

Full Length Research Paper

Standardization of *Elaeis guineensis* with respect to authenticity, assay and chemical constituent analysis

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In consideration of commercialization of formulations based on medicinal plants, quality control standards of various medicinal plants used in traditional medicine are becoming more important. An attempt for the standardization of *Elaeis guineensis* leaf has been carried out with respect to authenticity, assay and chemical constituent analysis. Many parameters of authentication involved in this study include gross morphology, microscopy of leaf and functional groups analysis by the fourier transform infrared (FTIR) method. Standardization involves the determination of minimum inhibitory concentration (MIC) of the extract whereby the chemical effects could be assessed and curative values established. The standardization of chemical constituent involves the quantification of lead molecules in *E. guineensis*. The MIC of the *E. guineensis* leaves extract was investigated using broth dilution method. The extract showed a MIC value of 6.25 mg/ml and same value was retained for different extracting time. The gas chromatography-mass spectrometry (GC-MS) method used for quantifications of 2,6,10,14,18,22-tetracosahexaene in the extract was rapid, accurate, precise, linear ($R^2 = 0.993$), rugged and robust. Hence this method is suitable for the quantification of 2,6,10,14,18,22-tetracosahexaene in *E. guineensis*.

Key words: *Elaeis guineensis*, standardization, microscopy, medicinal plants.

INTRODUCTION

Elaeis guineensis belonging to the family Palmae and tribe Coccoideae is a perennial monocot which originates from West Africa. *E. guineensis* was growing wild and was later developed into an agricultural crop. It was first introduced in Malaya as an ornamental plant in the early 1870's. In 1917, the first commercial planting took place in Tennamaram Estate in Selangor, laying the foundations for the vast oil palm plantations and palm oil industry in Malaysia (Chong et al., 2008). Oil palm is used for the treatment of cancer, headache, rheumatism, as an aphrodisiac, diuretic and liniment (Sasidharan et al., 2009). Besides that, the leaf extract and young petiole juice are applied to fresh wound. On the other hand, the fruit mesocarp oil and palm kernel oil are administered as poison antidote and used externally with several other herbs as lotion for skin diseases. Palm kernel

oil is applied to convulsant children to regulate their body temperature (Chong et al., 2008).

In consideration for the commercialization of formulations based on medicinal plants, quality control standards of various medicinal plants used in traditional medicine are becoming more important. Standardization as defined by American Herbal Products Associations refers to the body of information and controls necessary to produce materials of reasonable consistency. This is achieved through minimizing the inherent variation of natural product composition through quality assurance practices applied to agricultural and manufacturing process (Gaedcke et al., 2003). Hence the current study was carried out for the standardization of *E. guineensis* leaf with respect to authenticity, assay and chemical constituent analysis.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals and reagents used were of analytical grade,

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purchased from Sigma chemical co. (ST LOUIS, MQ, USA) and Merck (DARMSTADT, GERMANY).

Plant sample

Sample of *E. guineensis* was collected in August 2009, from Pulau Pinang, Malaysia and authenticated at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia where a sample (voucher number 11037) has been deposited.

Qualitative investigation

The macroscopic feature of the fresh leaves was determined using the methods of Evans, (1996). Anatomical sections and surface preparations of the fresh leaves sample for microscopy was carried out according to methods outlined by Brain and Turner (1975). Morphological examination of the *E. guineensis* leaf specimens was used to identify the structures of the leaf. Leaf tissue was fixed in 10% buffered formalin. After fixation, the tissue was dehydrated in a graded series of alcohol, cleared in xylene and embedded in paraffin wax. Multiple 5 mm sections from the block were mounted on slides and stained with 0.5% methylene blue and examined under a light microscope for identification of leaf structure.

Fourier transform infrared (FTIR) analytical methods

The methanol extracts of *Celeus spectabilis* was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra was recorded as KBr pellets on a Shimadzu FTIR Spectrometer 8000 series, between 4000 – 500 cm^{-1} . All determinations were performed in triplicate (Sasidharan et al., 2009).

