

ANTIMICROBIAL ACTIVITY OF *ELAEIS GUINEENSIS* LEAF EXTRACT AGAINST *GANODERMA BONINENSE* OF OIL PALM BASAL STEM ROT

ELAINE LEE, H.C. AND CHONG, K.P.*

Sustainable Palm Oil Research Unit (SPOR), School of Science and Technology, Universiti Malaysia Sabah,
88400 Kota Kinabalu, Sabah, Malaysia.

*Corresponding author's email: chongkp@ums.edu.my

Abstract

Basal stem rot (BSR) which is caused by *Ganoderma boninense* is the most serious disease faced by oil palm *Elaeis guineensis* in Malaysia. Hitherto, many control measures such as cultural practices, fungicides, and biocontrol agents were attempted, yet, the result were unsatisfactory. This study was conducted to investigate potential antimicrobial properties in oil palm leaf extract against *G. boninense*. Oil palm leaf were extracted with different solvents namely methanol, acetone, chloroform and petroleum ether. The antimicrobial activities and fungitoxicity of leaf extracts against *G. boninense* were expressed in inhibition of mycelia growth of *G. boninense* on Potato Dextrose Agar (PDA) incorporated with the four different solvent leaf extracts with range concentration of 0-40 mg mL⁻¹. Acetone leaf extract was found to be very fungitoxic to *G. boninense* at concentration of 40 mg mL⁻¹, the highest concentration tested in this study. Methanol and petroleum ether leaf extracts were having inhibitory effect with 40 mg mL⁻¹ in comparison to the absolute control. Further separation of active bioactive compounds was conducted using thin layer chromatography (TLC) bioassay for methanol and acetone leaf extracts. Acetone extract was found to possess good antifungal activity with properties of R_f 0.09, 0.21, 0.24, and 0.60 compared to methanol extract, which only showed inhibition at R_f 0.74 against *Aspergillus niger*. The R_f values suggested that potential antimicrobial compound might be mixtures of polar and non-polar properties. The results suggested oil palm leaf extract might potentially explore as an important allelopathic agent against *Ganoderma boninense*.

Key words: Antimicrobial, Oil palm leaf, *Elaeis guineensis*, Basal stem rot, *Ganoderma boninense*.

Introduction

Oil palm has been nominated as the “golden crop of Malaysia” since it generates profitable export earnings for the country and are truly nature’s gifts in alleviating poverty in Malaysia (Basiron, 2007). However, oil palm is subjected to a disastrous malady namely Basal Stem Rot (BSR) that has devastated thousands of hectares of plantings which led to deficit as high as RM 1.8 billion in oil palm industry for the past decades (Idris, 2012). BSR and its causal pathogen *Ganoderma boninense* were first reported in 1931 by Thompson which infected old aged oil palm trees that are older than 25 years old (Latiffah & Ho, 2005). In the 1960, the disease was found in younger palms of 10-15 years (Turner, 1981). Oil palm has an economic life span of 25-30 years. BSR can kill more than 80% of stands by the time they are halfway through normal economic life (Chong *et al.*, 2012a, 2012b). *G. boninense* initially infects the palm roots and gradually spread to the bole of the stem where they cause dry rot, which prevents absorption and transport of nutrients (Sanderson *et al.*, 2000; Naher *et al.*, 2012). By the time *Ganoderma* fruiting bodies are detected on the oil palm, about 50% of the internal tissues would have already rotted (Kandan *et al.*, 2010). The available control measures for BSR diseases, such as cultural practices, fungicides, and biocontrol agents were unsatisfactory due to the fact that *Ganoderma* has various resting stages like resistant mycelium, basidiospores, chlamydospores and pseudosclerotia (Susanto *et al.*, 2005; Izzati & Abdullah, 2008). With no known remedy at present, effects of *Ganoderma* infection on productivity decline in palm crops have been of considerable concern on economic importance in Malaysia oil palm industry. Rapid development of the oil palm industry since 1990s has

