hanafee Sidi Omar

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LIST OF SYMBOLS

α	alpha
m	meter
cm	centimeter
β	beta
µm	micrometer
ml	milliliter
g	gram
rpm	rotation per minute
‘c	celsius
mg	milligram
kg	kilogram
sem	standard error of the mean
mm	millimeter
%	percentage

LIST OF ABBREVIATIONS

ED	Erectile dysfunction
ARASC	Animal Research and Service Center
cAMP	Cyclic adenosine monophosphate
CCl_4	Carbon tetrachloride
cGMP	Cyclic guanosine monophodieterase
CN	Cavernous nerve
CNS	Cavernous nerve stimulation
EL	*Eurycoma longifolia*
FSH	Follicle stimulating hormone
GTP	Guanosine triphosphate
HCG	Human chorionic gonadotropin
HDL	High density lipoprotein
ICP	Intracavernosal pressure
LA	Linoleic acid
LAB	Lactic acid bacteria
LDL	Low density lipoprotein
LGM	Liquid chromatography mass spectrometry
MDR	Multi drug resistant
NO	Nitric oxide
NOS	Nitric oxide synthase
NS	*Nigella sativa*
NSM	*Nigella sativa* microemulsion
NSN	*Nigella sativa* nanoemulsion

NSO	*Nigella sativa* oil
PCS	Photon correlation spectrometer
PDE-5	Phosphodiesterase enzyme-5 inhibitor
SAP	Systemic arterial pressure
SN	Sodium nitroprusside
UK	United kingdom
WHO	World Health Organization
L-NAME	NG-nitro-L-arginine monomethyl ester

KESAN *Nigella sativa*, ASID LINOLEIK DAN *Eurycoma longifolia* PADA EREKSI PENIS ARNAB

ABSTRAK

Nigella sativa (NS) *a*nd *Eurycoma longifolia* (EL) memainkan peranan penting bukan sahaja pada kesuburan tetapi juga dalam aktiviti penjagaan gastrousus, anti-tumor, anti-kebimbangan, anti-bakteria, anti-inflamasi, anti-parasitik, anti-oksidasi and penurunan suhu badan. Tumbuhan ubatan traditional mempunyai agen penyembuhan utama dimana merupakan teknik yang selamat, atau dalam sesetengah kes, hanya satu-satunya rawatan yang berkesan. Tiada kajian setakat ini yang melaporkan tentang kedua-dua tumbuhan ubatan ini didalam ereksi penis, namun terdapat beberapa syarikat dan kajian menyatakan tentang potensi terutamanya keberkesanan terhadap sistem reproduksi. Maka, kajian ini mengkaji kesan tumbuhan ubatan ini pada ereksi penis arnab. Di dalam rawatan NS, haiwan telah dirawat dengan minyak *Nigella sativa*, mikroemulsi *Nigella sativa* dan nanoemulsi *Nigella sativa* dan *Eurycoma longifolia*. *Nigella sativa* telah diberikan secara segar kerana tiada ujian stabiliti dijalan pada penyediaan emulsi ini. Penentuan size partikel minyak/air *Nigella sativa* emulsi (NSE) juga dijalankan, ini penting terutamanya bagi keadah intravena. Data menunjukkan NSN ialah formulasi yang berkesan dibandingkan dengan NSO dan NSM bagi tempoh ereksi penis yang panjang. Walau bagaimanapun, pemberian EL secara bersendirian menunjukkan ereksi yang baik apabila dibandingkan dengan positif perencat PDE-5 sildenafil. NSN juga menunjukkan penurunan pada tekanan arteri sistemik didalam kehadiran activiti vasodilatasi tetapi tidak ketara. NSN memberikan peningkatan yang signifikan ($p<0.05$) dalam kedua-dua kumpulan sendirian dan dengan kehadiran sodium nitroprusside (SNP), manakala pemberian EL

secara bersendiri dilihat tidak menyebabkan sebarang perubahan dalam ereksi walaupun dengan kehadiran SNP dan NG-nitro-L-arginine monomethyl ester (L-NAME). NSN berkemungkinan mempunyai mekanisma ereksi tersendiri. Selain itu, kedua-dua agen menunjukkan respon segera selepas 5 minit pemberian secara oral NSN (30 mg/kg), EL (0.1 mg/kg) untuk kedua-dua ekstrak 50:50 dan 100:0.Keputusan kajian ini menyokong hipotesis bahawa kedua-dua tumbuhan ubatan ini mempunyai potensi untuk menyebabkan respon segera dalam ereksi penis dan peningkatan yang baik dalam ukuran panjang termasuk tempoh ereksi. Kajian ysng lebih lanjut terhadap mekanisma kedua-dua agen terhadap ereksi penis diperlukan untuk mendapatkan penjelasan yang lebih terperinci.

EFFECTS OF *Nigella sativa*, LINOLEIC ACID AND *Eurycoma longifolia* ON RABBIT PENILE ERECTION

ABSTRACT

The *Nigella sativa* (NS) *a*nd *Eurycoma longifolia* (EL) not plays role in fertility but gastroprotective, anti-tumor, anti-anxiety, anti-microbial, anti-inflammatory, anti-parasitic, anti-oxidant and anti-pyretic activities. No previous has thorough investigation on both medicinal plants giving spontaneous erection in penis, while studies claim on their potential as well as their effectiveness on reproduction system. Thus, this study investigated these plant's effect on penile erection of rabbit. In *Nigella sativa* treatment, animal was treated with *Nigella sativa* oil , *Nigella sativa* microemulsion and *Nigella sativa* nanoemulsion (NSN) and *Eurycoma longifolia* (EL). *Nigella sativa* were administered freshly to the rabbits cause no stability assessment done for this preparation. The determination of oil/water *Nigella sativa* emulsion drug particles size was done, mainly because intravenous introduction. Data expressed the NSN is effective formulation compared to NSO and NSM with longer duration in erection of penis. Although, EL alone shown greater erection when given alone in comparing with positive PDE-5 inhibitor sildenafil. NSN also appear decrease the systemic arterial pressure in the present of vasodilation activity but not adversely. NSN give significant increase ($p<0.05$) in both alone and in the present of sodium nitroprusside (SNP) while EL alone showed no changes in erection in present of SNP and NG-nitro-L-arginine monomethyl ester (L-NAME). NSN possibly have its own independent mechanism in erection. In other hand, both agents, show immediate respond after 5 minutes oral administration of NSN (30 mg/kg), EL (0.1 mg/kg) for both extraction of 50:50 and 100:0. The results of the present study support the

hypothesis that both *Nigella sativa and Eurycoma longifolia* has potential to induce immediate respond in erection of penile and greater improvement in length as well as duration of erection. Further study on mechanism of both agents on penile erection also need to be elucidate.

