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Review Article

Gnetum gnemon Linn. : A Comprehensive Review on its Biological, Pharmacological and Pharmacognostical Potentials.

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ABSTRACT

In recent era, the researchers and scientists from the developing as well as Western countries move on their research on plant and herbal products due to immense popularity. The *Gnetum gnemon* is an evergreen and perennial tree, native of the place from Northeast (NE) India to Fiji which is used in several traditional medicines to cure various diseases. *G. gnemon* is cultivated in many parts of the World mostly for its delicious fruits. It has been reported that the derivatives from its seed exerts possessed anti-microbial, anti-oxidant, anti-bacterial, angiogenesis-inhibitory, anti-aging and tyrosinase-inhibitory activities. In accordance to recent findings, it has been found that the *G. gnemon* contains bioactive compounds, such as saponins, tannins, and flavonoids, and stilbenoids. To provide a comprehensive overview of the traditional and ethno medicinal uses, phytochemistry and biological activities of *G. gnemon* with clinical and toxicity data possibly make recommendations for further research.

Keywords: Biological activity, bioactive compounds, ethno medicinal, *Gnetum gnemon.*

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance[1].

Folk medicine has been used for thousands of years with significant contributions made by its practitioners to human health, particularly as primary health care providers at the community level[2]. Traditional medicine uses the knowledge, skills and practices, beliefs and experiences endemic to its cultures, for well-being of the local people. It has reputed heritage, community acceptance and is based on the expertise gained by herbalists over a period of time[3].

One of the vital medicinal plants, broadly used therapeutically in the orient and becoming increasingly popular *Gnetum gnemon* is a member of the genus *Gnetum* indigenous to Southeast Asia and the western Pacific Ocean islands from Assam throughout Indonesia, Malaysia, Philippines and Fiji. They are occasionally called *padi oats* or *paddy oats*. This tiny tree is indigenous to Southeast Asia and "Melinjo" is the familiar Indonesian name for the plant. In Indonesia and Malaysia, Melinjo has been consumed as safety foodstuff for centuries and cultivated the plant as one of the common shade plants in different agro-forestry systems. Melinjo dimer resveratrol is a natural resveratrol isolated from the seeds of the melinjo, *Gnetum gnemon*.

METHODS

Google search was performed using the keywords "*Gnetum* and traditional medicine", "*Gnetum gnemon* and performed activity",” *Gnetum gnemon* ", etc. Search for published research articles were also done in PubMed. In addition, reference and bibliographies of numerous published articles were searched for the keyword of “*Gnetum*”. The back references of these articles were hand searched for any suitable articles. Only highly relevant articles were considered for the present review article. Fresh collection of the plants and ethnobotanical information was done by repeated survey in Karbi Anglong and Sonitpur districts of Assam, where the plant occurs in the primary forests. These informations have also been taken into consideration during the compilation.

TAXONOMY AND ECOLOGY

The name *Gnetum gnemon* was given by Carolus Linnaeus in 1767 to that Gymnosperm plant which revealed rather similarity with Angiosperm in the vegetative structure. In fact, he named the entire genus *Gnetum* (Family- Gnetaceae), showing *G. gnemon* as the type species. By origin, *Gnetum gnemon* is an Old World species and native of the place from Northeast (NE) India

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to Fiji[4-6] – the region which has been designated by Takhtajan[7], as the place where the angiosperm was either originated or cradled. Some of the characters which make *G. gnemon* unique amongst the flowering plants and make the species full of curiosity are the presence of true vessels in the secondary xylem, reticulate venation in the leaves and leaf anatomy resembling that of dorsiventral dicotyledounous leaves, G_2 karyogamy expressing a kind of double fertilization being a member of Gymnosperms[8], thin walled non-sclerenchymatous phellem, starch rich parenchyma, nest of branchy sclereids having calcium oxalate crystals and gelatinous phloem fibers in the bark[9], presence of non-lignified mesophyll fibers with dual function of mechanics and hydraulics, as well as presence of wider adaptability from medium to high rainfall zones, etc.[10] Mainly because of its peculiarity in vegetative structure in between Gymnosperm and Angiosperm, and its intrinsic relationship with human beings primarily as alternative food, *G. gnemon* is probably one of the most studied wild plants.

