In-vitro evaluation of anti-kinase, anti-phosphatase and cytotoxic activities of Mikania micrantha H.B.K. (asteraceae) from Malaysia

Article *in* Journal of Chemical and Pharmaceutical Sciences · January 2016

CITATIONS
9

READS
309

5 authors, including:

Gansau Jualang Azlan
Universiti Malaysia Sabah (UMS)
85 PUBLICATIONS **555** CITATIONS
SEE PROFILE

Azlinah Matawali
Universiti Malaysia Sabah (UMS)
9 PUBLICATIONS **24** CITATIONS
SEE PROFILE

Ping-Chin Lee
Universiti Malaysia Sabah (UMS)
103 PUBLICATIONS **681** CITATIONS
SEE PROFILE

Siew Eng How
Universiti Malaysia Sabah (UMS)
39 PUBLICATIONS **379** CITATIONS
SEE PROFILE

ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

In-vitro evaluation of anti-kinase, anti-phosphatase and cytotoxic activities of *Mikania micrantha* H.B.K. (asteraceae) from Malaysia

Azlinah Matawali[1], Lee Ping Chin[2], How Siew Eng[2], Lee Hong Boon[3] and Jualang Azlan Gansau[2*]
[1]Preparatory Centre of Science and Technology, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah.
[2]Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah.
[3]Drug Discovery Laboratory, Cancer Research Initiatives Foundation (CARIF), Subang Jaya, Selangor.
***Corresponding author: E-Mail: azlanajg@ums.edu.my,**
Phone: +6088-320000 (5729), +60168108368, Fax: +6088-435324

ABSTRACT

Mikania micrantha H.B.K (Asteraceae) is a creepy with soft stem weed which also known locally as Selaput Tunggul. Although being considered among worst Invasive Alien Weed (IAW) species in the world with less biologically importance, it still has patronage from traditional practitioners as the remedy to cure insects or snake bite. This study reports other promising medicinal properties of this plant species. Dried leaves were extracted with various solvent systems, concentrated under reduced pressure and later evaluated for its anti-kinase, anti-phosphatase and cytotoxic activities. Both anti-kinase and anti-phosphatase assays targeted protein MKK1, MSG5 and PP1 in mutated yeast strains namely as MKK1^{P386}, MKK1^{P386}_MSG5, PAY704-1 and PAY700-4, respectively. The crude methanolic extract has observed as the only inhibitor for PP1 screening assay. Liquid-liquid partition of this extract has confirmed the chloroform partition exhibited potential activity against PP1. Further separation of this partition extracts using column chromatography yielded 5 fractions namely as F1 to F5. Fraction F2 was later confirmed as the PP1 inhibitor, while fraction F1 observed as toxic. MTT assay of this plant extracts also showed good cytotoxic activity against HL60 cell line. This result has indicated that *M. micrantha* shows promise as the natural anticancer agent.

KEY WORDS: Signal transduction, Anti-cancer, HL-60, Malaysia, *Mikania micrantha*.

1. INTRODUCTION

Cancer is a disease of cells that proliferate inappropriately in the body partly due to the disturbance in protein kinase-mediated cell signaling pathways (Noble, 2004; Weinberg, 2007). Thus, targeting signal transduction pathway has become an effective therapeutics strategy in developing chemicals agents to treat cancer (Cobb and Ross, 2007). Protein phosphorylation is a major process in signal transduction pathway. Each protein typically acts by altering the conformation of the downstream protein in the series by activation or inhibition process of the protein, namely as protein kinases and protein phosphatases. Mitogen-Activated Protein Kinase (MAPK) cascades are abundant players in signal transduction which serve as highly conserved central regulators of growth, embryogenesis, cell death, differentiation, proliferation, stress responses and apoptosis (Mattison, 1999; English and Cobb, 2002; Nakagami, 2005). On the other hand, protein phosphatases are important regulators in glycogen metabolism, cell signaling and learning activity, acts as positive regulators of many hormonal responses, protein synthesis, muscle contraction, metabolism and transcription process (Shenolikar, 1994; Mitsuhashi, 2001; Watanabe, 2001; Bennett, 2006). Abnormalities from the control of kinase and phosphatases had been detected in various tumors progressions such as melanoma, breast carcinoma, pancreatic, prostate and ovarian cancer.