CHAPTER ONE

INTRODUCTION

Sexuality is a complex, multi-dimensional phenomenon that incorporates biological, physiological, interpersonal and behavioural dimensions. This series also involve neuronal, vascular and local genital changes. There is four phases of this event; excitement, plateau, orgasm and resolution. The excitement series into latency and tumescence, plateau into eredtion and rigidity, orgasm into emission and ejaculation and resolution into detumescence and refractoriness.

In the normal male sexual, the penile smooth-muscle relaxation will occur when the corpora cavernosa contain of a network of a smooth-muscle cells and endothelial cells surrounded tunica albuginea release nitric oxide (NO) by the enzyme NO synthase (NOS). It will cause the penile tonically contracted. When the NO diffuses into the smooth muscle, it will stimulates the guanyl cyclase. An increase in the cyclic GMP (cGMP) concentration stimulates the release of protein kinases. This causes the potassium channels to open and the calcium channels to close, producing hyperpolarization and, ultimately, smooth-muscle relaxation (figure 1.1) (Cellek, 2002; Ashok *et al.*, 2006). The relax smooth-muscle cell will allows the sinusiods to engorge with blood and causes the penis to become tumescent. As the sinusoids expand, they compress the subtunical venous plexus, which causes an erection.

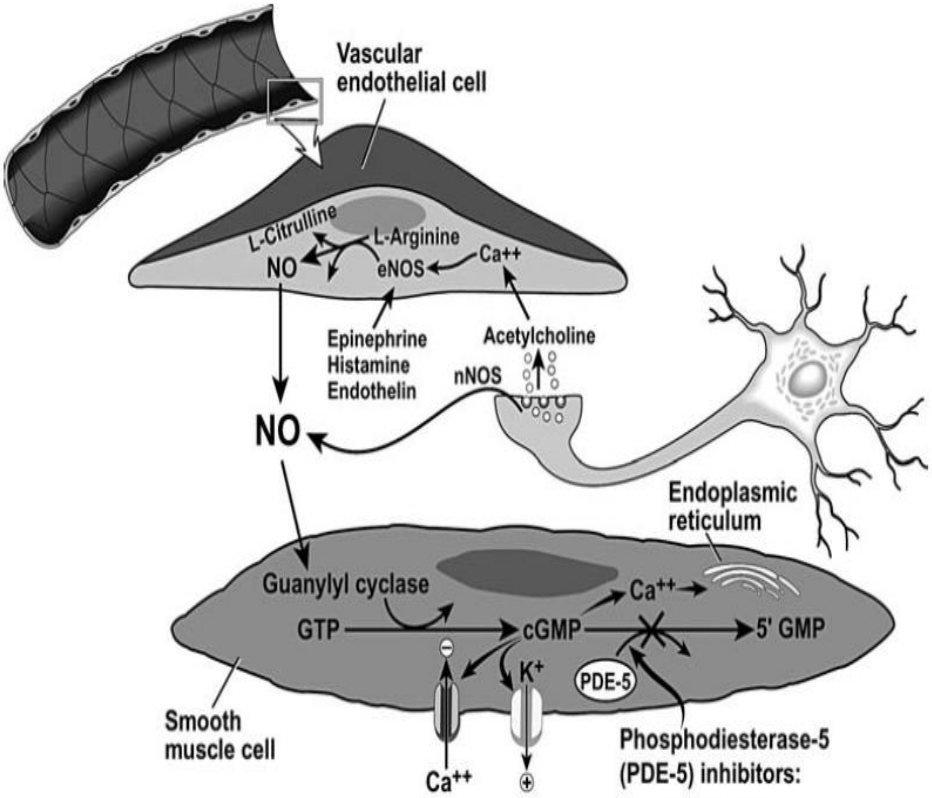

Figure 1.1: Physiological mechanism of normal erection. eNOS, endothelial NOS; GTP, guanosine triphosphate; nNOS, neuronal NOS; PDE, phosphodiesterase enzyme (Cellek, 2002; Ashok *et al.*, 2006).

In Malaysia, some people preferred to have the alternative ways in their sexual fulfillment. Some of the documented clinical trials on herbal medicines to treat fertility problems are *Nigella sativa*, *Eurycoma longifolia,* Badam, *Epimedium grandiflorum* (Horny goat weed), *Panax quiquelotius* (Ginseng), *Ginkgo biloba*, *Curculigo orchodes* (Xian Mao), *Centella asiatica* (Gotu Kola), flower pollen extracts, *Ficus carica, Achyrocline satureioides (Asteraceae), Anacardium ocidentale (Anacardiaceae), Anemopaegma arvense (Bignoniaceae), Aristolochia cymbifera (Aristolochiaceae), Arrabidaea chica (Bignoniaceae), Artocarpus integrifolia (Moraceae), Davilla rugosa (Dilleniaceae), Erythroxylum viceniifolium (Erythroxylaceae), Hippeastrum psittacinum (Amaryllidaceae), Mimosa pudica (Fabaceae), Mimosa tenuiflora (Fabaceae), Mucuna pruriensis*

(Fabaceae) ,Nymphaea ampla (Nymphaeaceae), Passiflora sp. (P. edulis, P. alata and P. caerulea), Paulinia cupana (Sapindaceae), Pfaffia paniculata (Amarantaceae), Schinus terebinthifolius (Anarcadiaceae), Trichilia catigua (Meliaceae)and Turnera diffusa (Turneraceae) (Yakubu *et al.*, 2005; Qinna *et al.*, 2009).

1.1 Anatomy of Penis

The penis is a specialized organ composed of complex vascular tissue responsive to neurological impulses that create penile rigidity. The penis is consists of two pairs of corpora cavernosa. The corpora cavernosa are separated cylinders, without visible septum and connected by free-vascular networks (Robert and Tom, 2005; Andersson, 2011). The corpora are made up of smooth muscle tissue and lined by endothelial cells. Corpus spongiosum that contains the urethra located inferior to corpora cavernosa and is contiguous with the glans penis. The corpora spongiosum is composed of similar spongy tissue but is surrounded by a less rigid thinner tunica albuginea resulting in less rigidity on activation.