Its tender parts, young leaves and inflorescences are harvested from the wild for consumption and accordingly the plant is conserved by the ethnic people in its natural habitats. Working on infra-specific diversity of *Gnetum gnemon*, Markgraf recognized five varieties, out of which *Gnetum gnemon* var. *brunonianum* (Griff.) Markgr. and *Gnetum gnemon* var. *griffithii* (Parl.) Markgr. occurred in NE India[6,11]. Both the varieties are shrubby Phenerophytes to small and medium sized tree and are integral component of low elevation rain Forest ecosystem. The plant can grow well in open and partially shaded situations of tropical and subtropical climate, freely draining land condition with slightly acidic to neutral or slightly alkaline soils[12]. They adopt well in the middle story strata as well as in open lands, and in NE India they are popularly known by Karbi name “Hanthu” or Assamese name “Letera” in Assam.

The common names of *Gnetum gnemon* are bago, melinjo, benjilo, bagoe, maninjau, voe, khalet peedae, phak, miang kaniang, liang, gam cay and bet[4]. It is found in Kingdom: Plantae, Division: Gnetophyta, Class: Gnetopsida, Order: Gnetales, Family: Gnetaceae, Genus: *Gnetum*, Species: *G. gnemon*, Binomial name: *Gnetum gnemon* [13].

GROWTH AND DEVELOPMENT

The plant is a small to medium sized evergreen tree with nearly conical crown and a stature of 10 to 15m. The stem is well branched and possessed cylindrical bole and a diameter upto 40 cm. Leaves are petiolate, ovate-oblong or elliptic, 10 to 20 cm long and 4 to 7 cm broad, reticulately veined, glabrous and shiny, dark green, apex acute to sub acuminate, margins entire, base acute and phylotaxy opposite; young leaves are reddish purple. Inflorescences are borne on young shoots and older branches. Being a Gymnospermous species, *G. gnemon* possessed aggregated cone or strobili at terminal stem axis in female plants and terminal 3-5 cm long axis of staminate strobili in male plants (Figure 1; Plate 1)[14]; only a few ovules in a spike develop into seeds. Seeds are naked, sessile, 1-3 cm long, ellipsoid in variety *brunonianum* and globose in variety *griffithii*. Female flowers consist of a nucellus, surrounded by three envelops; in mature seeds the middle envelope is stony and the outer most is fleshy.[15] Seed coat is thin and brittle, and separates readily from the seeds[4].

Growth Response activity of G. gnemon[16].

Harnessing and exploring the potentials of forest-based floral resources like *Gnetum gnemon,* with young leaves and immature flowers, were commonly eaten, to promote *ex-situ* conservation of the species. *Gnetum gnemon* could be successfully propagated through cuttings even without the use of rooting chemicals. To determine the growth response of bago, cuttings were immersed for an hour in the following rooting agents: ANAA (1 tbsp/li of water); IBA (500 ppm); pure coconut water and plain tap water. Data revealed that T-2 (ANAA) and T4 seedlings (control) had the maximum survival of 75% and 67% respectively. On the other hand, the leaf production in control was significantly higher than the other treatments. IBA i.e. treatment 1 had produced the highest average number of roots. Their study could accomplish that maximum survival was noted with the greenish cuttings carrying only a few brownish pigmentations and holding two nodes.

Reproductive biology of Gnetales[17].

Haycraft and Carmichael[17] suggested that Angiosperms and Gnetales (*Ephedra*, *Gnetum* and *Welwitschia*) represent the only seed plant that regularly produces bisexual cones. Although, the fertility and function of ovules formed on bisexual cones of Gnetales have remained uncertain. Some reports indicate that the ovules are sterile while others indicate that they may develop into seeds[18-21]. Their study demonstrates three different developmental patterns of ovules formed on bisexual cones of *Gnetum gnemon*. Type I ovules did not develop at all after pollination and represented the majority of ovules on each cone. Type II ovules enlarged slightly after pollination due to the enlargement of nucellar tissue. Type III ovules were typically found on the terminal whorl and developed into seed-like structures. The enlargement was due to proliferation of mega-gametophyte tissue. Sectioned material exhibited that mega gametophytes show altered development compared to those found in functional female ovules. None of the ovules studied contained embryos, and thus all were sterile. Densitometry of 4', 6-diamidino-2-phenylindole (DAPI) - stained sections showed that mega gametophyte nuclei formed in the sterile ovules are unreduced (diploid) and thus do not form viable female gametes.