Mikania micrantha (H.B.K) is an invasive weeds species belonging to the family of Asteraceae (Perez-Amador, 2010). It is a creepy plant with the soft stem, heart-shaped leaves, flowers contained many white to greenish-white florets, and locally known as 'Selaput Tunggul'. The seed dissemination of this plant relies on the wind power and might successfully germinate within 8 days (Fengjian, 2007). Previous studies reported that Mikania genera are rich with hydroalcoholic acid, coumarin, steroids, diterpenes and sesquiterpenes (Lobitz, 1997; Sola Veneziani and De Oliveira, 1999; Bighetti, 2005). This group of plants genus were used as medicinal herbs for anti-spasmodic, anti-ulcerogenic, anti-rheumatic, fever, influenza and respiratory diseases (Bighetti, 2005; Perez-Amador, 2010). For instance, *Mikania decora* had been reported with cytotoxic activity against various cancer cell lines (Aponte, 2011). Recent phytochemical studies on *M. micrantha* showed the presence of sesquiterpenes lactones and phenolic compounds (Cuenca, 1988; Wei, 2004). This plant had been reported to remedy the insects or snake bite (Lentz, 1998). Despite of these researches, none of the studies have been conducted in relation to biological activities of this plant against protein kinase and phosphatases. Hence, the objectives of this presented study were to determine the anti-kinase and anti-phosphatase activities of *M.micrantha*, as well as determining its cytotoxic activitiy toward HL-60 cancer cells.

2. MATERIALS AND METHODS

Plant material: The plant was collected from Membakut area, Sabah, Malaysia and deposited in Borneensis, Institute of Tropical Biology and Conservation (ITBC), University Malaysia Sabah (BORH number 0963). Samples

were washed thoroughly with tap water, air-dried, powdered and stored in air-tight containers at room temperature for extraction purposes.

Plant extraction and compound isolation: Ground sample was soaked in methanol with the ratio 1:10 to obtain the crude methanolic extracts (CME). The CME were further fractionated using different polarity of organic solvents namely as hexane, ethyl acetate, chloroform, chloroform:methanol (3:1), butanol and aqueous through liquid-liquid extraction methods following the method by Harborne (1998) with a slight modification. The six different fractions namely as hexane extracts (HE), ethyl acetate extracts (EAE), chloroform extracts (CE), chloroform:methanol extracts (CME), butanol extracts (BE) and aqueous Extracts (AE) were filtered through filter paper (Whatman paper no.1) and evaporated to dryness under vacuum using rotary evaporator at 40^0C (Heidolph, Germany) and freeze dried for aqueous extract. Upon test, the extracts are dissolved in methanol at concentration of 100mg/ml. Selected potential extract was further fractionated using gradient elution on silica gel column chromatography (Merck, 0.040-0.063mm, 230-400 mesh). Extraction yields of extracts and fractions were calculated as below;

$$\text{Yield (\%)} = \frac{\text{Dry weight of extracts or fractions}}{\text{Dry weight of plant powder or crude extracts}} \times 100$$

Test Microorganisms: Anti-kinase and anti-phosphatase screening assay were done using mutant yeast strains from Prof. Minoru Yoshida (University of Tokyo, Japan) and Prof. Micheal JR Stark (University of Dundee, Scotland). The MAPK Kinase (MKK1), MAPK Kinase Phosphatase (MSG5) and Protein Phosphatase 1 (PP1) screening system are using $MKK1^{P386}$, $MKK1^{P386}$_MSG5, PAY704-1 and PAY700-4 yeast strains, respectively. Table 1 showed the genotype of strains used in the screening assay.