The corpora cavernosa also known as penile erectile bodies are surrounded by a thick fibrous sheath and non-distensible (tunica albuginea), composed of elastic fibers and collagen that support and maintain the rigidity of erectile function. Surrounding the tunica albuginea is a second gossamer layer of fascia called Buck's fascia. Within these functional structures courses a complex vascular sinusoidal network of spongy tissue that activates erection (Pejman and Stanley, 2013).

Corpora cavernosa received the blood supply from the internal iliac arteries and courses to the internal pudendal arteries that terminate in the arterioles to the penis. These arterioles consist of the dorsal artery to the penis outside the tunica albuginea, the bulbo-urethral artery that travels within the corpus spongiosum lateral to the

urethra and the central cavernosal arteries that travel in the central portion of each of the paired corpora cavernosa and supply the blood for erection (Gerard and Bryan, 2006).

A proximal perineal branch of the pudendal artery provides blood supply to the perineal skin and scrotum. The dorsal artery of the penis is responsible for the blood supply of the penile skin and glans penis and contributes little to erectile function. On the other hand, the cavernosal arteries enter the corpora cavernosum at the hilum of each corpus cavernosum and further expand to multiple small helicine arteries that drain directly into the vascular lacunar spaces of the corpus cavernosum. Accessory pudendal arteries may also provide blood supply to the penis. These variable arteries may originate from the obturator artery, inferior pudendal artery, iliac trunk, or inferior gluteal artery and frequently lie close to the capsule of the prostate. Venous drainage of the penis is important both anatomically and functionally (Gerard and Bryan, 2006).

The lacunar spaces or vascular sinusoids of the corpora cavernosa drain through subtunical veins beneath the tunica albuginea into the emissary veins by way of the deep dorsal vein of the penis. The deep dorsal vein culminates in the periprostatic venous plexus superior and lateral to the prostate. The superficial dorsal penile vein lies above Buck's fascia and provides drainage predominantly for the penile skin culminating in the saphenous vein. The proximal penile shaft and proximal corpus cavernosum drains through veins exiting the crura of the corpora cavernosa termed the crural veins that join to form the internal pudendal vein. As with most venous systems, the venous drainage of the penis is variable and complex and has multiple intercommunications (Gerard and Bryan, 2006).

Sensation is not the only function provided by nerve supply to the penis control, but also vascular supply to the penis. Medial pre-optic in the hypothalamus

serves as the ultimate central nervous system to psychological and tactile stimuli. Erectile function is primarily controlled by a pair of sympathetic nerves from S2-4 nerve roots whereas ejaculation and emission are controlled by the sympathetic nerves from T1 I-L2 control detumescence. These autonomic nerve fibers form the pelvic plexus of nerves and enter the penis within the cavernous nerves that course lateral and inferior to the prostate. During nerve sparing radical prostatectomy, usually these nerves are preserved. Aside from the autonomic nervous system, peripheral nerves form sensory and motor elements through a reflex arc in the sacral spinal cord at Onuf's nucleus. Sensory element which is responsible for erectile function are maintained by peripheral nerves. (Gerard and Bryan, 2006). Refer to figure 1.2 to figure 1.4 for male organ reproduction structure.

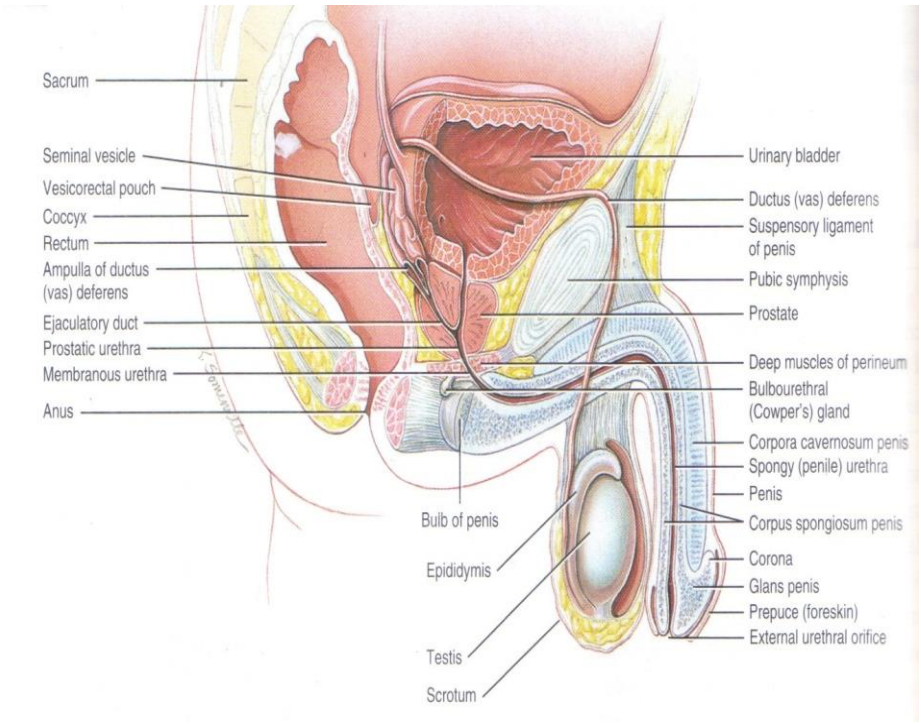

Figure 1.2: Sagittal section of male organ reproduction and surrounding structures (Gerard and Bryan, 2006).