caused an increasing output of by-products viz. oil palm fronds up to 100 kg ha⁻¹ (dry matter basis) were produced daily (Ishida & Abu Hassan, 1997). As a matter of fact, fronds are usually the part being harvested for roughage source or as compound feed for ruminants, the leaf and rachis are left behind (Chan, 1999). Conversely, oil palm leaf contains significant level of secondary metabolites than the frond which play major role in plant defensive mechanism as well as ethnopharmacology properties (Syahmi *et al.*, 2010). In the past research, several secondary metabolites has been determined from oil palm leaf such as flavonol, hydroxycinnamic acid, o-Diphenol, phenolic, tocopherol, carotene and sterols (Ng & Choo, 2010) and all these may potentially to be exploited for the management of fungal diseases (Chong *et al.*, 2008). Exploitation of naturally available chemicals from plants would be more realistic and ecologically sound method for plant protection. Hence the potential of this study can be viewed from the facts that there are excess of oil palm leaf available from the palm oil industry and they are treated as wastes and having no high value. This study was planned with the aim to examine the feasibility of using leaf of *E. guineensis* in managing the oil palm basal stem rot disease.

Materials and Methods

Preparation and extraction of plant material: Fresh oil palm leaf were collected from Field Laboratory of School of Science and Technology, Universiti Malaysia Sabah and washed under running tap water. Leaf samples were dried in oven at 40°C for 72 hours and blended into fine powder using a mechanical blender (Waring ® Commercial Blender). Powdered sample (100 g) were soaked in 300 mL of methanol and placed in a sonicator

(Branson ® 5510) for 10 minutes at temperature of 25°C followed by filtering through Whatman No. 1 filter paper and concentrated using a Rota Vapor™ (BUCHI) under reduced pressure at 40°C to obtain 1 mL of extract per 10 g of plant sample. The samples were extracted three times. Sample was also extracted with acetone, chloroform and petroleum ether in the same manner as followed for methanol. Aliquot were then kept in -20°C temperature for further use. Extraction yield was weighed and calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Dry weight of extract}}{\text{Dry weight of plant powder}} \times 100$$

In vitro antimicrobial assay: A series of concentrations of plant solvent crude extracts (0, 2, 10, 20, and 40 mg mL⁻¹) was incorporated into Potato Dextrose Agar (PDA). Crude extract was first dissolved in acetone:water (50:50, v/v) before incorporating into media. Agar without plant crude extracts, but containing identical concentration of acetone: water (50:50, v/v), served as negative controls. Another treatment in which no extract or solvent was added was served as absolute control. The *G. boninense* was taken from the edge of seven to eight days old culture using a sterile micropipette tip sized 0.8 cm and was introduced to the middle of the media. The growth of the pathogen was expressed in centimeter (cm) of diameter growth. Each extracts concentrations were assayed in triplicate and the mean values were calculated.

Thin layer chromatography bioassays: Acetone and methanol extract were further subjected to thin layer chromatography (TLC) for chemical profiling in order to detect the number of compounds of the extracts that displayed antifungal properties. Plant extracts were made to a concentration of 0.2 g mL⁻¹ by dissolving in respective solvents. Extracts (8 milligram or equivalent to 40 µL) were applied to 2 cm origins on TLC plates (Merck Kieselgel 60 F₂₅₄ silica gel). Plates were developed in a tank pre-equilibrated with solvent system of ethyl acetate: hexane (35:65, v/v) or (3:7, v/v). When the solvent reached 16 cm from the origin, the plates were taken out and air-dried followed by observation under UV light of 254 nm and 366 nm wavelength to observe the presence of fluorescence band (Chong, *et al.*, 2006). The plates were then sprayed with 7-10 days old spores of *Aspergillus niger* suspended in Potato Dextrose Broth (PDB) suspension and incubated in a moist chamber for two days at 20°C-22°C. Retention factor (R_f) for all bioactive and reactive bands were calculated and recorded.