Table.1. Genotype of yeast strains used in various type of screening assay

Screening assay	Strains	Genotype	References
Protein phosphatase type-1 (PP1)	PAY704-1	Mat**a** ade2-1 his3-11 leu2-3, 112trp1-1 ura3-1 can1-100 ssd1-d2 glc7::LEU2 trp1::GLC7::TRP1 Gal+	Andrews and Stark (2000)
	PAY700-4	Mat**a** ade2-1 his3-11 leu2-3,112trp1-1 ura3-1 can1-100 ssd1-d2 glc7::LEU2 trp1::glc7-10::TRP1 Gal+	Andrews and Stark (2000)
MAP kinase (MKK1)	$MKK1^{P386}$	Transformant from wild type 1788 with mutant type nNV7- $MKK1^{P386}$(GAL1p-$MKK1^{P386}$). MATa/MATa ura3/ura3 leu2/leu2 trp1/trp1 his4/his4 can1/can1 [pNV7-$MKK1^{P386}$].	Watanabe (1995)
MAPK phosphatase (MSG5)	$MKK1^{P386}$_MSG5	Mat**a** GAL1p-MKK1P386::TRP1 ura3 leu2 trp1 his4 can1[Pspg14-MSG5]	Watanabe (1995); Ho (2001)

Anti-kinase and anti-phosphatase assay: Sterile paper discs (Whatmann No.3) with the diameter of 6mm were impregnated with 20µl of extracts samples (100mg/ml) and left to dry under laminar flow cabinet. Sterile forceps used to transfer the disc onto the screening medium. Then, these dried paper discs impregnated with extract were placed on the inoculated agar surface for each type of screening tests. Both MKK1 and MSG5 yeast strain were incubated in broth culture at 28˚C with 220 rpm for 2 days for fermentation purposes and later were incubated on both glucose and galactose media at 28˚C for 5 days for screening assay (Gustin, 1998; Watanabe, 1995). Meanwhile, PP1 screening system had used YPD and YPD+1M sorbitol media. Both yeast strains were incubated at 28˚C, 220rpm and 3 days for fermentation and later incubated at 25˚C and 37˚C for 5 days for screening assay (Andrew and Stark, 2000). Zones of inhibition observed as measured and photographed for data analysis.

***In-vitro* cytotoxicity activity:** MTT assay was carried out to determine the cytotoxic activity of the samples (Lim, 2009). HL-60 human promyelocytic leukemia was obtained from the American Tissue Culture Collection (Virginia, USA) and maintained in RPMI 1640 medium (Invitrogen Corp.) supplemented with 10% Fetal Bovine Serum (FBS). Approximately 1.5 X 10^4 of HL-60 of exponentially growing cells in 80µl of medium were seeded in each well of the 96-well plate and were allowed to adhere overnight. Cells were then treated with each extract at both 20 and 100µg/ml concentrations giving the final volume of 100µl in each well. At the end of the incubation period (48 hr), 15 µl of 5 mg/ml MTT in phospahate buffered saline (PBS) was added and incubated for 4 hours. Medium and formazan product generated from the viable cells were aspirated and formazan formed was solubilized with 100µl of dimethyl sulphate (DMSO). Absorbance, as a measurement of viable cell number, was read at 570nm with Spectramax. The cell percentages viability formula is as follow;

$$\text{\% viability} = (\text{Abs of live cell count}/\text{Abs of total cell count}) \times 100$$

3. RESULTS AND DISCUSSION

Extraction yield: Alcohol is a universal solvent for preliminary extraction (Harborne, 1998). According to Fasihuddin and Hasmah (1993), most of the secondary metabolites such as flavonoids are polar and easily dissolve in the alcoholic solvent. Percentages yield of all extracts recorded as shown in Table 2. Based on the data, chloroform extraction produced higher yield (3.30%) while the lowest was aqueous extraction with only 0.50% yield.

Table.2. Extraction yield of *M. micrantha* in percentage (w/w)

Extracts	Percentage Yield (%)
Hexane (HE)	1.17
Ethyl acetate (EAE)	1.03
Choloroform (CE)	3.30
Chloroform:Methanol (3:1) (C:ME)	0.83
Buthanol (BE)	0.70
Aqueous (AE)	0.50
Column chromatography fractions	
CE.F1	1.50
CE.F2	0.90
CE.F3	2.30
CE.F4	2.90
CE.F5	1.80

