

INCORPORATION OF TONGKAT ALI AND GINSENG EXTRACTS FROM MASS PROPAGATED ROOTS DERIVED FROM BIOREACTOR TECHNOLOGY AS SUPPLEMENTS IN ENERGY CHOCOLATE CONFECTIONERY

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Abstract

Tongkat Ali (*Eurycoma longifolia*) and Ginseng (*Panax ginseng*) are well known herbs among Asians and have been sought after by Europeans and others for the benefits to health, especially as aphrodisiac and nourishing stimulants. They have high antioxidant level and were reported to be used in the treatment of type II diabetes, as well as for sexual dysfunction in men. Since Tongkat Ali and Ginseng are difficult to cultivate and have a long cultivation period, the bioreactor technology is the alternative method to produce huge amount of raw materials for the herbal industry and continuous supply of standardized raw materials that is not affected by geographical and environmental factors, soilless and free from pesticides and other contaminants. Tongkat Ali and Ginseng extracts from mass propagated roots derived from bioreactor technology have similar profiles as extracts derived from normal cultivation. Liquid Chromatography–Mass Spectrometer (LC-MS) profiles showed presence of active compounds in the Tongkat Ali and Ginseng extracts from the mass propagated roots. Cytotoxicity test using the brine shrimp (*Artemia salina* Leach) lethality assay, revealed that higher concentration of Tongkat Ali and Ginseng extracts from mass propagated roots did not kill or affect the brine shrimps, implying that the extracts were safe for consumption. Incorporation of combination of Tongkat Ali and Ginseng total extracts from mass propagated roots derived from bioreactor technology energy chocolate confectionery was accepted by the panelists in sensory evaluation and showed that the chocolate product has good potential as a carrier besides beverages and capsules.

Keywords: Tongkat Ali, Ginseng, chocolate, energy, bioreactor.

INTRODUCTION

The consumption of Tongkat Ali (*Eurycoma longifolia*) and Ginseng (*Panax ginseng*) are mostly through beverages and capsules. However, some manufacturers do not use the extracts in their products; whole powdered roots of Tongkat Ali are used instead, and this may affect the health of the consumers, especially on their vital organs. A simple, safer and easier consumption of Tongkat Ali and Ginseng extracts is sought and the bitter taste of Tongkat Ali extracts restricted the consumability among the consumers and they prefer if taken with beverages or in sweets.

Tongkat Ali and Ginseng are well known herbs among Asians and have been sought after by Europeans and others for the benefits to health, especially as aphrodisiacs and nourishing stimulants. They have high antioxidant level and were reported to be used in the treatment of type II diabetes, as well as for sexual dysfunction in men. Various parts of Tongkat Ali have been traditionally used for antimalarial, aphrodisiac, anti-diabetic, antimicrobial and antipyretic activities (Bhat & Karim, 2010). Tongkat Ali water extract increased the sexual activities and sperm count, motility and

viability in the treated rats (Mahanem et al., 2004). Tongkat Ali was found to increase testosterone and Dihydrotestosterone (DHT) in male rats, improved sperm concentration, motion and fertility. It also increased 60% ATP and produced rigid penile erection (Azimahtol, 2005).

Alike Tongkat Ali, both American ginseng (*Panax quinquefolius*) and Asian ginseng (*Panax ginseng*) roots are taken orally as aphrodisiacs, nourishing stimulants, and in the treatment of type II diabetes, as well as for sexual dysfunction in men. The root is most often available in dried form, either whole or sliced (Ang & Cheang, 2001). Recent studies have also shown that ginseng root suspension cultures could be induced for accumulation of phenolic compounds with high antioxidant properties useful for human health (Mohammad Babar Ali et al., 2005a; 2005b). Since Tongkat Ali and Ginseng are difficult to cultivate and have a long cultivation period, the bioreactor technology is the alternative method to produce huge amount of raw materials for the herbal industry. Bioreactors have also been used for the cultivation of hairy roots mainly as a system for secondary metabolite production. Furthermore, innovative processes have been proposed for producing secondary metabolites selectively by enzymatic reactions. This technology gives continuous supply of standardized raw materials that is not affected by geographical and environmental factors, soilless and free from pesticides and other contaminants. This project was carried out to incorporate the combination of Tongkat Ali and Ginseng total extracts from mass propagated roots derived from bioreactor technology into chocolate that acts as energy booster and blood circulation enhancer.

MATERIALS AND METHODS

Determination of active compounds in Tongkat Ali and Ginseng extracts from mass propagated roots derived from bioreactor technology was carried out using Liquid Chromatography-Mass Spectrometer (LC-MS) system.