Determination of minimum inhibitory concentration (MIC) of leave extract

Serial broth dilution technique was used to determine the MIC of the extract against *Staphylococcus aureus*. The stock solution of the plant extract was prepared to a concentration of 200 mg/ml. In serial dilution technique, 5 ml of the prepared stock solution was transferred to a test tube containing 5 ml of nutrient broth to give a concentration of 100 mg/ml, from which 5 ml was transferred to another test tube containing 5 ml of nutrient broth to give concentrations ranging from 100 to 0.1 mg/ml. After the preparation of the *S. aureus* suspensions (10^7 organism per ml), 500 μl of the bacteria suspensions was added to each broth dilution. After 24 h incubation at 37°C, the tubes were examined for growth. The MIC of the extract was taken as the lowest concentration that shows no growth. Growth was observed in those tubes where the concentration of the extract is below the inhibitory level and the broth medium was turbid (cloudy) when observed (NCCLS, 2002).

Gas chromatography–mass spectrometry (GC–MS) analysis

The GC-MS analysis was done on a thermo gas chromatograph mass spectrometer (Agilent GC/MS 6890N/5873I) equipped with column HP-5ms (30 m long, 0.25 mm i.d., film thickness 0.25 μm). The inlet temperature was 280°C. The oven temperature was programmed to 70°C for 2 min, with 20°C increases per min to 280°C, which was maintained for 20 min. The carrier gas was helium at a flow rate of 1.2 ml/min (splitless mode). MSD transfer

line heater was set to 280°C. The quadrupole mass spectrometer was scanned over the range 10 – 700 amu, with an ionising voltage of 70 eV, and an ion source temperature of 250°C. The compounds were identified by computer searches in commercial libraries of the National Institute of Standard and Technology (NIST) (Soetardjo et al., 2007).

RESULTS AND DISCUSSION

Herbarium

The authenticity of specimens using herbarium is becoming extensively important as the plant classification is constantly changing. Vouchers specimens help cross-reference these changes to previous plant material. Therefore, in this study, the herbarium of *E. guineensis* was prepared as shown in Figure 1. Since *E. guineensis* is recognized in the pharmaceutical industries, authenticity of the specimens is an important step for these industries to avoid the acceptance of wrong plant material for extracting drug which results in loss of billions of dollars. Therefore, for future *E. guineensis* collections, the correctly identified herbarium specimen can be used for comparison. In addition, herbarium specimen will help in the identification of field collected plant specimens by looking into the morphological or anatomical details.

Macroscopy and microscopy of leaf

Macroscopic and microscopic evaluation of the botanical materials for standardization has been suggested in most of the regulatory guidelines and pharmacopoeias (WHO, 1998). Therefore, in this study, macroscopic and microscopic evaluations were done for the leaf material due to the usage of this leaf extract for antimicrobial activity in our future study. The leaves of *E. guineensis* were subjected to macroscopical examination and observations were recorded. The proper examination of the leaves was carried out under sun light and artificial source similar to day light. The leaves of *E. guineensis* were observed to be dark green with 100 - 150 pair of leaflets, 60 - 120 cm long and 3.5 – 5 cm broad. The photomicrograph of the identifying features of the leaf is shown in Figure 2. Transverse section of the leaf of *E. guineensis* through the midrib revealed the presence of upper epidemis with straight anticlinal walls and elongated palisade cells underneath. There are spongy parenchymas cells conspicuously on the lower surface and in between are the xylem and phloem vessels.

FTIR fingerprinting

The use of FTIR fingerprinting for herbal extract tends to focus on identification and assessment of the stability of the chemical constituents functional groups observed by FTIR analysis. The results of FTIR finger print for the methanolic extract is shown in Figure 3. The results of



Figure 1. Herbarium of *E. guineensis*.



Figure 2. Transverse section of the leaf of *E. guineensis*.