Data analysis: Each treatment was replicated three times to obtain more accurate results. Mean values were subjected to Analysis of Variance (one-way ANOVA), and the Tukey test (SPSS statistical package version 20) was used to determine significant differences (p<0.05) between treatments.

Results and Discussion

Extraction yield: Choices of solvents play essential role in terms of the total extract yields (Table 1) and the ability of the respective solvent to extract the secondary

compounds for the oil palm leaf. In this study, methanol gave the highest yield (13.84%) compared to other solvents, followed by acetone (4.87%), chloroform (3.00%) and petroleum ether with the least yield (2.48%). Most bioactive compounds were commonly extracted using intermediate polarity solvent such as acetone, fewer in high like methanol and least in low polarity; hexane (Edziri *et al.*, 2010; Laouini *et al.*, 2012; Sayyad *et al.*, 2012). Furthermore, nearly all of the identified antimicrobial compounds from plants are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extract (Das *et al.*, 2010). Hence the most commonly used solvents for preliminary investigations of antimicrobial activity in plants are methanol, ethanol and water (Lourens *et al.*, 2004; Parekh *et al.*, 2006; Rojas *et al.*, 2006). However, there was report on extraction of bioactive compounds such as tannins and other phenolics were better in acetone than in methanol (Augustin, 1992). Similar result observed in study done by Masoko & Eloff (2006) whereby acetone was the best extractant followed by methanol. From the results Table 1, it showed contrast to the previous studies as methanol gave the highest yield (13.84%) followed by acetone extract (4.87%). Methanol was therefore the best extractant for this study compared to other solvents based on the quantity of plant material extracted. On another study, among the 20 different solvents evaluated, chloroform was found to be the best solvent for the extraction of non-polar biological active compounds (Harmala *et al.*, 1992). However, chloroform used in this study only obtained 3.00% of yield, slightly higher than petroleum ether (2.48%).

Table 1. Yield from different solvents for oil palm leaf extraction.

Solvents	Yield (g)	Yield (%)
Methanol	13.84	13.84
Acetone	4.87	4.87
Chloroform	3.00	3.00
Petroleum ether	2.48	2.48

Note: The initial weight of oil palm leaf powder was 100g

Inhibition of *Ganoderma* growth in media incorporated with oil palm leaf extract: *In vitro* bioassays were conducted to test the fungitoxicity and antimicrobial activities of the oil palm leaf extracts to *G. boninense*. Agar dilution method was chosen among all three bioassays tested in this study namely paper disc diffusion and agar well diffusion method as this method provide better distribution of extract in the agar which enhanced surface contact of fungal and plant extract, also, determination of minimum inhibitory concentration (MIC) can be done (Hadacek & Greger, 2000; Wiegand *et al.*, 2008). From the result, acetone extract of leaf of oil palm was found to possess good antifungal activity than methanol, chloroform and petroleum ether extracts against *G. boninense* although with lesser yield as shown in Table 1. This was comparable to absolute control and negative control. For acetone leaf extract concentration of 2-20 mg mL⁻¹ failed to completely stop the growth of the pathogen. However, at 40 mg mL⁻¹, *Ganoderma* was completely stopped up to two weeks of incubation (Fig. 1).

Growths of *G. boninense* both in absolute and negative control were not significant to each other indicate the toxicity was coming from the extracts and not from the solvent. Maximum growth (9 cm) was achieved by both absolute and negative control within the 14 days of incubation. Fungal growth rate was significant lower for those incorporated with acetone leaf extract in comparison to the absolute control. From day 4 till day 14 of the incubation, the fungal growth rate of *G. boninense* treated with the different concentrations of acetone extract was significantly lower than the mycelia growth in control. *G. boninense* were sensitive to all concentrations of acetone leaf extract. The similar trend was observed for methanol leaf extract. Whereas, for petroleum ether and chloroform leaf extract, only at higher concentration such as 10, 20 and 40 mg mL⁻¹, the fungal growth was significant lower throughout the 14 days as compared to absolute control. Higher concentration of crude leaf extracts had the higher ability to inhibit the growth of *G. boninense*.

