A survey on phytochemical and bioactivity of plant extracts from Malaysian forest reserves

Article *in* Journal of medicinal plant research · March 2010

CITATIONS
17

READS
561

10 authors, including:

Nardiah Rizwana
Universiti Kebangsaan Malaysia
7 PUBLICATIONS **20** CITATIONS
SEE PROFILE

Nazlina Ibrahim
Universiti Kebangsaan Malaysia
133 PUBLICATIONS **483** CITATIONS
SEE PROFILE

Shariffah Muzaimah Sy. Aripin
Malaysian Palm Oil Board
6 PUBLICATIONS **35** CITATIONS
SEE PROFILE

Ahmad Yaacob
Universiti Kebangsaan Malaysia
87 PUBLICATIONS **593** CITATIONS
SEE PROFILE

Journal of Medicinal Plants Research Vol. 4(3), pp. 203-210, 4 February 2010
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

A survey on phytochemical and bioactivity of plant extracts from Malaysian forest reserves

J. Nardiah Rizwana, I. Nazlina*, A. R. Mohd Razehar, A. Z. Siti Noraziah, C. Y. Ling, S. A. Shariffah Muzaimah, A. H. Farina, W. A. Yaacob, I. B. Ahmad and L. B. Din

Faculty of Science and Technology, Universiti Kebangsaan Malaysia, Bangi 43600, Selangor, Malaysia.

Accepted 30 December, 2009

Methanolic extracts of leaves from plants collected from two different forest reserves, namely, Gunung Stong, Dabong, Kelantan and Ulu Muda, Kedah in Malaysia were subjected to a study in phytochemical constituents screen, toxicity to brine shrimp, cytotoxicity to normal cells and antiviral activity. All the plant extracts contain saponins except for *Cyrtandra* cf*. anisophyllea*. *Goniothalamus macrophyllus* contains alkaloids, steroids/triterpenes and saponins. All the tested extracts were not toxic to the brine shrimp. In the cytotoxicity assay, the CTC_{50} values recorded for all the extracts ranged between 21.540 mg/L (*Galearia fulva*) to more than 1000 mg/L (*Cinnamomum javanicum, Cryptocarya costata, Scaphochlamys biloba, Diospyros frutescens, Galearia maingayi* and *Urophyllum blumeanum)*. From the CTC_{50} values, ten of the extracts were considered toxic to Vero cells. In the antiviral test carried out using 0.1 CTC_{50} of the various extracts, results showed that antiviral activity is effective when the cells were treated with the extracts and inoculated with measles virus at the same time. From this study, it was postulated that the most effective route for the extracts in preventing the killing of Vero cells by (MV) was through inactivation of virus particle or prevention of viral entry into the cells.

Keywords: Phytochemical constituent, toxicity to brine shrimp, cytotoxicity, antiviral activity.

INTRODUCTION

World Health Organisation (WHO) has defined medicinal plants as plants that contain properties or compounds that can be use for therapeutic purposes or those that synthesize metabolites to produce useful drugs (WHO, 2008). The development of antiviral drugs is a complicated task because of the toxicity that can affect both virus and the host cells. Plus, virus resistance to antiviral drugs through mutation especially RNA viruses such as measles virus (MV) makes it more difficult to produce new drugs (Kott et al., 1999). MV causes measles and every year almost one million children died of this disease (Rager-Zisman et al., 2003). MV is an enveloped, negative, single-stranded RNA virus belonging to the Paramyxoviridae family (Horikami and Moyer, 1995). Despite large vaccination campaigns, MV is still resisting eradication, and there is no available therapeutic treatment.

In Malaysia, 2,000 species from 14,500 flowering plants have been reported to contain medicinal properties and many have been scientifically proven (Jaganath and Ng, 2000). Plants that were shown to have antiviral properties include (*Cymbopogon nardus* (L.) Rendle) and *Datura stramonium* L. against bacteria, Newcastle disease virus and measle virus (Ahmad et al., 1993; Nurul Aini et al., 2006), *Chaetomium trilaterale* Chivers towards poliovirus (Ahmad and Romlah, 1993), *Melastoma malabathricum* against herpes simplex virus type-1 and measles virus (Nazlina et al., 2008). Plant-derived compounds offer potential source of new antimicrobial, anticancer and anti-HIV agents among other pharmaceuticals (Gurib-Fakim et al., 2005). Malaysian is wealthy in her plant diversity and potential antiviral drugs are still waiting to be explored. This study focuses on plants that were collected in Gunung Stong, Kelantan and Ulu Muda, Kedah both

*Corresponding author. E-mail: drnazlina@gmail.com.

Kedah both states of Malaysia with the hope of exploring new compounds for anti-measles therapy with low or no toxicity. The plant chemical content and the antiviral mode of action of the plant extracts were determined in this study.