The constituents of bioreactor produced Tongkat Ali root samples (0.02 g powder) were extracted with 10 ml of 95% methanol for days at 40 °C. The extract was sonicated for 20 min. The suspensions were filtered through a 0.45 µm PTFE syringe filter (Whatman, UK) prior to injection into the Liquid Chromatography-Mass Spectrometer (LC-MS) system.

The constituents of bioreactor produced Ginseng root samples (0.02 g powder) were extracted with hot 95% methanol. The extract was further partitioned with ethyl acetate and water. The aqueous layer was extracted with butanol, and this final extract separated via silica gel chromatography. The elution solvent consisted of a mixture of CH₂Cl₂:MeOH:H₂O in varying ratios from 50:10:1 to 5:5:1.

Cytotoxicity tests of Tongkat Ali and Ginseng extracts were carried out using the brine shrimp (*Artemia salina* Leach) lethality assay. Powdered Tongkat Ali or Ginseng root samples (each at 25 g) from mass propagated roots derived from bioreactor were individually extracted with water (500 ml) and then filtered. Filtrates were concentrated in a rotary vacuum evaporator and stored in the refrigerator. Brine shrimp dried eggs were hatched using method as described by Seri Chempaka et al. (2006) and Zainah et al. (2005). Tongkat Ali and Ginseng extracts were first diluted to 10 mg/ml with sea-salt (Sigma) solution. The brine shrimp lethality test was carried out using the 96-well microplates method as described by Solis (1993).

Incorporation of Tongkat Ali and ginseng extracts in chocolate confectionery was carried out with concentration of 25 mg per 10 gm chocolate respectively. The extracts were obtained by boiling powdered Tongkat Ali or Ginseng roots from mass propagated roots derived from bioreactor (5 g) in 200 ml distilled water for 15 min and filtered. The extract was mixed with a food grade carrier in proportion 1:2 and dried in a dryer (50 °C) for 2 h. The dried extract was kept in airtight glass bottles in refrigerator. Both Tongkat Ali and Ginseng extracts were combined and mixed in the melted chocolate until smooth before poured into the moulds.

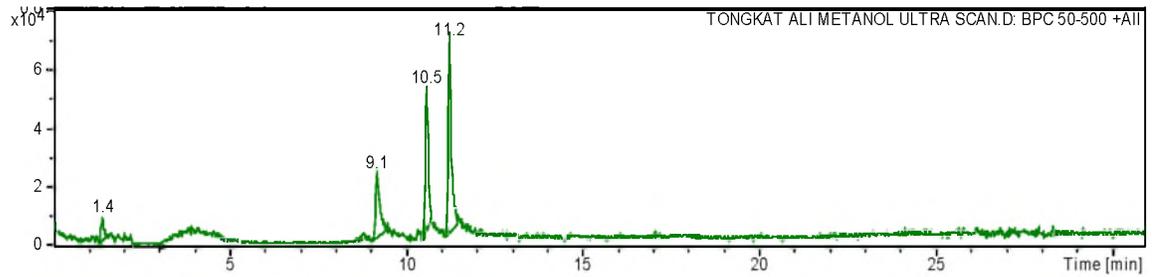
Acceptability of the Tongkat Ali and Ginseng chocolates were determined through sensory evaluation using 30 members (male and female) of untrained panelists comprising staff of Malaysian Nuclear Agency. A 5-points hedonic rating scale was used with number 5 as the most acceptable and number 1 as the most unacceptable. The attributes evaluated were colour, aroma, hardness, sweetness, chewiness and overall acceptance on samples with 0%, 0.2% and 0.4% Tongkat Ali and Ginseng extracts. Statistical analysis using ANOVA test (SAS Institute, 1991) and comparisons were made by Duncan's multiple range tests.

RESULTS AND DISCUSSION

Tongkat Ali and Ginseng extracts from mass propagated roots derived from bioreactor technology (Figure 1) have similar profiles as extracts derived from normal cultivation. Liquid Chromatography–Mass Spectrometer (LC-MS) profiles showed presence of active compounds in the Tongkat Ali and Ginseng extracts from the mass propagated roots (Figure 2 and Figure 3).



Figure 1: Tongkat Ali and Ginseng roots from mass propagated roots derived from bioreactor technology: A–Tongkat Ali tissue culture, B–Tongkat Ali roots in lab scale bioreactor, C–1000 L Air-Lift Bioreactor System, D–Ginseng roots from air-lift bioreactor.