The possible antimicrobial activity may due to presence of preformed antifungal metabolites in oil palm

leaf (Ng & Choo, 2010; Sasidharan *et al.*, 2012). Those components have been reported previously to have significant antimicrobial activity (Chong *et al.*, 2008; Syahmi *et al.*, 2010; Jaffri *et al.*, 2011; Rosalina Tan *et al.*, 2011). Furthermore, previous report by Sasidharan *et al.* (2012) had revealed the methanolic extraction of the leaf of oil palm comprised tannins, alkaloids, reducing sugars, steroids, saponins, terpenoids and flavonoids in the extract. The presence of flavonol, hydroxycinnamic acid, phenolic and o-Diphenol in oil palm leaf has also been reported by Ng & Choo (2010). Characterization of the oil palm leaf compound involved in biochemical and molecular defense against pathogen has been conducted by Vijayarathna *et al.* (2012) using FTIR analysis and demonstrated eight functional groups which were (OH) stretching vibrations from phenols present in the extract, carboxylic acid groups, aromatic, alkene C=C, primary alcohol and phenol groups. Therefore, there may be possibility of these compounds to be responsible for the antimicrobial against *Ganoderma* observed in the current study.

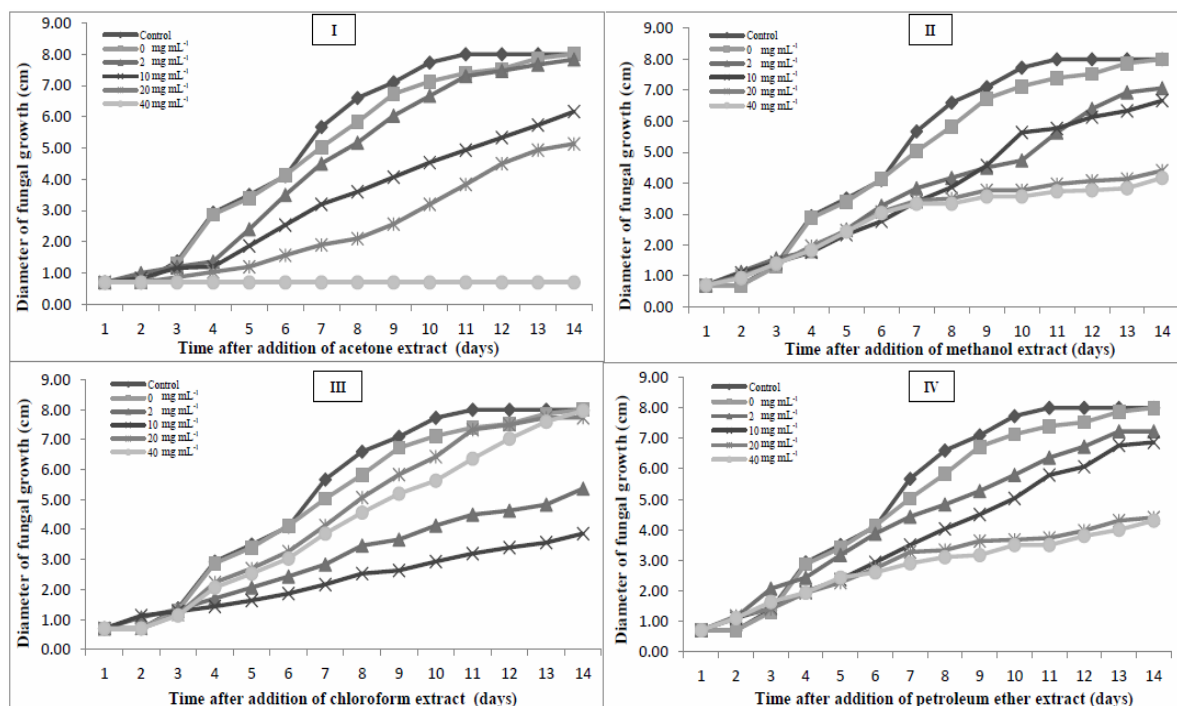


Fig. 1. Comparison of *Ganoderma boninense* mycelia growth in different concentrations oil palm leaf extract (I: Acetone extract; II: Methanol extract; III: Chloroform; IV: Petroleum ether) for 14 days of incubation.

TLC bioautographic: To further investigate properties that may contribute to *In vitro* inhibition of *G. boninense* growth as in *In vitro* bioassays, a thin layer chromatography (TLC) bioassay was conducted. Each extract (40 μ L or 8mg) was loaded on the origin of the TLC plate. Ethyl acetate and hexane were used as the mobile phase to develop the TLC plate at different ratio. Varying the ratio of the mobile phase would affect the R_f value of the separated compounds. Polarity difference between compounds and solvent systems, make the compounds that have relatively high R_f values in polar