Anti-kinase activity: Anti-kinase activity was performed using MKK1 and MSG5 screening assays. Glucose and galactose media were used in MKK1 screening system. $MKK1^{P386}$ yeast strain was able to grow on glucose-containing medium but unlikely on galactose. Therefore, the growth of this mutant yeast could be compared in the medium that contains glucose and galactose. The presence of inhibitor however detected when there are none inhibitory zones on glucose media yet the yeast strain growth around the paper disc on galactose media. Toxic extracts showed inhibitory zones on glucose plate, but no growth observed on galactose plate (Doi, 1994; Watanabe, 1995; Gustin, 1998). $MKK1^{P386}$ inhibits the growth of the yeast cells when overexpressed by strong GAL1 promoter. Therefore, induction with the addition of galactose in the media can cause this gene the overexpression. The presence of galactose induced the GAL1 promoter and hence resulted in the overexpression of $MKK1^{P386}$, resulting in growth of inhibitory effect on the yeast cells. As a result, if there is any potential inhibitor that inhibits $MKK1^{P386}$, the yeast able to grow because it would arrest the Pkc pathway at Bck1, MKK1 or Mpk1 (Watanabe, 1995).

In order to specify the targets on MKK1 inhibitory activities only, MSG5 screening assay should be conducted (Watanabe, 1995). Glucose and galactose media was also used in this system. $MKK1^{P386}$_MSG5 yeast strain will grow as normal as wild type strain on both glucose and galactose media. MSG5 inhibitor displayed inhibitory zones only on galactose, unlike toxic extracts that reacted on both screening media (Doi, 1994, Watanabe, 1995, Gustin, 1998). In MSG5 inhibitor screening, yeast mutant $MKK1^{P3386}$ will be transformed with the plasmid carrying MSG5 producing $MKK1^{P386}$_MSG5 yeast strain. MSG5 belongs to the novel subclass of protein phosphatases whose substrates are MAP kinase family members. Overexpression of MSG5 suppresses the toxic effect caused by hyperactivation of the Mpk1 pathway by dephosphorylating and inactivating Mpk1 MAPK. Thus, in MSG5 screening assay, mutant yeast strain grew in glucose plate as the GAL1 promoter is not induced. However, galactose plate also has yeast growth because of the overexpression of MSG5 will inhibit its target; Mpk1 consequently suppresses the growth inhibitory effect as a result of overexpression of $MKK1^{P386}$ gene (Doi, 1994; Watanabe, 1995). If the extract potential to both MKK1 and MSG5 screening assay, thus it can be assumed that the extract specifically as MKK1 inhibitor. However, results showed no significant activities for all extracts and fractions.

Anti-phosphatase activities: Anti-phosphatase activity was performed using PP1 screening test. In anti-phosphatase screening assay, type-1 protein serine/threonine phosphatase (PP1) has been targeted. About eight different growth conditions have been prepared by manipulating type of media (YPD and YPD+1M sorbitol), yeast strain (PAY704-1 and PAY700-4) and temperature (28^0C and 37^0C). In normal condition, both strains will be growing on the media with the exception of PAY700-4 on YPD media at 37^0C. The presence of inhibitor will mimic this property; inhibitory zones will be detected on wild type strain, PAY700-4 using YPD media at 37^0C (Andrew and Stark, 2000). The potential inhibitor is able to mimic the properties of a temperature-sensitive PP1 mutant (glc7-10) at its restrictive temperature. glc 7-10 causes a cell cycle arrest and a cell wall integrity defect at 37^oC, but proliferation and cell integrity is rescued by addition of 1M of sorbitol. A potential inhibitor that acts on the wild-type GLC7 should, therefore, exhibit similar characteristics; cell proliferation and cell integrity is rescued by addition of 1M of sorbitol and, therefore, no inhibition zone at the wild type strain with 1M of sorbitol (Sasoon, 1999, Andrew and Stark, 2000). Catalytic subunit of type I protein serine/threonine phosphatase (PP1) in Saccharomyces cerevisiae is encoded by GLC7. Isolation of temperature-sensitive glc7 allele; glc7-10 exhibits a G_2/M arrest at the restrictive temperature. At

37^0C, this gene caused defects in kinetochore functions. Additions of ATP at both 37^0C and 26^0C will decrease the microtubule-binding activity. After ATP depletion by apyrase, microtubule-binding activity was restored in wild type strain. However, this activity was not restored in glc7-10 at 37^0C extracts and marginally restored in glc 7-10 at 26^0C (Sasoon, 1999, Andrew and Stark, 2000).