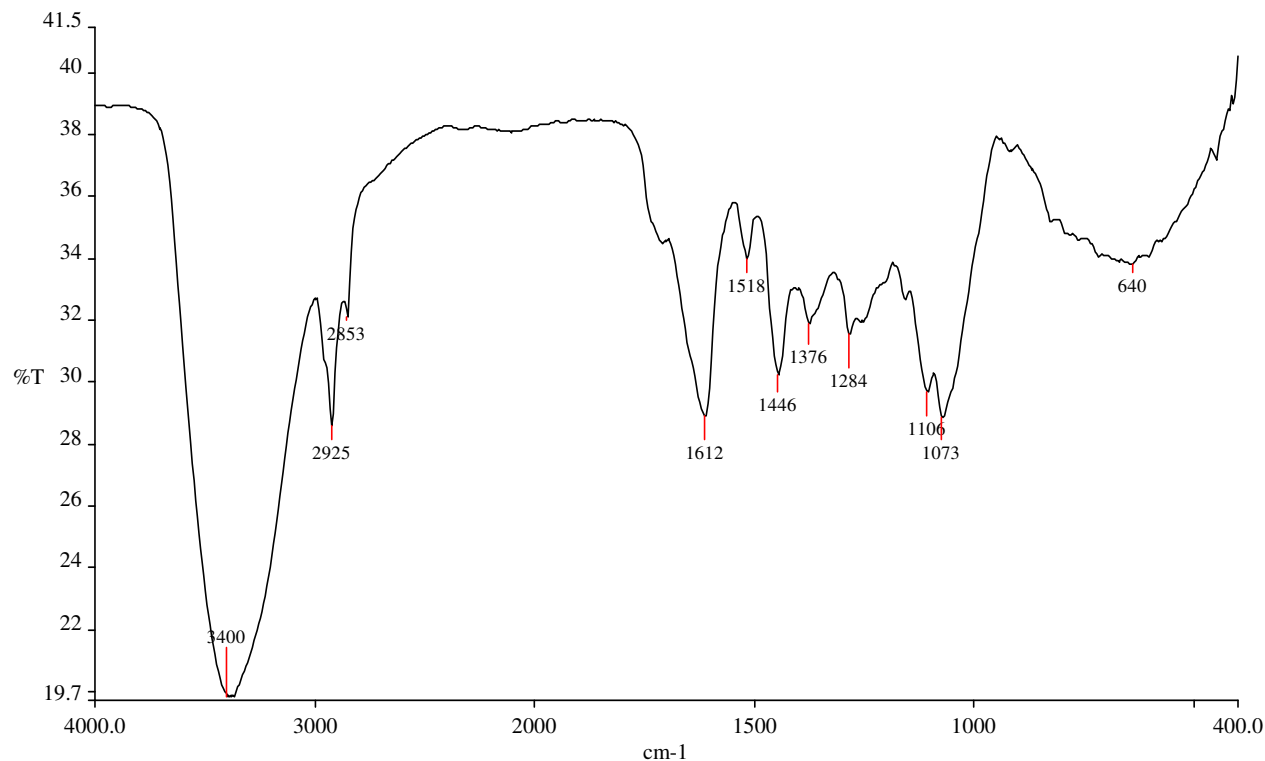


Figure 3. FTIR spectra of methanolic extract of *E. guineensis* (KBr disc), cm^{-1} .

functional group analysis using FTIR had demonstrated the existence of various functional groups in the *E. guineensis* extract. This was verified by the peaks formed during the FTIR study (Figure 3). Ten major peaks in the range of 1000 - 1700 and 2800 - 3400 cm^{-1} were observed in the FTIR spectra. Therefore, for future *E. guineensis* methanolic extraction, this FTIR spectra can be used for comparison. Whereby, the further extraction should be standardized to these FTIR fingerprint. Obviously, the FTIR fingerprint can be used to ensure that the functional groups in the new extract are present in reproducible manner although new extraction has been done. In this way, FTIR fingerprints give information that assist in manufacturing control and assure batch-to-batch consistency of the extraction.

MIC

Consistency in biological activity is an essential requirement for the effective use of therapeutic agents. It ensures that the extract remains with its therapeutic parameters throughout the shelf life assigned to the extract. Therefore it is crucial to carry out research that confirms that the extract retains its efficacy and biological activity before it is consumed by the pharmaceutical industries. Hence in this study, the antimicrobial activity by determining the MIC value was used for this purpose. The MIC of the *E. guineensis* leaves extracts was

investigated by using broth dilution method against pathogenic bacteria *S. aureus*. The extracts showed a MIC value of 6.25 mg/ml and retained the same value for the three different extracting times. The retained MIC values demonstrate the established chemical effects and curative values of this extract.

Standardization of chemical constituent

Along with authentication of species identity and assay, chemical constituent standardization is also required for quality control in the use of plants materials for pharmaceutical purposes. In this study, the *E. guineensis* leaf extract was identified as extract containing no constituents documented as being determinant or relevant for efficacy, or as having pharmacological or clinical relevance. In this case, chemically defined constituent (markers) without known therapeutic activity may be used for control purposes. This marker may be used to monitor good manufacturing practice or as an indication for the content of the extract. The standard curve is plotted using the area of the peak obtained in the GCMS chromatogram against the concentration of 2,6,10,14,18,22-tetracosahexaene. The GCMS profile and calibration curve of the compound is given in Figures 4 and 5. This could be applied for the standardization and validation of *E. guineensis* leaf extract in terms of 2,6,10,14,18,22-tetracosahexaene as a chemical marker.