solvents will have low R_f values in non-polar solvents (Suleiman *et al.*, 2010). Methanol extract developed in ethyl acetate: hexane (35:65) was separated into 13 spots whereas acetone extract developed in ethyl acetate: hexane (3:7) was separated into 11 spots (Figs. 2 & 3). The antifungal activity of the methanol and acetone crude extract was examined using a bioautography sprayed with *Aspergillus niger*. Weak inhibition zones were observed when the *A. niger* failed to grow in the area containing these bioactive compounds. Acetone extract was found to possess good antifungal activity at R_f 0.09, 0.21, 0.24, and

0.60 (Fig. 2) compared to methanol extract which only showed inhibition zone at R_f 0.74 (Fig. 3). Higher R_f values indicated that the compound might be of less polar or non-polar characteristics when developed in this system. In comparison to the current work, bioautography studies done by Masoko and Eloff (2006) on *Combretaceae* species revealed most of the antifungal compounds against *A. fumigatus* were relatively non-polar and therefore did not separate well in the polar eluent ethyl acetate: methanol: water instead have better separation in non-polar eluent which were combination of benzene: ethanol: ammonium hydroxide. Due to the properties of the solvent system used was of least polar thus, most of the polar components were failed to separate and retained at the origin of the TLC plates. Few compounds appeared to present at the origin of the plate resulting in clear inhibition zone for both TLC bioassays. The polar bioactive compounds contained in the oil palm leaf may probably contribute to this scenario. These potential compounds could be of saponins as saponins are one of the preformed antimicrobial compounds extracted mostly with acetone and methanol (Osbourne, 1996; Masoko & Eloff, 2006). Saponins molecules have been

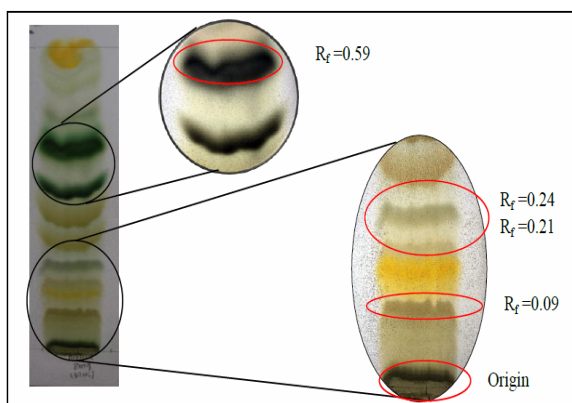


Fig. 2. Detection of inhibitors using TLC bioassay of acetone leaf extract against *A. niger*. Extract with active ingredient (AI) of 8 mg (or 40 μ L) were applied to 2 cm length on origin. Weak antifungal activities (circled in red) were observed. Solvent system used ethyl acetate: hexane (3:7, v/v).