Mass spectrum : Tongkat Ali in methanol

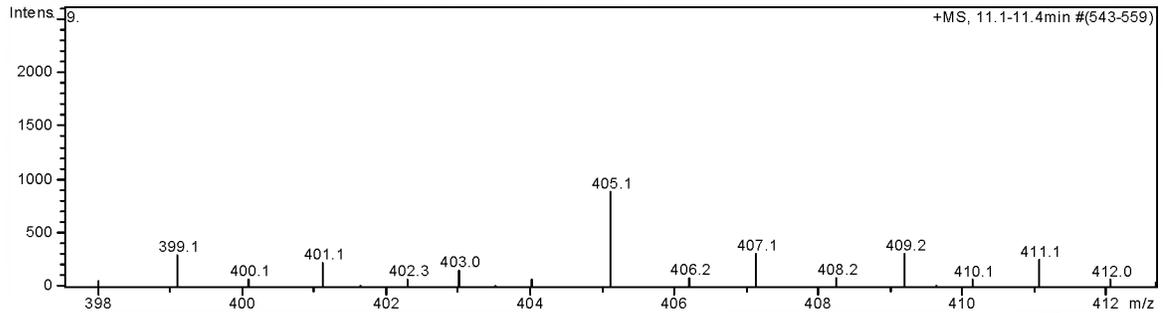


Figure 2: Presence of active compounds in the Tongkat Ali root extract from the mass propagation roots in Liquid Chromatography Mass Spectrometer (LC-MS) profiling.

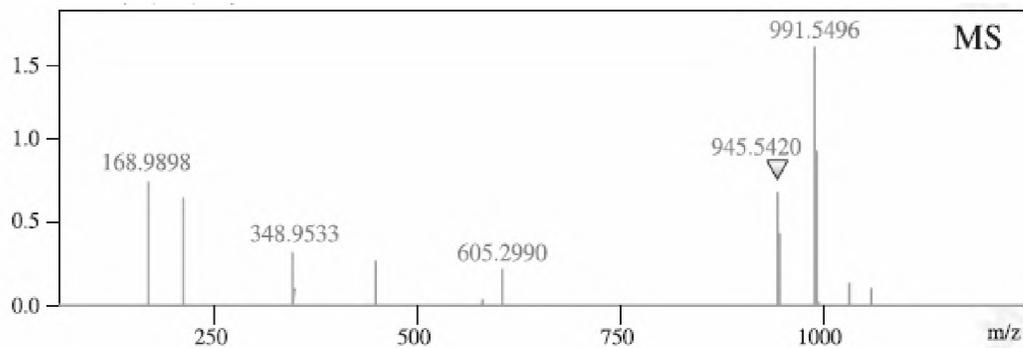
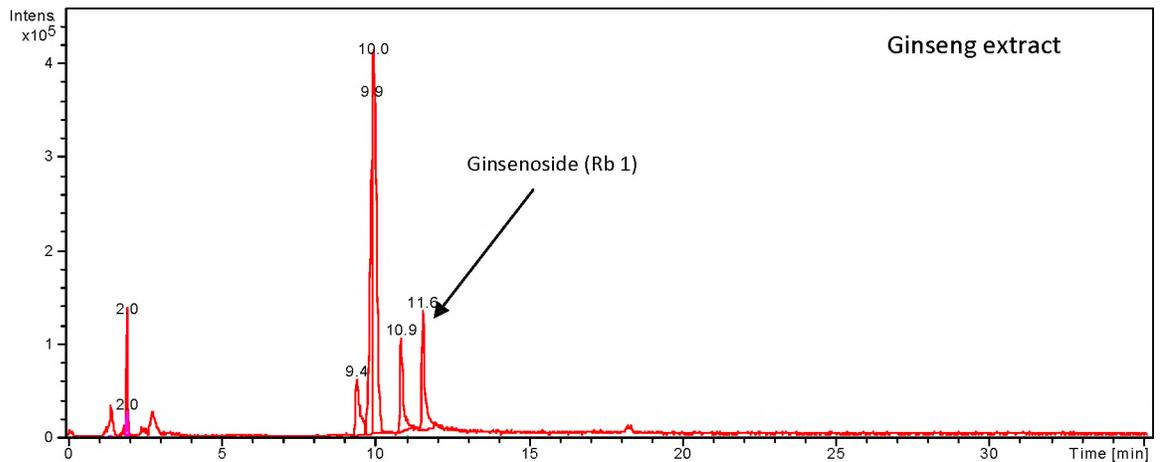


Figure 3: Presence of active compound in the Ginseng extract from the mass propagation roots in Liquid Chromatography Mass Spectrometre (LC-MS) profiling. The quantitative analysis with the optimized LC-MS method demonstrated that the various batches of Ginseng bioactive extract did not fall within a narrow range of each chemical constituent.