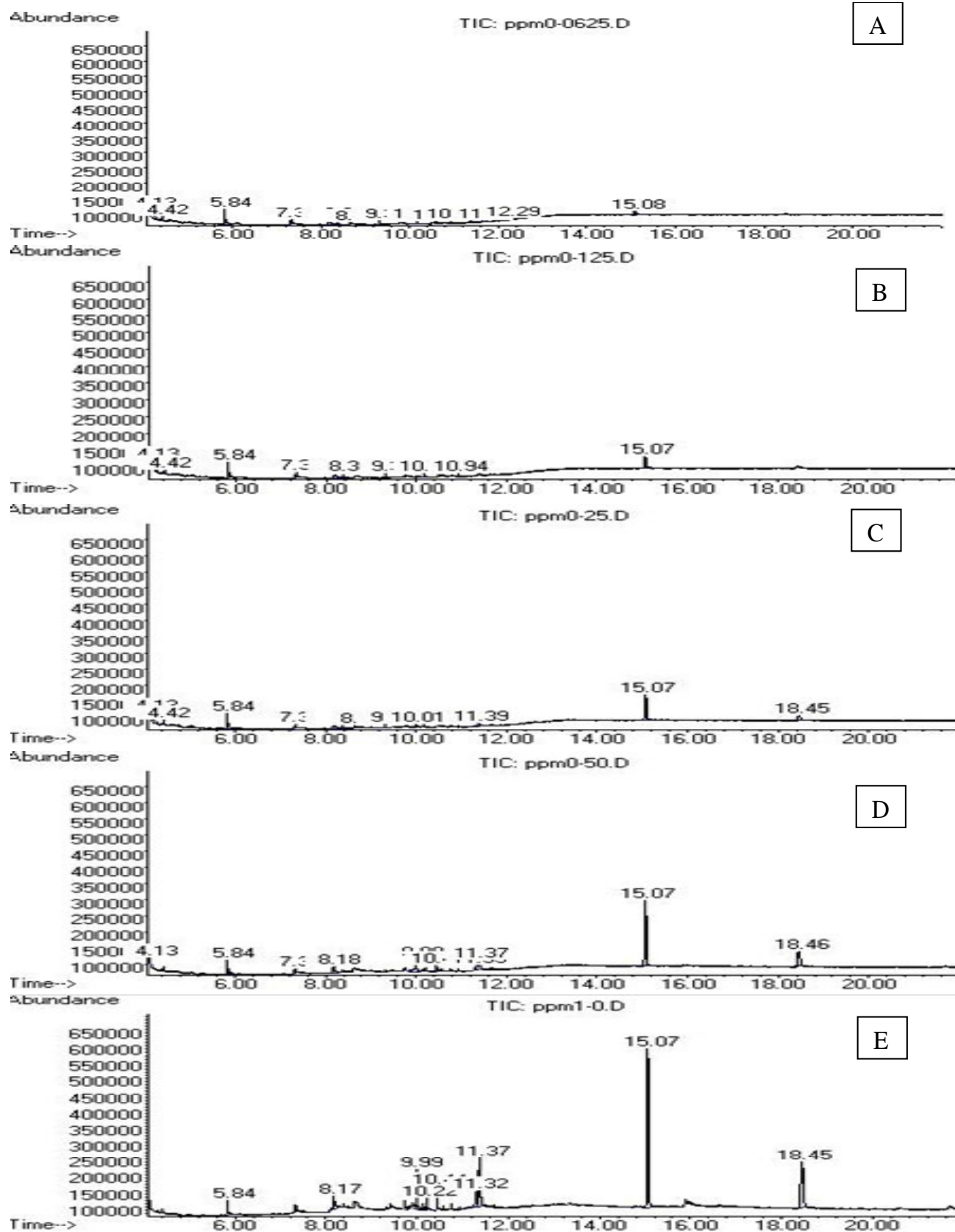


Figure 4. GCMS profile of 2,6,10,14,18,22-tetracosahexaene at retention time 15.07. A = 0.0625 mg/ml, B = 0.1250 mg/ml, C = 0.2500 mg/ml, D = 0.500 mg/ml, E = 1.000 mg/ml.