Conclusion

This study has successfully identified novel roles of oil palm leaf extract and their efficacies in controlling the growth of a very challenging plant pathogen, *G. boninense*. Acetone extract of leaf of oil palm possess better antifungal activity among the four, with the lowest tested concentration; 2 mg mL⁻¹ can inhibit the growth of *G. boninense* while 40 mg mL⁻¹ totally killed the pathogen, *In vitro*. The highest tested concentration of methanol and petroleum ether extracts; 40 mg mL⁻¹ only inhibited the growth of *G. boninense* in comparison to control. The identification of the compound of interest which causing the inhibition to *Ganoderma* need further investigation. This report suggests the potential of oil palm leaf extract as a natural biofungicide in controlling *Ganoderma boninense*.

implicated as determinants of a plant's resistance to fungal attack. A number of other properties are also associated with these compounds, including pesticides and molluscicidal activity; allelopathic action; and antinutritional effects (Osbourne, 1996).

The beneficial medicinal effect of plant materials basically results from the secondary products present in the plant and is not usually attributed to a single compound but a combination of the metabolites (Parekh *et al.*, 2006). Hence, the non-activity of the extracts against *A. niger* by using bioautography assays could be explained by a weak activity of the extracts against the fungal, with the disruption of synergism between active constituents caused by TLC separation, or the low concentration of the active compounds in the crude leaf extract under the tested conditions. There were few cases where fungi did not grow well on certain parts of the bioautograms, making it difficult to evaluate the number of inhibition bands. Mild activity of these extracts in bioautography may possibly be explained by evaporation of the active compounds during removal of the TLC elutes or by the disruption of synergism between active constituents caused by TLC separation (Schmourlo *et al.*, 2004).

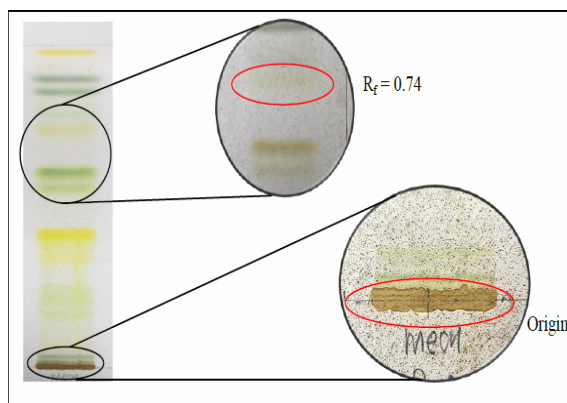


Fig. 3. Detection of inhibitors using TLC bioassay of methanol leaf extract against *A. niger*. Extract with active ingredient (AI) of 8 mg (or 40 μ L) were applied to 2 cm length on origin. Weak antifungal activities (circled in red) were observed. Solvent system used ethyl acetate: hexane (3:7, v/v). Note the higher R_f of the zones of inhibition than in Fig. 2.

Reference

- Augustin, S. 1992. Quantitative methods for the estimation of tannins in plant tissues. In: *Plant Polyphenol: Synthesis, Properties and Significance*. (Eds.): Hemingway, R.W., P.E. Laks and S.J. Branham. Plenum Press, New York, pp. 271-272.
- Basiron, Y. 2007. Palm oil production through sustainable plantations. *Eur. J. Lipid. Sci. Technol.*, 109: 289-295.
- Chan, K.W. 1999. Biomass in the oil palm industry. In: *Oil Palm and the Environment: A Malaysian Perspective*. (Eds.): Gurmit, S., K.H. Lim, L. Teo and K.D. Lee. Malaysian Palm Oil Growers' Council, Kuala Lumpur, Malaysia, pp. 41-53.
- Chong, K.P., J.W. Mansfield, M.H. Bennett, and J.F. Rossiter. 2006. Detection of antifungal compounds in *Arabidopsis thaliana* and *Brassica oleracea* by thin layer chromatography. *ASEAN J. Sci. and Tech. for Development*, 23 (1): 123-131.