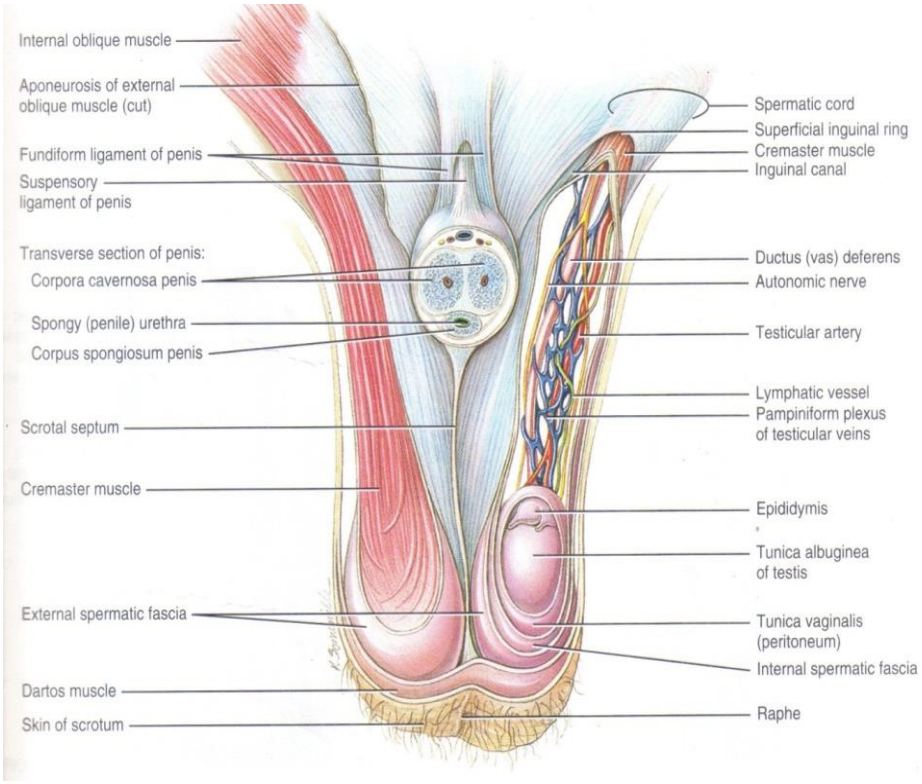

Figure 1.3: Anterior view of scrotum and testes and transverse section of penis (Gerard and Bryan, 2006).

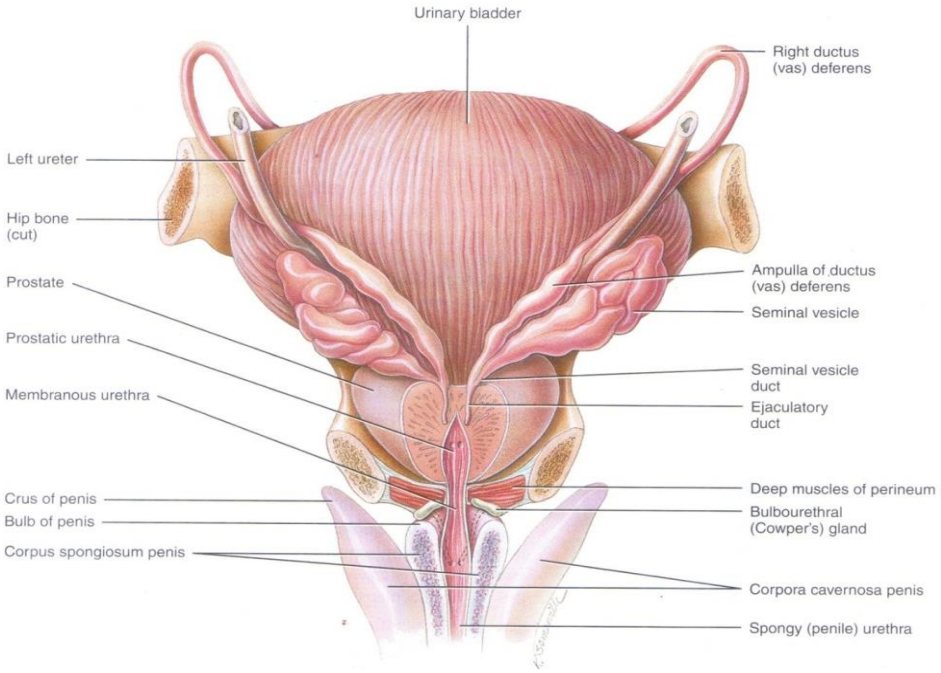

Figure 1.4: Posterior view of male accessory organs of reproduction (Gerard and Bryan, 2006).

1.1.1 Physiology of Erection

Excitatory stimuli will first pass the autonomic nervous system through a series of neurotransmitters that subsequently will produce erections. Neurotransmitters (acetylcholine) and vasoactive intestinal polypeptide (VIP) are two main components that associated with erectile function. In the corpora cavernosa, nitric oxide (NO) is produced from the precursor L-arginine by the enzyme nitric oxide synthase (NOS), is the major component for erection. It functions through the cyclic guanosine monophosphate (cGMP) system and the activation of PDE-5 and cyclic adenosine monophosphate (cAMP) mediated pathway. The secondary transmitter eGMP is ultimately responsible for smooth muscle relaxation in the corpus cavernosum and producing erection. The relaxation is mediated by calcium efflux from the smooth muscle cell (Lue, 2000; Andersson, 2001).

After the tactile stimulus received by CNS, secretion of NO is initiated and smooth muscle sinusoids relaxation of the corpus cavernosum occur. Initial stimuli produce dilation and relaxation of the central cavernosal artery and the helicine arterials that produce increased influx of blood to the lacunar spaces of the corpora cavernosa. As this increased flow and downstream relaxation continues, the lacunar spaces or sinusoids fill with blood, increase in size, and produce decreases in venous outflow by compressing the subtunical venous channels against the tunica albuginea producing a high-pressure rigid erection. Rigidity is increased to a level beyond that of the abdominal aorta from the contraction of the perineal muscles, especially the bulbocavernosus and ischiocavernosus muscles

1.2 Erectile Dysfunction

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain an erection adequate for satisfactory sexual activity. After premature ejaculation, it is the most common disorder of sexual function in men, affecting nearly 30 million individuals in the Asia (Pejman and Stanley, 2013). Some peoples might be confused with the term of ED, where they evaluate their ED condition by the size of penis and making sure their size is five to seven inches long and four to six inches in circumferences (Alan *et al.*, 2006).

Toshinobu *et. al.,* (2012) reported that the majority of infertility problem caused by sexual attitudes and interests, sexual partners and especially masturbation among young peoples. Erectile dysfunction is multifactorial and has been typically classified by the primary presumed cause: vasculogenic, psychogenic, neurogenic and endocrinology disease (McVary, 2007). Any condition or injury that impairs the transmission of impulses along the psychogenic or reflexogenic neurological pathway may be associated with neurogenic erectile dysfunction. The penis is innervated by the dorsal penile and perineal nerves. These nerves are a continuation of sympathetic and parasympathetic autonomic nerves as well as sensory and motor somatic nerves. The somatic sensory system is responsible for the specialized structures that transmit information about the external environment. There are four major classes of somatic sensation: pain, temperature, position sense and touch-pressure sensation (table 1.2 and table 1.3) (Bleustein *et al.*, 2002; Ian, 2013; Agarwal *et al.*, 2006).