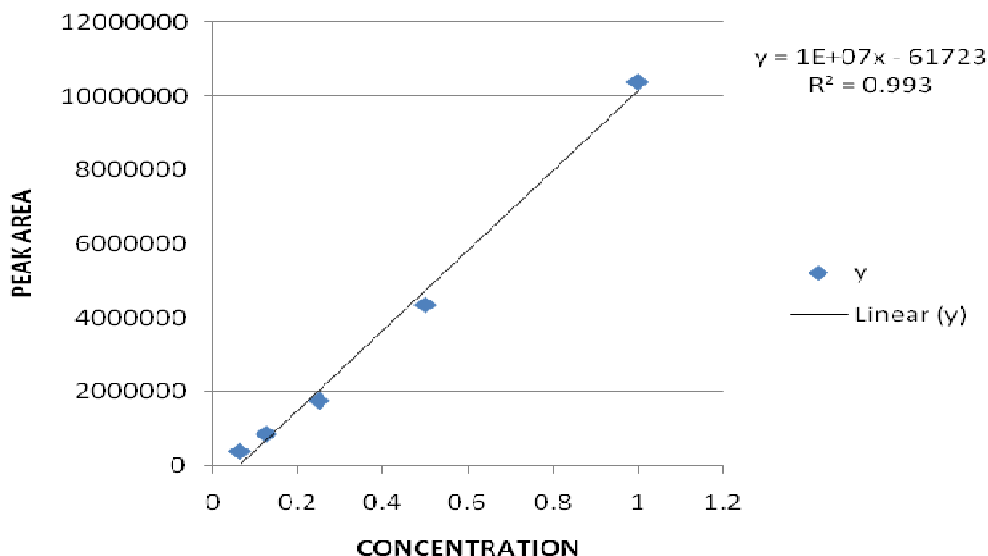


Figure 5. Calibration curve of 2,6,10,14,18,22-tetracosahexaene by GCMS.

Conclusion

The results obtained in the present investigation are encouraging and will be used as reference data for the standardization of *E. guineensis* and the formulations containing *E. guineensis* as a main ingredient. The plant is collected from wild sources and varies in constituents and efficacy due to geographical diversity. Improper collection and storage conditions lead to contamination by microorganism and heavy metals. Standardization is the prime need of time because it establishes quality and identifies profile that can be used for the purpose of safety monitoring and overall quality assurance of herbal medicines. There is an urgent need for evaluation and analysis of herbal drugs using modern sophisticated techniques of standardization.

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REFERENCES

- Brain KR, Turner TD (1975). The practical evaluation of phytopharmaceuticals. Wright Sciencetechnica, Bristol, pp. 81-82.
- Chong KH, Zuraini Z, Sasidharan S, Kalnisha Devi PV, Yoga Latha L, Ramanathan S (2008). Antimicrobial activity of *Elaeis guineensis* leaf. Pharmacologyonline, 3: 379-386.
- Evans WC (1996). Trease and Evans' Pharmacognosy. 14th Ed. W. B. Saunders Company limited, London, pp. 545-546.
- Gaedcke F, Steinhoff B, Blasius H (2003). Herbal Medicinal Products: Scientific and Regulatory Basis for Development, Quality Assurance and Marketing Authorisation By (Wissenschaftliche, Stuttgart). CRC Press, Boca Raton, Florida, USA, pp. 37-52.
- NCCLS (National Committee for Clinical Laboratory Standards) (2002). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Fourth Ed. Approved standard. NCCLS document M100-S12. Wayne, PA, NC-275 CLS.
- Quality Control Methods for Medicinal Plants Materials (1998). Document No. WHO/PHARMA/92.559/rev.1. World Health Organization: Geneva, Switzerland.
- Sasidharan S, Darah I, Jain K (2009). Effects of season on the yield and quality of extracts from *Gracilaria changii* (Gracilariaceae, Rhodophyta). Sains Malays. 38: 935-937.
- Sasidharan S, Sharmini R, Vijayarathna S, Yoga Latha L, Vijenthir R, Amala R, Amutha S (2009). Antioxidant and hepatoprotective activity of methanolic extracts of *Elaeis guineensis* Jacq leaf. Pharmacologyonline, 3: 84-90.
- Soetardjo S, Chan JP, Noor AM, Lachimanan YL, Sreenivasan S (2007). Chemical composition and biological activity of the *Centipeda minima* (Asteraceae). Mal. J. Nutr. 13: 81-87.