The anti-phosphatase properties of Mikania micrantha are summarized in Table 3. The CME and CE of this plant showed good anti-phosphatase activity. Based on the data, CME inhibitory zones were found on two conditions; PAY704-1 strain, YPD media at 37^0C and PAY700-4 strain, YPD +1S media at 37^0C. Thus, it was considered as an inhibitor to GLC-7. This inhibitory type is able to inhibit the catalytic domain that reduces the function of Glc7 protein in the cell integrity pathway irrespective to the mutation of the glc7-10. In contrast with that, CE was categorized as inhibitor insensitive to glc7-10 catalytic domain change. This inhibitor, on the other hand, was able to inhibit normal Glc7 that is reversible the addition of sorbitol or not without affecting the mutant Glc7. It was not preferred much compared to the former. CE was chosen to be further fractionating through column chromatography to yield another 5 fractions. Among them, fraction 2 (F2) showed potential activities as inhibitor to GLC-7 with inhibitory activities about 13mm (PAY704-1, YPD media, 37^0C) and 15mm (PAY700-4, YPD+1S media, 37^0C). In contrast with that, fraction 1 (F1) showed toxic activities during the screening assay as some inhibitory activities were detected on media plate at 28^0C which indicating the ability of this fraction to kill even normal yeast cells.

Table.3. Bioactivities of *M. micrantha* against PP1 screening assay

Extracts		Yeast-based screening method								Remarks
		GLC								
		PAY704-1				PAY700-4				
		YPD	YPD+1S	YPD	YPD+1S	YPD	YPD+1S	YPD	YPD+1S	
		28^0C	28^0C	37^0C	37^0C	28^0C	28^0C	37^0C	37^0C	
CME		0	0	20.25±3.3	0	0	0	0	9.5±1.0	Potential
HE		0	0	0	0	0	0	α	0	NA
EAE		0	0	0	0	0	0	α	0	NA
CE		0	0	9.5±0.70	0	0	0	α	0	Potential
CE	F1	0	0	31	13	8	0	α	30	Toxic
	F2	0	0	13	0	0	0	α	15	Potential
	F3	0	0	0	0	0	0	α	0	NA
	F4	0	0	0	0	0	0	α	0	NA
	F5	0	0	0	0	0	0	α	0	NA
C:ME		0	0	0	0	0	0	α	0	NA
BE		0	0	0	0	0	0	α	0	NA
AME		0	0	0	0	0	0	α	0	NA

Notes:
α: No yeast growth observed on medium prepared
0: No inhibition zones
F: Colum chromatography fraction
NA: No activity
Concentrations of extracts: 100mg/ml
Diameter of paper disc: 6mm
Negative control: absolute methanol

In-vitro* cytotoxicity activity of *M. micrantha: Cytotoxicity assay use in this study is microtetrazolium (MTT) assay. MTT is 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide that widely utilize for assessment of cytotoxicity, cell viability and proliferation studies in cell biology (Stockert, 2012). MTT salt (yellowish aqueous solution) was metabolically reduced by viable cells to yield a violet-blue formazan that is lipid soluble. The amount of MTT-formazan is directly proportional to the number of living cells (Rejon, 2009). About four extracts from *M. micrantha* (crude methanolic, CE, CE.F1, and CE.F2) were selected for cytotoxic test against HL-60 cells. The MTT assay shows that apoptosis occurred when the cells treated with CE, CE.F1 and CE.F2 (Figure 1). The crude extract of *Mikania micrantha* however did not show any apoptosis activity. Cells viability was vigorously reduced even at lower concentration (20µg/ml) CE.F1 and CE.F2 extracts indicating that both semi-purified extracts have strong inhibitory effect against HL-60 cells. This preliminary study of *M. micrantha* served as the platform for other thorough studies of this plant healing properties.