These stimuli are transmitted in the autonomic nervous system through both large (Aa and Ab) and small (Ad and C) caliber nerves. Currently, many tests are available to evaluate the sensory afferent nerves from the penile skin (Bleustein *et al.*, 2002; Ian, 2013).

Table 1.1: The predisposing, precipitating and maintaining factors of penile erection (Ian, 2013).

Predisposing factors	i. Educational issues (e.g; poor sexual education) ii. Cultural Issues (e.g; restricted upbringing) iii. Traumatic experience iv. Lifestyle issues (e.g; marital, financial stress)
Precipitating factors	i. Organic disorders ii. Extra – marital affairs iii. Unreasonable expectations iv. Depression and anxiety v. Loss of partner (e.g; Widower's syndrome)
Maintaining factors	i. Performance related anxiety ii. Diminished attraction to partner iii. Eductional issues (e.g; poor sexual education) iv. Fear of intimacy

Table 1.2: Neurogenic causes of erectile dysfunction (Ian, 2013).

Diseases of the central nervous system	i. Multiple sclerosis ii. Spinal cord injury iii. Depression iv. Parkinson's disease v. Cerebrovascular disease
Disease of the peripheral nervous system	i. Cauda equine compression ii. Prolapsed inter-vertebral disc iii. Peripheral neuropathy (e.g; diabetes, alcohol) iv. Surgical injury to pelvic

1.3 Traditional Medicine for Erectile Dysfunction

Traditional medicine has been established since centuries among the native and local people. According to World Health Organization, traditional medicine can be defined as the sum of all knowledge and practices, whether explicable or not, used in diagnosing, preventing, and eliminating physical, mental or societal imbalances. It relies exclusively on observation and practical experience passed down to generations through verbal or writing (Hui-Meng *et al.*, 2007). Thousands of works to examine herbs as folklore traditional healing have been carried out, in order to summarize the

latest scientific research on the benefit and prevention of any potential side effects (Arif, 2000).

The drawback in herbal research is the difficulty to identify the active ingredients and to maintain efficacy. The inconsistencies of medicinal properties of a plant or herb is resulted from the environmental factors such as the cultivation area and soil, therefore, made the dosages of various batches differ greatly in the treatment of erectile dysfunction (ED) (Hui-Meng *et al.*, 2007).

ED is a common sexual problem in Asia and other countries faced mainly among the aging males. There were a lots of studies conducted to understand this disease. In one multinational study of men aged 40-70 years in four countries (Japan, Malaysia, Italy and Brazil) it was reported that the overall age-specific prevalence of moderate or complete ED was 9% for men aged 40 to 44 years, 12% for 45 to 49 years, 18% for 50 to 54 years, 29% for 55 to 59 years, 38% for 60 to 64 years, and 54% for those 65 to 70 years. Problems such as heavy smoking, heart disease, diabetes, lower urinary tract symptoms, and depression may increase the risk of ED by 10% per year of age. On the other hand parameters such as education, physical activity, and alcohol drinking were inversely contributed to ED (Alfredo *et al.*, 2003; Nicolosi *et al.*, 2003). In several Asian countries, prevalence of self-reported ED were reported as 17.7% in Taiwan, 28.3% in China and 32.2% in Korea. Although the prevalence is relatively high, the number of ED patients seeking for treatment is low, with few reported to be willing to try the traditional remedies for treatment of ED (Wah and Hui, 2007). In 2006, study of aging men was conducted with cooperation of Malaysian Society of Andrology. The result indicated that in Klang Valley, almost 60% of men between the age of 40 and 70 suffered from erectile dysfunction. The sexual problems faced by men above the age of 40 were an early indication of subsequent development of

chronic diseases such as diabetes or cardiovascular problem (El-Sakka *et al.*, 2009; Kanchan *et al.*, 2009).

1.3.1 Medicinal Plant Therapies for Male Dysfunction

Current treatments for physical disorders of ED include oral medication, intracavernosal injection, vacuum pumps and penile prosthesis whereas psychotherapeutic approach is applied for patient with psychological disorder.

Some well-established oral medications available for ED treatment are yohimbine and cantharidin (Spanish fly) from natural sources, besides synthetic selective inhibitors, such as sildenafil (Viagra®), tadalafil (Cialis®), vardenafil (Levitra®), udenafil (Zydena®) and lodenafil (Helleva®). The efficacy of PDE-5 inhibitors is significant as compared to placebo however side effects are reported such as nasal congestion headache, flushing and dyspepsia (Humberto *et al.*, 2010; Wang *et al.*, 2001).

Yohimbine is an indole alkaloid with a 2-adrenergic blocking activity. It is originated from African tree (*Corynanthe yohimbe*) bark. It was marketed with brand name Aphrodex in Africa until 1973. Studies have reported several side-effects of yohimbine such as anxiety, hypertension, manic symptoms and interactions with used medications. (Cinara *et al.*, 2012).

Cantharidin is a lactone which presents in Spanish flies (Cantharides) and beetles. According to Asian and European Pharmacopoeias, the dried form of the compound is consumed for the treatment of impotence. Cantharides acted by causing the vascular congestion which in turn causing irritation of urethra and inflammation of the penile tissue. However, due to the reported toxic effects, the uses of Spanish flies were banned (Cinara *et al.*, 2012).