Study of non lignified fibers in leaves of G. gnemon*[10].*

George[22], Rodin[23] and Martens[24]; stated that the leaves of *G. gnemon* had an extensive anastomosing network of thick-walled cellulosic fibers that permeate mesophyll tissues. In their study, the mesophyll fibers of *Gnetum gnemon* were mainly highlighted due to their unusual method of development and cell wall structure. In case of mesophyll differentiation, laticifers cell type appeared prior to fibers and more or less homologous to major veins. The most distinctive feature of the fibers were

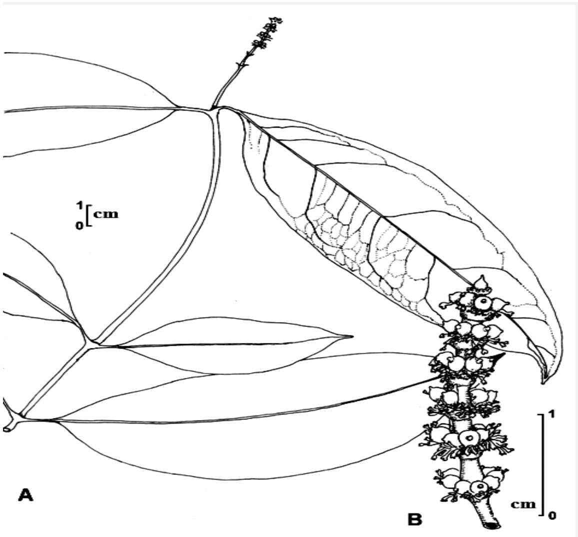

Figure 1: Habit Sketch of *Gnetum gnemon* Linn. A: A twig; B: An inflorescence

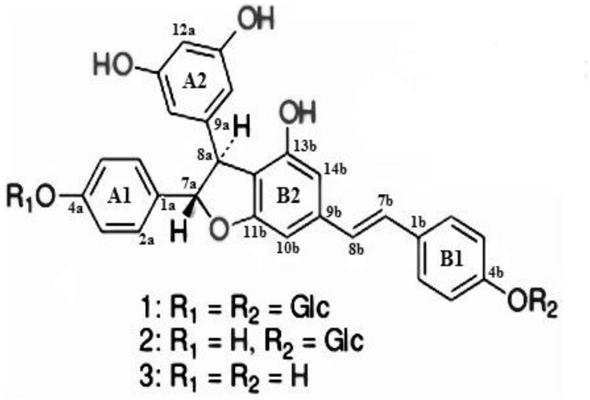

Figure 2: Dimer resveratrol structure

early binucleate and sometimes become four-nucleate. Because of their intrusive growth, the path of fibers become irregular, little branched and infuses other mesophyll layers. When sclereids were little developed, fibers become the major mechanical structure of the mature leaf. Once the expansion was complete, the full-fledged fibers formed a cellulosic, but unlignified secondary walls were non-lamellate and almost obstruct the cell lumen. These fibers were divergent with the gelatinous (tension) fibers developed aberrantly in stems of *Gnetum.* Besides mechanical purpose, fibers may also have a hydraulic role to preserve a highly hydrated internal leaf atmosphere.

Gelatinous fibers in the stem, on the other hand, are characteristically multinucleate, almost wholly cellulosic G-layer (Sg) of the secondary wall, with its shallow microfibrillar organization and weak attachment to the primary and outer secondary wall layer (S_1) – all these features are suggestive of cell type homologies between Gnetales and dicotyledons[25].

PHYTOCHEMISTRY

Several bioactive compounds are found in *G. gnemon*, such as saponins, tannins and flavonoids[26,27]. Melinjo seed contains an abundance of resveratrol (stilbenoid) mainly in the form of dimers (gnetin C). Melinjo dimer resveratrol has been studied for more than 10 years, principally by Japanese scientists. There is already a good level of clinical documentation and new studies are continuing to emerge. The dimer Resveratrol Structure is shown in the (Figure 2)[13].