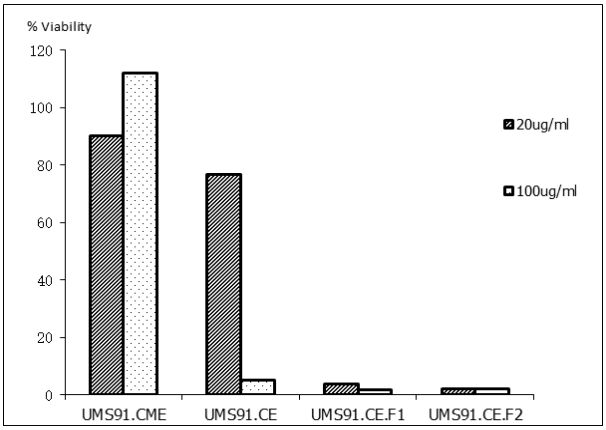

Figure.1. The comparison of viability percentages of various extracts of *M. micrantha*

4. CONCLUSION

In conclusion, *M. micrantha* collected from Malaysia demonstrates significant activity against *glc7* in PP1 inhibitor screening system. In addition, the extracts fractions showed good growth inhibitory activity to the cancer cell lines tested. These preliminary results suggests the need for further studies on *M. micrantha* extract in order to further identify, isolate characterize and elucidate the phytocompound(s) using spectroscopic methods including IR, NMR and MS.

5. ACKNOWLEDGEMENTS

The authors of this research project would like to acknowledge Prof. Minoru Yoshida (University of Tokyo, Japan) and Prof. Micheal J. R. Stark (University of Dundee, Scotland) for providing the yeast strains. This project was funded by The Ministry of Science, Technology and Innovation (MOSTI) under Sciencefund (Grant number 02-01-10-SF0107).

REFERENCES

Andrew P.D, and Stark M.J.R, Type 1 protein phosphatase is required for maintenance of cell wall integrity, morphogenesis and cell cycle progression in *Saccharomyces cerevisiae*, Journal of Cell Science, 113, 2000, 507-520.

Aponte J.C, Jin Z, Vaisberg A.J, Castillo D, Malaga E, Lewis W.H, Sauvain M, Gilman R.H, and Hammond G.B, Cytotoxic and anti-infective phenolic compounds isolated from *Mikani decora* and *Cremastosperma microcarpum*, Planta Med., 77 (14), 2011, 1597-1599.

Bennett D, Lyulcheva E, and Alphey L, Towards a comprehensive analysis of the protein phosphatise 1 interactome in *Drosophilla*, Journal of Molecular Biology, 364, 2006, 196-212.

Bighetti A.E, Antonio M.A, Kohn L.K, Rehder V.L.G, Foglio M.A, Possenti A, Vilela L, and Carvalho J.E, Antiulcerogenic activity of a crude hydroalcoholic extract and coumarin isolated from *Mikania levigata* Schultz Bip, Phytomedicine, 12, 2005, 72-77.

Cobb M. H, and Ross E. M, Principles of cell signaling, In Lewin B, Cassimeris L, Lingappa V.R, and Plopper G, (ed.), Cells, Jones and Bartlett Publishers, USA, 2007.

Cuenca M.D.R, Bordon A, and Catalan C.A.N, Sesquiterpene lactones from *Mikania micrantha*, Journal of Natural Products, 51 (3), 1988, 625-626.

Doi K, Gartner A, Ammerer G, Errede B, Shinkawa H, Sugimoto K. dan Matsumoto K, MSG5, a novel protein phosphatase promotes adaptation to pheromone response in *S. Cerevisiae*, European Molecular Biology Organization Journal, 13, 1994, 61-70.

English J. M, and Cobb M.H, Pharmacological inhibitors of MAPK pathways, Trends in Pharmacological Sciences, 23 (1), 2002, 40-45.

Fasihuddin A, and Hasmah R, Kimia Hasilan Semulajadi dan Tumbuhan Ubatan, Dewan Bahasa dan Pustaka, Kuala Lumpur, 1993.

Fengjian Y, Zhonghua Z, Wenjie W, Yuangang Z, Huafeng C, Jing J, Yu G, and Naijing Z, Anatomical and physiological differences of eight species from Asteraceae, Acta Ecologica Sinica, 27 (2), 2007, 442-449.

Gustin M.C, Albertyn J, Alexander M, and Davenport K, MAP kinase pathways in yeast *Saccharomyces cerevisiae*, Microbiology and Molecular Biology Reviews, 62, 1998, 1264-1300.