Several natural products (theophylline and methylxanthines caffeine) were reported to act like non-selective PDE inhibitors, but some flavonoids and their derivatives (quercetin from *Allium cepa*, pyrano-isoflavones from *Eriosema kraussianum* - kraussianone 1 and 2); alkaloids (neferin from *Nelumbo nucifera*, berberine from *Berberis aristata*, papaverine from *Papaver somniferum* – used in association with Prostaglandin-E1 to injections intracavernosal), saponins (steroidal saponins from *Allium tuberosum*), coumarins (osthole from *Angelica pubescens*) and terpenes (forskolin from *Coleus forskohlii*) showed similar effects to PDE-5 inhibitors. In 1982, papaverine, a synthetic chemical was introduced to ED patients. It acts through smooth muscles relaxation of penile cavernosal which in turn will induce erection. Along with expand of knowledge, the erection mechanism was properly understood and management of erection was improved. In treatment of ED, normally repeated injections are needed to initiate and maintain the erection. Vasoactive compound (phentolamine, prostaglandine E-1) and the oral synthetic chemicals (sildenafil-citrate, apomorphine SL, vildenafil, taladafil) on the other hand, were able to produce instant erection. Although there are many drugs available in the market, their expensive costs have made ED sufferers shifted to alternative treatment such as traditional massages which later worsen the nerves damage as it is performed by unauthorized practitioners (Fatimi, 2010). Blood transfusion and resuscitation using penile corpora and penile venous stripping surgery were also introduced as alternative treatment, however the techniques showing risks of inducing tissue and nerve injuries (Ahmad *et al.,* 2005; Geng-Long., 2010).

1.4 *Nigella sativa*

Since centuries, medicinal plants have been utilized as therapeutic agent in treatment of diseases. In majority of people, it does not only serves as therapeutic alternative but also safer choices, and the only effective treatment in some cases. Based on World Health Organization (WHO) data, about up to 80 % of people still rely on natural remedies for their health care need (Al-Sa'aidi *et al.*, 2009; New WHO guidelines to promote proper use of alternative medicines (22 June 2004; Zainab, 2013).

N. sativa is a herbal medicine that belongs to *Ranunculacea* (Buttercup) family and have been employed for spice and food preservative for more than 2000 years. With the increasing of its popularity in recent years, many products are marketed in muslim and non-muslim businesses. This medicinal plant is indispensable constituent of medicinal and food formulation for middle eastern Mediterranean region, South Europe and several Asian countries. It is also important valuable object of the Greco Arab/Eastern system of Medicine's Pharmacopoeia with interesting ethonobotanical and enthnopharmacological data (Munira *et al.*, 2013; Zainab, 2013). In Yemen, this plant mainly in the areas of Marib, Sadah and Taiz. It is also been sold as a condiment, additive on bread and for cheese (Ghanya *et al.*, 2009).

The most interesting fact, this plant is not only important in Islam referred by the prophet Mohammad S.A.W as having healing power, but it also mentioned in Holy Bible (Munira *et al.*, 2013).

The *N. sativa* has variously called such as a nutmeg flower, fennel flower, roman coriander, black caraway and black cumin. In Latin, it is referred as *N. sativa Linn.*, Arabic name Showneez and Habbatus sauda, German as Zwibelsame, Nigella and Schwarzkümmel, Chinese as Pei hei zhong cao, Gujarati name is Kalonji Jeeru,

Kalounji, Bengali as a Mota Kalajira, French as Cheveux de Vénus, Nigelle, Hindi as Kalonji, Persian as Siah Dana; Punjabi called as Kalvanji, Sanskrit for Upakunchika and Urdu as Kalonji and Kannada name is Karijirige Marathi; name is Kalaunji Jire. It should be noted that in Iran and parts of Asia and the Middle East, black cumin is the name given to another species *Bunium persicum* (Apiaceae) (Peter, 2001; Ali and Blunden, 2003; Hassan, 2004; Ahmad *et al.*, 2008; Mohammad, 2008).

1.4.1 Pharmacognostical Description of the *Nigella sativa*

The intact seeds produced little aroma but they give a peppery smell with hints of oregano when rubbed. In Indian, it is used in cooking and some confectioneries. It is used in indigenous medicine (Bahman *et al.*, 2003; Hala, G. M. and Regine, S. 2006). It is grow up to 45cm in the height with 2.5-5.0 cm long, linear-lanceolate leaves. The leaves are arranged alternately. The petiole is strongly broadened at the base, light green, only present in basal leaves, measures 1-6 cm long, ribbed and minutely hairy. The stipules are absent. The leaf measures up to 7 cm x 5 cm in outline, bi-,tri- or even multi-pinnately dissected into short, thin sublinear, divergent, slightly pilose lobes, which are normally green but sometimes turn reddish brown (Ziya, A. and Tambe, S. 2013).

The flower are delicate and usually pale blue and white in colour with 5 - 10 petals 2.0 - 2.5 cm across, solitary on long penduncles with a well-developed yellow-brown taproot and numerous feeder roots (figure 1.1). The fruit is large and inflated capsule composed of 3 - 7 united follicles, each containing numerous seed (figure 1.2). The seed are black, flattened, oblong and angular, funnel shaped 0.1 cm wide and 0.2 cm long. The plant starts flowering and fruiting from January and April. The cultivation area of the plant usually on dry soil between November to April and the

seed takes about 10-15 days to germinate. Propagation of the plant can be made from the callus culture *in vitro* from leaf, stem and roof explants from aseptically grow seedling (Abbas *et. al,* 2011). The stem is profusely branched, subterete, ribbed, and sometimes hollow when old and light to dark green.

The seed are small dicotyledonous, trigonus, angular, regulose-tubercular, size ranging from 2 - 3.5 x 1 – 2 mm, dark black coating and white internal, slightly aromatic (some with carrot-like smell) and bitter taste. Its embryo is minute, embedded in copious and fatty endosperm (Ayob, 2003).

Figure 1.5: *Nigella sativa* flower (*Nigella sativa*. (http://en.wikipedia.org/wiki/ Nigella sativa)

Figure 1.6: *Nigella sativa* seeds (*Nigella sativa*. (http://en.wikipedia.org/wiki/ Nigella sativa))

1.4.2 Phytochemical Studies

The *Nigella sativa* contain active ingredients such as thymoquinone (30-48%), thymohydroquinone, dithymoquinone and p-cymene (7-15%), carvacrol (6-12%), 4-terpineol (2-7%), t-anethole (1-4%) and sesquiterpene longifolene (1-8%), α-pinene and thymol etc (Saha and Bhupendar, 2011; Aftab *et al.*, 2013). Apart from that, several nutritional components such as carbohydrates (24.9%), fats (28.5 %) (Aftab et al., 2013), vitamins, mineral elements (Cu, Zn, P, Fe), proteins (26.7 %) including eight essential amino acids were characterized. Different types of alkaloids such as isoquinoline alkaloid (nigellicimine N-oxide, nigellicimine and pyrazole alkaloids) or indazole ring bearing alkaloids which include nigellidine and nigellicin have been reported from the seed (Hala and Regine, 2006).