Cytotoxicity test using the brine shrimp (*Artemia salina* Leach) lethality assay revealed that higher concentration of Tongkat Ali and Ginseng extracts from mass propagated roots did not kill or affect the brine shrimps, implying that the extracts were safe for consumption. Early studies on Tongkat Ali reported that the level of acute oral LD50 of Tongkat Ali aqueous extracts is greater than 1000 mg/kg BW in male and female mice. According to WHO protocol on the acute oral toxicity classification, this compound is unlikely to possess hazardous substance therefore safe to be used as herbal preparation (Azimahtol, 2002). There was no significant cytotoxic effect of water extracts obtained from the root of Tongkat Ali was detected on MDBK (kidney) normal cell line (Nurhanan et. al., 2005). In numerous clinical trials, the dosage of crude root has ranged from 0.5 to 3 g/day and the dose of extracts has generally ranged from 100 to 400 mg (<http://www.drugs.com/npp/ginseng.html>) and 200 mg for Tongkat Ali (http://www.raysahelian.com/tongkat_ali.html). Malaysian Cocoa Board recommended that application of 0.2% of Tongkat Ali powder extract was found to be suitable in plain chocolate.

Sensory evaluation as in Figure 4 illustrates the results of the initial sensory evaluation carried out for the products. Samples with higher percentage of Tongkat Ali and Ginseng extracts (0.2%) were most acceptable for most of the attributes followed by samples with 0.4% and control (0%). Chocolate is the most suitable carrier that can mask the bitter taste of Tongkat Ali extract (as compared to biscuits and cakes) and combination with ginseng extract gives the chocolate a unique and exotic taste. The availability of extracts of Tongkat Ali and Ginseng will be easier for industries due to the bioreactor technology that can produce huge amounts of raw materials and guaranteed the continuous supply of the herbal extracts. The chocolate product has good potential as a carrier for introducing unique ingredients, for example herbal extracts from soilless cultivation for the advancement of the food industry.

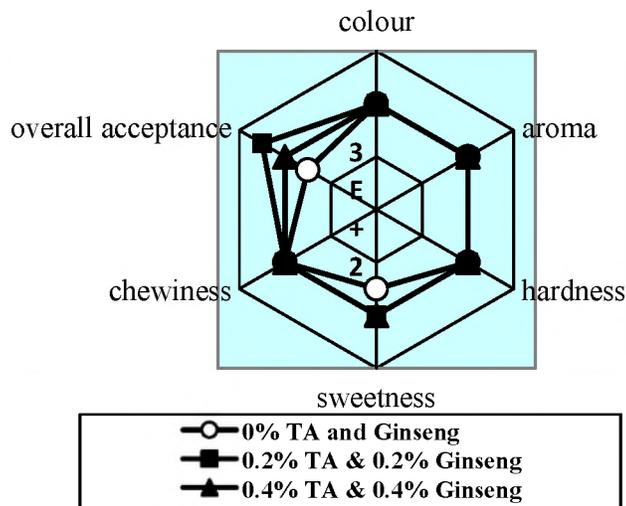


Figure 4: The acceptance of chocolates with different percentage of Tongkat Ali and Ginseng extracts. Samples with 0.2% of Tongkat Ali and Ginseng extracts were most acceptable for most of the attributes followed by samples with 0.4% and control (0%).

Benefits of these products:

- New chocolate confectionery product using extracts from bioreactor technology, which is rapid, robust and reproducible for continuous production (Figure 5).
- Soilless raw materials and free from pesticide.
- Affordable healthy product for the consumer.
- Simple and easy to consume and no bitter taste of herbal extracts.

- Continuously supply of raw materials from bioreactor with consistent quality and quantity for food and herbal industries.
- Additional profits for industries that currently involved in plant cell, tissue, organ culture, food industries, nutraceutical and pharmaceutical industries.
- Suitable value added product for commercial chocolate production
- Reduce cost of production of raw materials and production line spacing.
- High efficiency of chocolate confectionery product.
- Less space requirement for production and manpower.
- In house production and job opportunity for housewives.
- Customers' satisfaction as they do not create secondary risks to consumers.



Figure 5: Energy chocolate confectionery supplemented with Tongkat Ali and Ginseng extracts from mass propagated roots derived from bioreactor technology.

CONCLUSION

Chocolate is the most suitable carrier that can mask the bitter taste of Tongkat Ali extract and combination with ginseng, an energy chocolate confectionery was developed and act as energy booster and blood circulation enhancer. Application of 0.2% of Tongkat Ali and Ginseng extracts from mass propagated roots derived from bioreactor technology in chocolate was most suitable and acceptable.

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