ETHNOBOTANICAL USES

Agroforestry: *Gnetum gnemon* is widely planted as a home garden tree or for field boarders, agroforestry and soil improvement purposes in many places of southeast Asia.

Food: The young leaves, inflorescneces and tender tips of *Gnetum gnemon* are edible and used as a vegetable in Assam, mostly by Karbi tribe. Tender leaves are one of the commonly traded vegetables in the ethnic people dominated markets in the entire NE India; Karbis, the Mongoloid race with linguistically Kuki-Chin sub-group, considered themselves as the children of “Hanthu” (*Gnetum gnemon*)[28]. Study revealed that unlike other species of *Gnetum*, *Gnetum gnemon* possesses no tannin in the leaves, but the leaves are not infested by diseases and insect pests, and that defensive capacity is believed to be because of some leaf borne fine crystals[10]. In Java, it is an important home industry where the seed is heated, the hush is broken and hot kernel is pounded into a flat cake. The cakes are sun-dried, graded and packed for sale. A crsip snack (emping) is prepared by soaking up the cakes in boiling water.

Wood: The weed is used for making boxes, tool handles, as well as soft timber. The bark fibres are processed into rope making, fishing net and high grade paper products. The inner bark is used for the famous Sumba bow string.

Other uses: It has a beneficial association with mycorrhizae (*Scleroderma sinnamariense*) which makes phosphorus and some micro-element from soil readily available for plant growth and development. *Gnetum gnemon* has been used in folk medicines for the treatments of arthritis, bronchitis and asthma[29].

BIOLOGICAL ACTIVITY OF *GNETUM GNEMON*

Antimicrobial activity of *G. gnemon*[30].

Gnetum gnemon is commonly used as raw material for making '*emping'* and as supplementary soup material[4-6] in many places of SE Asia. Several bioactive compounds are found in melinjo, such as saponins, tannins, and flavonoids[26,27,30,31]. It was reported that, stilbenoids from melinjo showed moderate antimicrobial activity via a diphenyl-picrilhydrazil-hydrate (DPPH) radical scavenging effect, including lipase and α-amylase inhibition activity[32]. In accordance to those findings, a study was conducted by Bloomfield[33] to perform antimicrobial activity of the melinjo seed extract and melinjo peel extract using the well-diffusion method with minor modification following Yasni et al.,2009 for *Staphylococcus aureus* ATCC 25953, *Bacillus cereus* ATCC 10876, *Pseudomonas aeruginosa* ATCC 07853 and *Aspergillus flavus* IPBCC 88.030. [34] Antibacterial activity melinjo seed and melinjo peel extract, could inhibit the growth of tested bacteria as seen in the inhibition area, supported by (i) the MIC and MBC values (0.26~1.46%; 1.02~6.04% respectively), (ii) anti spore results (5% w/v of extract), (iii) having the equivalent inhibition capacity as compared to antibiotics (penicillin G and streptomycin 10 ppm). According to Parhusip[35] and Rogers[36], destruction of bacterial cell membrane may also cause the extraction of minerals from cell inner side such as, calcium (Ca) and potassium (K). It was observed under scanning electron microscopy (SEM), and confirmed by the presence of ions (Ca^{2+} and K^{+}) outside of the cells, detected by Atomic absorption spectroscopy (AAS). Finally, from these comprehensive data it was interpreted that melinjo extract has real potential for use in food preservation against selected pathogenic bacteria.

Anti-toxicity activities of G. gnemon[37].