MATERIALS AND METHODS

Plant identification and preparation of extracts

Leaves from various plants were collected from Gunung Stong, Dabong, Kelantan and Ulu Muda, Kedah in Malaysia. The list of plants' names, plant family, their specimen voucher numbers and their traditional uses are shown in Table 1. Extracts were prepared from dried leaves soaked in methanol for at least seven consecutive days and later dried using a rotary evaporator (Buchi). The alkaloids, steroids/triterpenes and saponins contents were determined using simple qualitative methods of Sofowora (1984) and Harborne (1993).

Brine shrimp lethality bioassay

The toxicity of the different extracts was tested by lethality to *Artemia salina* brine shrimp according to Meyer et al. (1982). Concentrations of 10, 100 and 1000 ppm of each active extract were tested. The number of dead larvae was recorded and used to calculate the 50% lethal concentration (LC_{50}) and 95% confidence intervals were determined from the 24 h counts using the Finney probability analysis software. LC_{50} values greater than 1000 ppm were considered inactive and not toxic.

Cytotoxicity test

Cytotoxicity testing was done according to Marini et al. (1998) with modification. Plant extracts were dissolved in DMSO to prepare a stock extract of 10^5 mg/L. Serial doubling dilutions were prepared in Dubelco's Minimal Essential Medium (DMEM) with 2% foetal bovine serum (FBS) (GIBCO). Diluted extracts (200 µl) were treated to confluent Vero cells (2.5 x 10^5 cells/ml) grown in 96-well microtitre plate (Costar). After 48 h of incubation, the microtitre plates were processed and stained with 2% Eosin B. The dye was then dissolved with 100 µl cell lysis buffer and the amount of dye retained in each well was measured with microplate reader (Labsystems Multiscan Multisoft, Finland) at 540 nm. The CTC_{50} values were calculated graphically from the optical density readout from each well after normalisation against empty wells. CTC_{50} value was presented as the percentage of survived cells compared to control cells.

Antiviral assay

MV from Edmonston strain at 1000 $TCID_{50}$ (Serum Institute of India Ltd.) was used in this study. Confluent Vero cells were treated with MV using three different treatments for detection of antiviral activity in each plant extracts according to Nurul Aini et al. (2006) that is, I. virus (V) were inoculated to the cells (C) 1 h before treatment with extracts (E), that is (V+C)+E; II. Cells were treated with extract 1 day before inoculation with virus, that is (C+E)+V and III. Virus and extracts were added concomitantly to the cells, that is C + (V+E). For the antiviral tests, the extracts were diluted to 0.1 CTC_{50}. Plates were developed according to the method discussed in the cytotoxicity testing.

RESULTS

Phytochemical constituents

All the plant extracts except for *Cyrtandra anisophyllea* cf. contain saponins at different concentrations (Table 2). In this study, only two plant extracts, that is, *Urophyllum blumeanum* and *Goniothalamus macrophyllus* contain alkaloids. Five extracts contain steroids/triterpenes at low levels and *G. macrophyllus* contains all three chemical groups with moderate amounts of alkaloids and saponins. On the contrary, *C. anisophyllea* does not contain any of the chemicals tested.

Toxicity to brine shrimp

In this study, all 30 plant extracts (20 extracts from Gunung Stong and 10 extracts from Ulu Muda) were not toxic to brine shrimp with the LC_{50} values are more than 1000 mg/L (Table 2).

Cytotoxicity to Vero cells

Cytotoxicity of the plant extracts to Vero cells were screened before the evaluation of antiviral activity and recorded in Table 2. According to Marini et al. (1998), any plants that have CTC_{50} values less than 208 µg/ml are considered toxic to cells. In this study, six plants from Ulu Muda were found to be cytotoxic, that is, *Connarus cochinchinensis, Dioscorea esculenta, Euonymus wrayi, Hodgsonia macrocarpa, Kopsia pauciflora* and *Lasianthus densifolius* and four plant extracts from Gunung Stong (*Gluta elegans, Saurauia roxburghii, Galearia maingayi* and *Tabernaemontana corymbosa)* have CTC_{50} lower than 208 µg/ml (Table 2). It is a known fact that *G. elegans* is a toxic plant and can cause edema and inflammation if touched (Wiart, 2002) and this is confirmed in this study. The other plants extracts have higher CTC_{50} values. Some of the plants used in folklore medicine showed higher values in CTC_{50} in this study confirming the safety of these plants for human use (Wiart, 2002).

Antiviral activity of the plant extracts

In the first treatment, cells were infected with virus for 24 h and treated with plant extracts. *Poikilospermum suaveolens* and *Psueduvaria macrophylla* showed moderate antiviral activity, three plant extracts (*Saurauia roxburghii, Tabernaemontana corymbosa* and *Kopsia pauciflora*) have mild antiviral activity and the rest of extracts demonstrated very mild or no antiviral activity.