- Chong, K.H., Z. Zuraini, S. Sasidharan, P.V. Kalnisha Devi, L. Yoga Latha and S. Ramanathan. 2008. Antimicrobial activity of *Elaeis guineensis* leaf. *Pharmacol. online*, 3: 379-386.
- Chong, K.P., M. Atong and S. Rossall. 2012a. The role of syringic acid in the interaction between oil palm and *Ganoderma boninense*, the causal agent of basal stem rot. *Plant Pathol.*, 61(5): 953-963.
- Chong, K.P., M. Atong and S. Rossall. 2012b. The susceptibility of different varieties of oil palm seedlings to *Ganoderma boninense* infection. *Pak. J. Bot.*, 44(6): 2001-2004.
- Das, K., R.K.S. Tiwari and D.K. Shrivastava. 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. *J. Med. Plants Res.*, 4(2): 101-111.
- Edziri, H., M.A. Mahjoub, S. Ammar, N. Mighri, A. Bouraoui and Z. Mighri. 2010. Anti inflammatory and antioxidant activities of some extracts and pure natural products isolated from *Rhustripartitum* (Ucria). *Med. Chem. Res.*, 19: 271-282.
- Hadacek, F. and H. Greger. 2000. Testing of antifungal natural products: Methodologies, comparability of results and assay of choice. *Phytochem. Anal.*, 11: 137-147.
- Harmala, P., H. Vuorela, K. Tornquist and R. Hiltunen. 1992. Choice of solvent in the extraction of *Angelica archangelica* roots with reference to calcium blocking activity. *Plant. Med.*, 58(2): 176-183.
- Idris, A.S. 2012. *Ganoderma* disease of oil palm in Malaysia: Latest technologies on detection, control and management. *17th International Conference. On Oil Palm and Expopalma*, 25-28 September 2012, Convention Centre, Cartagena De Indias, Colombia.
- Ishida, M. and O. Abu Hassan. 1997. Utilization of oil palm frond as cattle feed. *Jpn. Agric. Res. Q.*, 31: 41-47.
- Izzati, N. and F. Abdullah. 2008. Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. *Plant Protect. Sci.*, 44: 101-107.
- Jaffri, J.M., S. Mohamed, N. Rohimi, I.N. Ahmad, M.M. Noordin and Y.A. Manap. 2011. Antihypertensive and cardiovascular effects of catechin-rich oil palm (*Elaeis guineensis*) leaf extract in nitric oxide-deficient rats. *J. Med. Food*, 14(7/8): 775-783.
- Kandan, A., R. Bhaskaran and R. Samiyappan. 2010. *Ganoderma*- A basal stem rot disease of coconut palm in South Asia and Asia Pacific regions. *Arch. Phytopath. Plant Protect.*, 43: 1445-1149.
- Laouini, S.E., L. Segni, M.R. Ouahrani, N. Gherraf and S. Mokni. 2012. Phytochemical analysis, antioxidant and antimicrobial activities of leaf extract of date palm grown in Algeria. *J. Fund. App. Sci.*, 4(2): 48-58.
- Latiffah, Z. and Y.W. Ho. 2005. Morphological characteristics and somatic incompatibility of *Ganoderma* from infected oil palm from three inland estates. *Malays. J. Microbiol.*, 1(2): 46-52.
- Lourens, A.C.U., D. Reddy, K.H.C. Baser, A.M. Viljoen and S.F. Van Vuuren. 2004. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. *J. Ethnopharmacol.*, 9: 253-258.
- Masoko, P. and J.N. Eloff. 2006. Bioautography indicates the multiplicity of antifungal compounds from twenty-four southern African *Combretum* species (Combretaceae). *Afr. J. Biotechnol.*, 5(18): 1625-1647.