Fruit, seeds, leaves, and flowers of *G. gnemon* are edible. Tatefuji et al., 2014 suggested that the seeds are abundant in resveratrol dimers such as gnetin C and its glucosides, gnemonoside A and gnemonoside D, and also encompass trans-resveratrol and its glucoside (trans-piceid)[23,37]. Although melinjo has been consumed in Indonesia for a very long time, the safety of melinjo seed extract (MSE) was assessed using acute oral toxicity, repeated dose toxicity, and micronucleus testing in rats. In the acute and subchronic toxicity studies, the groups administered with the MSE powder orally were not considered to be toxic, compared with the control group. The no observed adverse effect level (NOAEL) was determined as 1000 mg/kg/day. A genotoxicity test (rat bone marrow micronucleus test) was negative for MSE powder at levels up to 4000 mg/kg/day. These toxicological results might furnish supportive evidence of safety of melinjo seeds indicating that the melinjo seeds should be safe as a food or food ingredient.

Antioxidant activities of G.gnemon.

Three phenolic derivatives were isolated from stem bark of *G. gnemon*, namely 3,4-dimethoxychlorogenic acid (1), resveratrol (2), and 3-methoxyresveratrol (3). Isolation and structure elucidation of these phenolic compounds were carried out by chromatographic method and interpretation of spectroscopic data, comprising UV, IR, 1H and 13C NMR 1D and 2D, and FAB MS[38].

Wazir et al., 2011 analysed biological activities of *G. gnemon* to determine the total phenolic and antioxidants of the plant[39]. Folin-Ciocalteu method was used for the determination of total phenolic content of the plant extracts. The antioxidant activities of the plant extracts was determined by using DPPH and FRAP assays, respectively. However, the experimental data proved that there were no correlation between total phenolics and both antioxidant assays tested.

Two active antioxidant protein fractions (Gg-AOPI and Gg-AOPII) were isolated and characterized from *G. gnemon* seed[31]. The purification of the active protein fractions was done using a precipitation method and ion exchange chromatographic techniques to identify the potent antioxidant and free radical scavenging activities. The antioxidant or free radical scavenging effect of fractions were explored by employing *in vitro* assay systems involving the linoleic acid autoxidation inhibition, free radical scavenging effect on α,α-diphenyl-β-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), reducing power, chelating capacity of metal ions Cu^{2+} and Fe^{2+}, and protections against hydroxyl radical-mediated DNA damage[31].

Four stilbene derivatives, gnemonols K and L (resveratrol trimers), M (isorhapontigenin dimer), and gnemonoside K (glucoside of resveratrol trimer) together with eleven known stilbenoids and a lignan were isolated from the acetone, methanol and 70% methanol soluble parts of the root of *Gnetum gnemon* (Gnetaceae). The structures of the isolates were determined by spectral analysis. Free radical scavenging activity of the stilbenoids on lipid peroxide inhibition and super oxide were also investigated which is indicative of their antioxidant activity[40]. Iliya et al., 2003 also reported that three stilbene trimers viz. gnemonols D, E, F were isolated from the root of *G. gnemon*[40]. The structures were analysed spectroscopically, and examined for the antioxidant activity on lipid peroxide inhibition and super oxide scavenging.

Ex-vivo cell culture activity of *G. gnemon*[26].

The fruits and seeds of *G. gnemon* were consumed for their nutrient values in addition to its use in traditional medicines. To examine the regulatory actions on ileal immune responses of the fruit, investigation was carried out on T-helper (Th) cytokine production, i.e., interleukin-2 (IL-2), IL-4, IL-5, and interferon-gamma (IFN-γ), in cultured Peyer's Patch (PP) cells from mice. The results of the study revealed that oral administration of 50% ethanol extract of *G. gnemon* fruit potentiated production of Th cytokines such as IL-2 and IFN-γ in PP cells irrespective of Con-A stimulation, whereas, no effect on the production of the Th2 cytokines IL-4 and IL-5 was observed. Kato et al., 2011 reported two new stilbene glucosides compounds gnemonoside L (5) and gnemonoside M (7) along with the previously identified stilbenoids resveratrol (1), isorhapontigenin (2), gnetin C (3), gnemonoside D (4), and gnetin E (6) from the active EtOAc (ethyl acetate) fraction[26]. The known compounds 1, 2, 3, 4, and 6 were identified by analyses of NMR data. Among these tested isolated compounds, only new stilbenoid gnemonoside M (7) strongly elevated Th1 cytokine production in cultured PP cells at 10 mg/kg/day. This finding suggested that gnemonoside M (7) was one of the active constituents of melinjo fruit potentiated T-cell-dependent immune responses in the ileal mucosa.