Harborne J.B, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, 1998.

Lentz D.L, Clark A.M, Hufford C.D, Meurer-Grimes B, Passreiter C.M, Cordero J, Ibrahimi O, and Okunade A.L, Antimicrobial properties of Honduran medicinal plants, Journal of Ethnopharmacology, 63, 1998, 253-263.

Lim S.H, Wu Liangxing, Kevin Burgess, and L.H.B, New cytotoxic rosamine derivatives selectively accumulate in the mitochondria of cancer cells, Anti-Cancer Drugs, 20, 2009, 461-468.

Lobitz G.O, Tamayo-Castillo G, and Merport I, Diterpenes and sesquiterpenes from *Mikania banisteriae*, Phytochemistry, 46 (1), 1997, 161-164.

Mattison C.P, Spencer S.S, Kresge K.A, Lee J, and Ota I.M, Differential regulation of the cell wall integrity mitogen-activated protein kinase pathway in budding yeast by the protein tyrosine phosphatases Ptp2 and Ptp3, American Society for Microbiology, 19 (11), 1999, 7651-7660.

Mitsuhashi S, Matsuura N, Ubukata M, Oikawa H, Shima H, and Kikuchi K, Tautomycetin is a novel and specific inhibitor of serine/threonine protein phosphatase type 1, PP1, Biochemical and Biophysical Research Communication, 287, 2001, 328-331.

Nakagami H, Pitzschke A, and Hirt H, Emerging MAP kinase pathways in plant stress signaling, Trend in Plant Sciences, 10 (7), 2005, 340-346.

Noble M.E.M, Endicott J.A, and Johnson L.N, Protein kinase inhibitors: insight into drug design from structure, Drug Discover, 303, 2004, 1800-1805.

Perez-Amador M.C, Munoz Ocotero V, Ibarra Balcazar R, and Garcia Jimenez F, Phytochemical and pharmacological studies on *Mikania micarantha* H.B.K (Asteraceae), International Journal of Experimental Botany, 79, 2010, 77-80.

Rejon G, Caamal-Fuantes E, Cantillo-Ciau Z, Cedillo-Rivera R, Flores-Guido J, and Moo-Puc R, *In vitro* cytotoxic activity of nine plants used in Mayan traditional medicine, Journal of Ethnopharmacology, 121 (3), 2009, 462-465.

Sasoon I, Severin F.F, Andrews P.D, Taba M, Kaplan K.B, Ashford A.J, Stark M.J.R, Sorger P.K, and Hyman A.A, Regulation of Saccharomyces cerevisiae kinetochores by the type 1 phosphatase Glc7 p, Genes and Development, 13, 1999, 545-555.

Shenolikar S, Protein serine/threonine phosphatases-new avenues for cell regulation, Annual Reviews of Cell Biology, 10, 1994, 55-86.

Sola Veneziani R.C, and De Oliveira C.R, Constituents of *Mikania glomerata*, Biochem System Ecol., 27, 1999, 99-102.

Stockert J.C, Blazquez-Castro A, Cantet M, Horobin R.W, and Villanueva A, MTT assay for cell viability: Intracellular localization of the formazan product is in lipid droplets, Acta Histochemica, 114, 2012, 785-796.

Watanabe T, Huang H, Horiuchi A, Da Cruz Silva E.F, Hsieh-Wilson L, Allen P.B, Shenolikar S, Greengard P, and Nairn A.C, Protein phosphatase 1 regulation by inhibitors and targeting subunits, PNAS, 98 (6), 2001, 3080-3085.

Watanabe Y, Irie K, and Matsumoto K, Yeast RLM1 encodes a serum response factor-like protein that may function downstream of the Mpk1 (Slt2) mitogen-activated protein kinase pathway, Molecular and Cellular Biology, 15, 1995, 5740-5749.

Wei X, Huang H, Ping Wu, Cao H, and Ye W, Phenolic constituents from *Mikania micrantha*, Biochemical Systematics and Ecology, 2004, 1091-1096.

Weinberg R.A, Cancer-principles and overview, In Lewin B, Cassimeris L, Lingappa V.R, and Plopper G, (eds.), Cells, Jones and Bartlett publishers, USA, 2007.