In addition, this medicinal plant possessed a high content of unsaturated fatty acids, mainly linoleic acid (55.6%) followed by oleic acid (23.4%), eicodadienoic acid (3%) and dihomolinoleic acid (10%) and the saturated fatty acid such as palmitic acid

and stearic acid amount to about 30% or less (Rahmatollah *et al.*, 2012; Ko and Yilmaz, 2005).

1.4.3 Traditional Uses

Nigella sativa are mainly used as a carminative, stimulant, aromatic, and flavouring in curries. Some health problems (loss of appetite, indigestion, diarrhoea, amenorrhoea, dysmenorrhoea, dropsy, irregular menstruation and skin eruptions) were treated by tincture preparation from the seed (Mohamed, 2005).

1.4.4 Pharmacological Uses

Mohamad *et al.* (2009) and Samir (2007) was reported that *Nigella sativa* and their thymoquinone had beneficial effects on bone and joint diseases. Animal studies shows that extracts of *Nigella sativa* seeds exhibited anti-oxidant, anti-parasitic, anti-inflammatory, anti-tumor, anti-anxiety, anti-microbial, diuretic, galactagogue, anthelmintic, diaphoretic and gastroprotective activities.

1.4.4(a) Antibacterial Effect

Five samples of *Nigella sativa* seeds which were cultivated from different regions was analysed in the *in vitro* study on inhibition growth of *Escherichia coli, S. aureus, Pseudomonas aureoginosa, Enterobacter, Proteus, Klebsiella* for its clinical importance due to the cause of numerous disease. The experiment proved that the extracts of *N. sativa*; contain flavonoids, alkaloids, thymoquinone and tannins. The extract also shows effective inhibition growth of these microorganisms and the highest inhibition effect was against the growth of *Enterobacteriaceae* (Zainab, 2013).

These antibacterial effects were screened at three concentrations (0.5, 1.0 and 2.0%) using the agar diffusion method against seventeen spoilages, pathogenic and lactic acid bacteria (LAB). Antibacterial effects were observed in all tested oils against all the bacteria analysed. The oils at concentration of 2.0% showed most effectiveness compared to other concentrations. *Aeromonas hydrophila* showed the most sensitivity to the oils whereas *Yersinia enterocolitica* showed the most resistant effect. Generally, lactic acid bacteria exhibited stronger resistance compared to pathogenic and spoilage bacteria against *N. sativa* oils. Thus, the oil may be used as an antimicrobial agent to prevent spoilage in food products (Padmaa, 2010; Chowdhury *et al.*, 1998).

1.4.4(b) Antifungal Effect

It was reported in *in vitro* study of the methanolic extract of black seeds exhibits potent inhibition of fungus growth against *Candida parapsilosis*, and *Issatchenkia orientalis* with IC_{50} value of 4.846 µg/ml and 6.795µg/ml, respectively. The ethanolic extract also showed significant anti-fungal activity against fungus strain *Issatchenkia orientali* with IC_{50} value of 5.805 µg/ml (Zainab, 2013).

Previously *in vivo* antifungal effect of *N. sativa* in mice was conducted. *Candida albicans* were inoculated into the mice to produce colonies in the kidneys, spleen and liver. After 24 hrs of inoculation, 6.6 mL/kg doses of aqueous extract which is equivalent to 5 mg of estimated protein were administered daily for three days. The analysis showed inhibition of the organisms growth in kidneys (fivefold), liver (eight fold) and spleen (eleven fold). These findings were confirmed by histopathological examination of the respective organs. Hence, it suggested that the aqueous extract of *N. sativa* seeds can be a potent agent against candidiasis thus validated its traditional use to treat infection (Anwar-ul, 2004).

1.4.4(c) Antiparasitic Effect

Aqueous extract of *Nigella sativa* seeds are reported to possess anti-parasitic activity against trophozoites of *Entamoeba histolytica* in two clinical settings in Iraq. The oil of the seed also showed activity againts *Entamoeba histolytica* trophozoites, prophylactic and therapeutic effects on murine toxoplasmosis (*Toxoplasma gondii*). The aqueous extract also showed potential in treating *Trichomonas vaginalis* infected mice through inhibition of the trophozoites motility. The ethanol, chloroform and aqueous extracts at serial doses (50, 100, 200 and 400 µL/kg) also showed anti-malarial activities against *Plasmodium berghei* in mice when administered orally and intraperitoneally. *N. sativa* alcoholic extract exhibited potent anti-cutaneous leishmanial activity (Fatemeh *et al.*, 2014; Muhammet *et al.*, 2005; EI Shenawy *et al.*, 2008).

1.4.4(d) Anticancer Effect

Nigella sativa oil was reported as antineoplastic agent in various *in vitro* and *in vivo* carcinogenesis models. The seed, extracts, oil and its active compounds (thymoquinone and α-hederin) exert significant anticancer properties in *in vitro* and *in vivo* models. The anti-neoplasms of the plant extracts were postulated through its antioxidant and anti-inflammatory activities. Through molecular structure modification of thymoquinone and α-hederin, safer and more effective drugs anti-neoplastic can be produced. Moreover, a combination with commercial chemotherapeutic agents may be possible (Hala and Regine, 2006; Zainab, 2013).
N. sativa gum and oil (thymoquinone and DIM) were studied for their cytotoxicity against several parental and multi-drug resistant (MDR) human tumor cell lines. From the result, it was found that both gum and oil exhibited cytotoxic effect for all of the

analysed cell lines (IC_{50}'s ranging from 78 to 393 micron) with high sensitivity to the herbal preparations. *N. sativa* gum and oil were proven superior than the drugs DOX and ETP by overcome the resistant of the cell lines without alteration of the 3H-taxol cellular accumulation. TQ or DIM also were not affected by the inclusion of radical scavenger DMSO (0.5% v/v) in cytotoxicity compared to DOX which suppressed up to 39%. The results suggested the potential of *N. sativa* herbal preparation as antitumor agent against selected human tumor cell lines (Kourosh, 2010).