Inhibitory effect of G. gnemon on tyrosinase activity and melanin biosynthesis[41].

Melanogenesis is the process primarily responsible for the production of melanin, where tyrosinase is the key enzyme responsible for melanogenesis[42]. As early as in 2003 Kenji et al., 2003 observed that resveratrol inhibited tyrosinase activity, but was less effective than gnetol; therefore, the number of hydroxy substituents seems to play an important role in the inhibitory potency of hydroxystilbene compounds for tyrosinase activity[43]. Later study[41] to investigate the *in vitro* inhibitory effects of isolated compound gnetin C on tyrosinase activity and melanin biosynthesis in murine B16 cells revealed similar inhibitory effects of gnetin C and resveratrol on tyrosinase activity and melanin biosynthesis. The IC_{50} values for gnetin C and resveratrol against tyrosinase activity were 7.0 and 7.2 μM, whereas melanin biosynthesis values were 7.6 and 7.3 μM, respectively. Hence, as per their study, gnetin C may be a potent stuff for use as a skin-whitening agent and for the hindrance of hyperpigmentation. However, to estimate the direct effects of gnetin C and resveratrol on tyrosinase activity, B16 cell lysates were used as a source of murine tyrosinase. Resveratrol showed 64.1% inhibition at 16 μM, while gnetin C only exhibited 25.2% enzyme inhibition at same concentration. The IC_{25} values for gnetin C and resveratrol were 15.5 and 4.0 μM, respectively. The effects of gnetin C may be due to mechanisms other than the direct inhibition of tyrosinase activity.

Study of beneficial effects of MSE in healthy adult males[44].

Kato et al.,2009 reported that melinjo seed extract (MSE) contains various stilbenoids including *trans*-resveratrol, gnetin C, gnetin L, gnemonoside A, gnemonoside C, and gnemonoside D.[32] Moreover, human studies revealed that *trans*-resveratrol was beneficial in the management of diabetes and cardiovascular diseases[45,46], although, its mechanism of action in human remains unknown. The effects of MSE on human were studied using healthy volunteers and various biomarkers in association with metabolic syndrome were evaluated as well. The study was a randomized, double-blinded, 30 males aged 35–70 years with ≤10% flow-mediated dilatation received placebo or 750 mg MSE powder for 8 weeks, and twenty-nine males (45±8.8 years old) completed the trial. The results showed that MSE significantly reduced serum uric acid at 4 and 8 weeks (n=14 and n=15, respectively) compared with the placebo control. To elucidate the exact mechanism of MSE for diminishing the uric acid level, xanthine oxidase inhibitory activity, angiotensin II type 1 (AT1) receptor binding inhibition rate, and agonistic activities for PPARα and PPARγ were also investigated. MSE exhibited that trans-resveratrol and gnetin C, significantly inhibited AT1 receptor binding and showed mild agonistic activities for PPARα and PPARγ.

Evaluation of pharmacokinetics and safety profile of G. gnemon in human[47].

Tani et al., 2014 reported that the fruit and seed of *G. gnemon* were resveratrol derivative-rich materials, mainly Melinjo seed extract (MSE) contains trans-resveratrol (tRV); isorhapontigenin; gnetin C, gnemonosides A, C, and D, and gnetin L. 47 Pharmacokinetics of resveratrol derivatives were investigated by determining the plasma concentration profile of tRV, dihydroresveratrol, isorhapontigenin, gnetin C, and gnetin L in healthy volunteers after oral administration of 1000 mg of MSE powder and compared with those after oral dosing of tRV powder carrying 4.8 mg of tRV only, identical to the content in 1000mg MSE powder. In addition, using one compartmental model, the persistence of plasma concentrations of tRV and gnetin C after repeated oral administration, were also examined. Plasma concentrations of tRV with enzymatic hydrolysis were maintained over 24 h, having a t_{max} of and a mean residence time (MRT) of 14 h, which was 5 and 2 times higher than those for tRV powder intake, respectively. Plasma concentrations of gnetin C with hydrolysis was maintained for >96 h in plasma with an MRT 36 h. Plasma tRV and gnetin C concentrations with hydrolysis were in good agreement with the theoretical curves with repeated doses once daily for 28 days. For the safety assessment of MSE powder between, before and after each oral administration of 1000, 2000, and 5000 mg of MSE powder suggests that MSE is safe, well tolerated up to the oral dosing of 5000 mg with no serious adverse events.