Extract were added to the cells a day before virus inoculation in the second treatment. No extract showed

Table 1. Plant name and family, specimen voucher number, their local names, traditional uses and plants' origin.

Plant name/ Family	Specimen voucher number	Local name	Traditional uses	Origin
Saurauia roxburghii Actinidiaceae	SK557/03	Kelapong, Lengadir	Firewood, fruit is edible (Faridah Hanum and van de Maesen, 1997)	Gunung Stong, Kelantan
Gluta elegans Anacardiaceae	SB1-28	Pokok Rengas Air	Has antitumor, antifungal and antibacterial activities; can cause allergy and inflammation (Wiart, 2002)	Gunung Stong, Kelantan
Goniothalamus macrophyllus Annonaceae	SK 522/03	Gajah beranak; Penawar hitam	For high blood pressure, fever and post-natal blues treatment (Brandis, 1978)	Gunung Stong, Kelantan
Psueduvaria macrophylla Annonaceae	SA1-3	No literature report on local name	Treatment for diarrhea (Mat-Salleh and Latiff, 2002)	Gunung Stong, Kelantan
Kopsia pauciflora Apocynaceae	SK 468	Jelutong; Pulai	Roots used for nose ulcer trearment (Mat-Salleh and Latif, 2002)	Ulu Muda, Kedah
Tabernaemontana corymbosa Apocynaceae	SA1-6	Jelutong Badak	Ulcer and post-natal treatments (Burkill, 1965)	Gunung Stong, Kelantan
Poikilospermum suaveolens Cecropiaceae	SK535/03	No literature report on local name	Sap used for stomach ulcer (Chua, 1996)	Gunung Stong, Kelantan
Euonymus wrayi Celastraceae	SK 497	No literature report on local name	No reports on traditional uses	Ulu Muda, Kedah
Connarus cochinchinensis Connaraceae	SK 482	Sembilat	As part of tuberculosis treatment	Ulu Muda, Kedah
Hodgsonia macrocarpa Cucurbitaceae	SK 479	Akar kepayang	Leaves for treating bleeding nose and to reduce body heat	Ulu Muda, Kedah
Dioscorea esculenta Dioscoreaceae	SK 465	Ubi torak	Food (bubur caca)	Ulu Muda, Kedah
Shorea curtisii Dipterocarpaceae	SB 1-4	Meranti merah	Timber	Gunung Stong, Kelantan
Diospyros frutescens Ebenaceae	SA3-19	Buah tubai; Kayu malam	Fish poison (Burkill, 1965)	Gunung Stong, Kelantan
Cephalomappa malloticarpa Euphorbiaceae	SA3-8	Bantas; Mingaram; Perupuk batu	Wood for house construction	Gunung Stong, Kelantan
Drypetes pendula Euphorbiaceae	SA2-2	Lidah lidah	Toxic latex, timber	Gunung Stong, Kelantan
Cyrtandra cf. *anisophyllea* Gesneriaceae	SK 553/03	Meroyan	No reports on traditional uses	Gunung Stong, Kelantan
Cinnamomum javanicum Lauraceae	SK 464	Kura bengkak; Lawang kecil	Roots used for post-natal blues (Burkill, 1965) and spleen treatments (Burkill and Haniff, 1930)	Ulu Muda, Kedah
Cryptocarya costata Lauraceae	SK 474	No literature report on local name	No reports on traditional uses	Ulu Muda, Kedah
Saraca declinata Leguminosea	SK 461	Tudung periuk; Kapih beruk	Roots used for post-natal treatment (Mat-Salleh and Latiff, 2002)	Ulu Muda, Kedah
Magnolia villosa Magnoliaceae	SK 487	Cempaka hutan	Poisonous latex that can cause allergy (Burkill,1965)	Ulu Muda, Kedah

Table 1. Contd.

Galearia fulva Pandaceae	SA2-17	Ekor Tupai; Serga	Treatment for gonorrhea (Burkill, 1965)	Gunung Stong, Kelantan
Galearia maingayi Pandaceae	SK 505/03	Minyak berok	No reports on traditional uses	Gunung Stong, Kelantan
Microdesmis caseariifolia Pandaceae	SA3-2	Cherek Rimba; Sigoh; Rambeh	No reports on traditional uses	Gunung Stong, Kelantan
Lasianthus densifolius Rubiaceae	SK 485	No literature report on local name	No reports on traditional uses	Ulu Muda, Kedah
Rothmannia macrophylla Rubiaceae	SK538/03	Hidung babi	Produce dye	Gunung Stong, Kelantan
Urophyllum blumeanum Rubiaceae	SK 531/03	No literature report on local name	Treatment for malaria (Wiart, 2002)	Gunung Stong, Kelantan
Madhuca kingiana Sapotaceae	SB 3-40	Pokok nyatoh	No reports on traditional uses	Gunung Stong, Kelantan
Smilax megacarpa Smilacaceae	SK 545/03	Akar rebana; Lampu bukit	Fruits use for post-natal treatment (Brandis 1978)	Gunung Stong, Kelantan
Callicarpa candicans Verbenaceae	SA1-9	Tampang besi	For treatment of cuts (Burkill, 1965) and, the leaves concoction for the treatment of diarrhea (Fasihuddin, 1993)	Gunung Stong, Kelantan
Scaphochlamys biloba Zingiberaceae	SK 512/03	Halia	No reports on traditional uses	Gunung Stong, Kelantan