Farah and Begum, (2003) reported the effect of aqueous (WE) and alcohol (AE) extracts of *N. sativa* on MCF-7 breast cancer cells in combination with H_2O_2 as an oxidative stressor. The alcohol extract and its combinations (Mix of WE and AE, AE and H_2O_2 + AE and) were able to completely inactivate the MCF-7 cells with LC_{50} ranged from 377.16 - 573.79 respectively. H_2O_2 alone effectively inactivated MCF-7 cells (LC_{50} = 460.94) whereas WE + H_2O_2, WE + AE + H_2O_2, and WE showed the least potency (LC_{50} were 725.79, 765.94, and 940.5) respectively. This showed the opportunities of *N. sativa* as promising agent in the breast cancer treatment.

1.4.4(e) Anti- hepatotoxicity Effect

Liver is the largest organ in the body and primarily concerned with the metabolic activity of organisms. It is responsible for biotransformation and detoxifying the chemical substances in the blood and in this process it is exposed to high concentration of toxicants and toxic metabolites making it susceptible to injury. The liver damage caused by pathogen as well as chemical agents. Lack of hepatoprotective drug available has encouraged scientists to explore the herbal sources as hepatoprotective agent.

The *N. sativa* oil was reported to be able to significantly decrease the elevated serum levels of liver enzymes and improved the state of oxidative stress induced by carbon tetrachloride (CCl_4). The alcoholic extract was able to alleviate the oxidative stress and hepatotoxic effects associated with naphthalene in rats and improve some biological markers related to liver disease (Munira *et al.*, 2013). The protective roles *N. sativa* was attributed to the presence of vitamin E and flavonoids in the seed. The seed also exerted hepatoprotective effect against $AlCl_3$ induced toxicity *in vivo* using rabbits (Mahmoud, 2002).

1.4.4(f) Hypocholesterolemic and Antiatherogenic Properties

Antiatherogenic effect of *N. sativa* powder and oil were reported due to their ability to decrease low density lipoprotein cholesterol (LDL), serum triglycerides, total cholesterol and LDL cholesterol level significantly in *in vivo* model. In addition, it also significantly increased the high-density lipoprotein cholesterol (HDL) reduce uric acid levels after 2, 4, 6 and 8 weeks treatment compared to the positive control group (Asgary *et al.*, 2012; Badary *et al.*, 2000; Maznah et al, 2009).

1.4.4(g) Effect on Fertility

According to Zainab (2013), administration of *N. sativa* oil to hyperlipidemic rats was able to improve their reproductive efficiency and produced additional protection against hyperlipidemia induced infertility**.** The effect was caused by unsaturated fatty acid (linoleic acid) which present in the oil.

Administration at doses 0.5 ml/day of *N. sativa* oil for two months in normal and hyperlipidemic rats were able to increase the seminal vesicles mass, sperm count,

motility, plasma testosterone level and lower the sperm abnormalities (Foresta *et al.*, 2004).

Total of 85% unsaturated fatty acid, the *N. sativa* oil contain rich source of linoleic acid (LA) and omega-6 fatty acid. Estrogen has been used as main treatment for menopausal symptoms. *N. sativa* has shown a great number of 23 sterol which can improves some symptoms associated with menopause (Fadwa *et al.*, 2006). No studies have been done to determine specific impact of LA and other component of *N. sativa* on reproductive performance.

A study conducted on two set of group of man. A group is given *N. sativa* seed oil as supplement and the other are place under placebo. Both groups are compared with its motility, morphology and sperms count of each man. Studies conducted favour for the group which has taken the supplement as they had a significantly higher sperm counts, better sperm motility and sperm morphology as well as improved pH levels compared to the placebo group. The study concluded that daily intake of 5 ml *N. sativa* oil for two month improve abnormal semen quality in fertile men without any adverse effect (Peter, 2001).

Other studies also reported that treatment of *N. sativa* oil in hypercholesterolemia induced rats increased their reproductive performance, weight of seminal vesicle, level of testosterone, sperm motility, and sperm quality. Administration of *N. sativa* oil for a period of 53 days orally has also shown improved male rat fertility. This agrees well with the report that black seeds contain alkaloids and phenols, which could stimulate the secretion of testosterone and follicle stimulating hormone (FSH). The increased levels of testosterone and FSH in testicular tissue have been shown to increase sperm concentration (Ng *et al.*, 2014).

1.5 *Eurycoma longifolia*

Eurycoma longifolia from the family Simaroubaceae is widely found in the jungles of Malaysia, Borneo, Sumatra and Indochina. It is known as Pasak bumi in Indonesia (Lily and Sapto, 2011). There are several popular names for this *Eurycoma longifolia* includes Payung Ali, Tongkat Ali Hitam, Tongkat Baginda, Penawar Pahit, Setunjang Bumi, Bedara Pahit, Pokok Syurga, Pokok Jelas, Long Jack, Natural Viagra, Local Ginseng, Malaysian Ginseng, Cay Ba Binh, Ian-Don, And Jelaih (Rajeev and Karim, 2010).

It is commonly addressed as Tongkat Ali which literally means Ali's walking stick in Malaysia, the tree is a medium size slender shrub which can grow up to 10 meters, with little branches and reddish brown petioles. This medicinal plant is slow growing, with maximum height of 15-18 m and produces fruits after nearly 2-3 years of cultivation (figure 1.3 and figure 1.4). The fruits are oblong, hard with 2-3 cm long, green in color and becomes dark red after ripening. The leaves are compound of even pinnates with maximum 1 meter long. Each compound leaf consists of 30-40 leaflets and each leaflet is about 5-20 cm long and 1.5-6 cm wide. The flowers are hermaphrodites with small petals and very fine pubescent. The plant may take up to 25 years for complete maturation, however the roots are usually harvested after 4 years of cultivation (Rajeev and Karim, 2010).

All the tree part has bitter characteristic. The root is infamously harvested as source of traditional medicine to treat fever, after birth medication, for healing of wounds, bleeding gums, boils, ulcer and syphilis (Ang and Cheang, 2001; Ang *et al.*, 2003; Rajeev and Karim, 2010).

Figure 1.7: *Eurycoma longifolia* (Tongkat Ali) plant (Tongkat ali (*Eurycoma longifolia* jack) herbal medicine (http://www.medicalhealthguide.com/articles/tongkatali.htm))

(a) (b)

Figure 1.8: (a) *Eurycoma longifolia* Root (Cultivated) and (b) *Eurycoma longifolia* Root (Wild) (Tongkat ali (*Eurycoma longifolia* jack) herbal medicine (http://www.medicalhealthguide.com/articles/tongkatali.htm))