Inhibitory effect of G. gnemon on multiple angiogenesis[48].

Angiogenesis is a favourable target for cancer prevention and treatment[49]. The antiangiogenic effects of *G. gnemon* seed extract and its resveratrol derivative constituents, such as gnetin C (GC), gnetin L (GL), gnemonoside A (GMA), gnemonoside C (GMC), and gnemonoside D (GMD) was evaluated[48]. They have observed that the proliferation and tube formation of human umbilical vein endothelial cells (HUVEC) stimulated with vascular endothelial growth factor and basic fibroblast growth factor were markedly inhibited in an ethanol extract of melinjo seeds (EEMS) and the two gnetins. The results revealed that the inhibitory effects of GC and GL were much stronger than those of resveratrol. On the other hand, GMC and GMD inhibited only proliferation, whereas, GMA had nearly no effect on the two endothelial cell functions. Reduction in the cell viability of tube-forming HUVEC, with assisting ERK1/2 inactivation, and suppression of HUVEC migration was observed in EEMS and GC. Moreover, it was also recorded that dietary intake of EEMS notably inhibited tumor angiogenesis in a mouse dorsal air sac assay. Finally, EEMS and its resveratrol derivatives, especially GC, suppressed multiple angiogenesis-related endothelial cell functions and/or tumor angiogenesis, stipulating that the melinjo seeds and the natural resveratrol derivatives may be convenient for cancer prevention and treatment.

Evaluation of Nutritional value of G. gnemon[49].

Data obtained from the various nutritional analyses of *G. gnemon* seed flour showed that the seed flour abundant in protein (19.0g/100g), crude fibre (8.66g/100g), carbohydrates (64.1%), total dietary fibre (14.5%) and encompassed adequate amounts of essential amino acids, fatty acids and minerals. Total phenols (15.1 and 12.6mg GAE/100g), tannins (35.6 and 16.1mg CE/100g) and flavonoids (709 and 81.6mg CEQ/100g) were also estimated. FTIR analysis data demonstrated that absorption bands for major functional groups such as:

amines, amides, amino acids stipulate the presence of protein, amines, amides, amino acids, while other absorption bands specified the presence of bio-molecules like polysaccharides, carboxylic acids and lipids. Consequently, *G. gnemon* seed is found to be a rich source of nutrients, and can be efficiently used as a basic raw material to develop novel nutritious functional foods.

Anti –senescence activity of G. gnemon[50].

The seeds of this species mainly contain dimeric stilbenoid compounds [gnetin C (1), gnemonoside A (2), and gnemonoside D (3)] along with *trans*-resveratrol (4). Anti-aging, anticancer, and antidiabetic effects, as well as being a calorie restriction mimetic of *trans*-Resveratrol have been reported[51]. SIRT1 trans-Resveratrol (4) was identified as a SIRT1- activating molecule, which has been recognized as a key modulator of vascular endothelial homeostasis, regulating angiogenesis, endothelial senescence, and dysfunction[52-54]. A study was conducted to investigate the effects of these four main stilbenoid derivatives of *G. gnemon* seed endosperm ethanolic extract on endothelial senescence. Administration of the *G. gnemon* ethanolic extract increased SIRT1 and decreased endothelial senescence showed in streptozotocin-induced diabetic mice. An *in vitro* study was carried on the four main stilbenoid derivatives of *Gnetum gnemon* seed on the endothelial senescent phenotype, premature endothelial senescence was induced by treating human umbilical vein endothelial cells (HUVEC) with hydrogen peroxide (H_2O_2) (100 μmol/L) for 1h. Treatment with *trans*-resveratrol (4) inhibited the senescent phenotype, which increased the expression of endothelial nitric oxide synthase and SIRT1, whereas 1–3 had no effect. These results suggested that the ethanolic extract of *Gnetum gnemon* seeds inhibited endothelial senescence, suggesting that *trans*-resveratrol (4) plays a critical role in the prevention of endothelial senescence.