- Naher, L., S.G. Tan, U.K. Yusuf, C.L. Ho and S. Siddiquee. 2012. Activities of chitinase enzymes in the oil palm (*Elaeis guineensis* Jacq.) in interactions with pathogenic and non-pathogenic fungi. *Plant Omics J.*, 5(4): 333-336.
- Ng, M.H. and Y.M. Choo. 2010. Determination of antioxidants in oil palm leaves (*Elaeis guineensis*). *Am. J. Applied Sci.*, 7(9): 1243-1247.
- Osbourn, A.E. 1996. Preformed antimicrobial compounds and plant defense against fungal attack. *Plant cell*, 8(10): 1821-1831.
- Parekh, J., N. Karathia and S. Chanda. 2006. Screening of some traditionally used medicinal plants for potential antibacterial activity. *Indian J. Pharm. Sci.*, 68(6): 832-834.
- Rojas, J.J., V.J. Ochoa, S.A. Ocampo and J.F. Monoz. 2006. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in treatment of non-nosocomial infections. *BMC Complement. Alternat. Med.*, 6: 2.
- Rosalina Tan, R.T., S. Mohamed, G.F. Samaneh, M.M. Noordin, Y.M. Goh and M.Y.A. Manap. 2011. Polyphenol rich oil palm leaf extract reduce hyperglycaemia and lipid oxidation in STZ rats. *Int. Food Res. J.*, 18: 179-188.
- Sanderson, F.R., C.A. Pilotti and P.D. Bridge. 2000. Basidiospores: Their influence on out thinking regarding a control strategy for basal stem rot of oil palm. In: *Ganoderma Disease of Perennial Crops*. (Eds.): Flood, J., P.D. Bridge and M. Holderness. CABI Publishing, Wallingford.
- Sasidharan, S., S. Logeswara and L.Y. Latha. 2012. Wound healing activity of *Elaeis guineensis* leaf extract ointment. *Int. J. Mol. Sci.*, 13: 336-347.
- Sayyad, S.F., D.S. Randive, S.M. Jagtap, S.R. Chaudhari and B.P. Panda. 2012. Preparation and evaluation of fermented Ayurvedic formulation: Arjunarishta. *J. Applied Pharm. Sci.*, 2(5): 122-124.
- Schmourlo, G., R.R. Mendonca-Filho, C.S. Alviano and S.S. Costa. 2004. Screening of antifungal agents using ethanol precipitation and bioautography of medicinal food plants. *J. Ethnopharmacol.*, 96(3): 563-568.
- Suleiman, M.M., L.J. McGaw, V. Naidoo and J.N. Eloff. 2010. Detection of antimicrobial compounds by bioautography of different extracts of leaf of selected South African tree species. *Afr. J. Tradit. Complement. Altern. Med.*, 7(1): 64-78.
- Susanto, A., P. Sudharto and R.Y. Purba. 2005. Enhancing biological control of basal stem rot disease (*Ganoderma boninense*) in oil palm plantations. *Mycopathologia*, 159(1): 153-157.
- Syahmi, A.R.M., S. Vijayarathna, S. Sasidharan, L.Y. Latha, Y.P Kwan, Y.L. Lau, L.N. Shin and Y. Chen. 2010. Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq., (oil palm leaf) methanol extract. *Molecules*, 15(11): 8111-8121.
- Turner, P.D. 1981. *Oil Palm Diseases and Disorders*. Oxford University Press, UK.
- Vijayarathna, S., Z. Zakaria, Y. Chen, L.Y. Latha, J.R. Kanwar and S. Sasidharan. 2012. The antimicrobial efficacy of *Elaeis guineensis*: Characterization, *In vitro* and *In vivo* studies. *Molecules*, 17: 4860-4877.
- Wiegand, I., K. Hilpert and R.E. Hancock. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. Protoc.*, 3(2): 163-175.