Biological activity of stilbenoids isolated from the seeds of G. gnemon[55].

A new stilbenoid 1, named gnetin L, along with previously identified five stilbenoids viz. gnetin C (2), gnemonosides A (3), C (4), and D (5), and resveratrol (6) were isolated. The results revealed that 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of all of these stilbenoids are similar to that of ascorbic acid and Dl-alpha-tocopherol. Among the constituents, gnetin L (1) and C (2) possessed moderate inhibitory effect against porcine pancreatic lipase, and C (2) showed relatively weak inhibition against porcine pancreatic α-amylase as well as moderate antimicrobial activity against food microorganisms. From these results, except for 6, seed extract has the potential to scavenge active oxygen as an antioxidant, suppress fat absorption, control the level of sugar in the blood, improve intestinal bacterial flora by depressing the multiplication of harmful enterobacteria and also accord to the extension of the shelf life of food[45].

Anti-Quorum Sensing Properties of G. gnemon[56].

Different parts of *Piper nigrum*, *Piper betle* and *G. gnemon* had been used as food sources in the Southeast Asia region for centuries. These plants serve not only as foods, but they are also used as medicinal plants, although little is known about the anti-QS properties of these plants. Their study furnishes a new insight on the anti-QS potential of *Piper nigrum*, *Piper betle* and *G. gnemon* extracts. Mainly three plant extracts of these plants viz. hexane, chloroform and methanol were assessed in bioassays involving *Escherichia coli* [pSB401], *E. coli* [pSB1075], *Pseudomonas aeruginosa* PA01 and *Chromobacterium violaceum* CV026. The results suggested that the different extracts of these three plants have anti-QS capacity. Consequently, the results obtained from this study also showed that the extracts of *P. betle* had the most effective inhibition against QS as compared to *P. nigrum* and *G. gnemon* as arbitrated by the bioassays. After all, these plants that serve as food sources in Malaysia, the compounds derived have lower possibility of causing any unwanted reaction in the human system. Though the anti-QS activity of these plants had yet to be tested, future work should emphasize on isolation and characterization of the anti-QS compounds.

CONCLUSION

The relation of *G. gnemon* with human culture probably is prehistoric, as this species of Miocene and Pliocene origin[57] maintained a close proximity with the aboriginal human tribes and races as evidenced in the regions from Assam to SE Asia. Since long this species has been a site of attraction because of its endless botanical curiosity, particularly in the evolutionary studies. Despite its own history of over three million years, *G. gnemon* is still maintaining its life span as one of the important constituents of tropical and subtropical rain forest ecosystems and also in cultivated form in several other environments.

Gnetum gnemon is a versatile medicinal plant with a wide range of ethnobotanical utilizations, which are primarily based on the diverse patterns of secondary metabolites found in the plant. Stilbenoids were found to be responsible for the pharmacological effects of *G. gnemon* like inhibitory effect on tyrosinase activity, melanin biosynthesis and on multiple angiogenesis. Additionally, clinical studies suggested that stilbenoids were beneficial in the management of diabetes and cardiovascular diseases. Resveratrol, a polyphenol biphenyl, and multiple hydroxyl groups and its derivatives such as resveratrol oligomers polyphenol have been emerging to be promising new sources of natural antioxidant[58,59]. Resveratrol is also known to protect the plant while being invaded by vermin, or while climate is severe[60]. *Gnetum gnemon* resveratrol (in dimer form) seemed to be effective in preventing arteriosclerosis, cancer, Alzheimer's diseases and other life style diseases. In addition to *G. gnemon* resveratrol's anti-oxidative and antiviral activities, its probable role in reduction of visceral fat and increase stamina in human beings[61] have attracted the global markets. Hence, from the available scientific data pertaining to the nutritive potency and antitoxicity effect of this plant, it can be inferred that *G.*

gnemon is a safe food and can be utilized effectively as a basic raw material to develop a novel nutritious functional food. Exploration of its newer pharmacological properties in addition to the reported activities can fill up the gap for development of a novel compound with medicinal as well as nutritive properties.

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