pronounced activity against MV in this second treatment except for *P. suaveolens*. In the third treatment, six extracts exhibit the highest antiviral activity by this route, that is, *Connarus cochinchinensis, Dioscorea esculenta, Euonymus wrayi, Hogsonia macrocarpa, Kopsia pauciflora* (all from Ulu Muda) and *Tabernaemontana corymbosa* (from Gunung Stong). Five extracts from Gunung Stong showed no antiviral activity by this route, that is, *P. suaveolens, Saurauia roxburghii, Callicarpa candicans, Rothmannia macrophylla* and *Drypetes pendula.* Other extracts showed mild to moderate activity against MV. The mode of action for guanidine hydrochloride as synthetic antiviral in this study was in the first and second treatment which can be related to the blocking of viral RNA synthesis (Klein et al., 2000). However, in this study, guanidine hydrochloride is found to be cytotoxic thus unsuitable as antiviral drug.

DISCUSSION

Saponins are secondary metabolites that have similar chemical character to glycosides. They are believed to be useful for the human diet in controlling cholesterol, but some (including those produced by the soapberry) are very poisonous if swallowed, are able to lyse red blood cells and cause urticaria or skin rash in many people (Mohamad et al., 2001). According to Ong (2004), saponins can also be used as an anti-inflammatory agent and in treatment for tuberculosis.

Alkaloids are organic chemical compounds which contain nitrogen heterocyclic and morphine is the first alkaloid used in medicine as an analgesic drug (Cowan, 1999). Most of these compounds are toxic and do not have smell or taste. Alkaloids can increase nutrient absorption and blood circulation, reduce pain and stimulate nerve system as it has narcotic effect (Ong, 2004). *Goniothalamus macrophyllus* has been reported in treatment for high blood pressure, fever and post-natal blues (Brandis, 1978) while *Urophyllum blumeanum* was used for malaria treatment (Wiart, 2002).

According to research by Amoros et al. (1987), saponins inhibit virus attachment to the cells. They suggested that this active biological compound can be use to compete with virus for the binding site at the cell's receptor. *Saurauia roxburghii* has highest concentration of saponins (4+) but it only show mild activities in first treatment and no activities in second and third treatments. The result showed no link between antiviral activities and saponins indicating that there are other components in the extracts that have an influence to saponin activity (Micol et al., 2005).

Plants use steroids to avoid being eaten by vertebrate animals. These active compounds have four carbon rings called 'steroid backbone' and classified as cardiac glycoside because of it specific response to the heart (Mohamad et al., 2001). Steroids were used as allergy, arthritis and coronary failure therapy; control in menstrual

Table 2. Phytochemical constituents, toxicity to shrimp, CTC_{50} values and antiviral activity of plants from Gunung Stong, Dabong, Kelantan and Ulu Muda, Kedah.

Plant name/ Family	Alkaloid	Steroids/ triterpene	Saponins	Toxicity to shrimp (mg/L)	Cytotoxicity (Vero) CTC_{50} (mg/L)	Treatment		
						I	II	III
Sauraua roxburghii Actinidiaceae	-	-	4+	>1000	165.95	2+	-	-
Gluta elegans Anacardiaceae	-	-	1+	>1000	192.75	-	-	3+
Goniothalamus macrophyllus Annonaceae	2+	+	2+	>1000	758.46	+	-	3+
Psueduvaria macrophylla Annonaceae	-	-	2+	>1000	584.34	3+	-	3+
Kopsia pauciflora Apocynaceae	-	-	2+	>1000	88	2+	+	4+
Tabernaemontana corymbosa Apocynaceae	-	-	1+	>1000	36.87	2+	-	4+
Poikilospermum suaveolens Cecropiaceae	-	-	1+	>1000	575.44	3+	2+	-
Euonymus wrayi Celastraceae	-	-	+	>1000	135	+	-	4+
Connarus cochinchinensis Connaraceae	-	-	+	>1000	104.5	-	1+	4+
Hodgsonia macrocarpa Cucurbitaceae	-	-	+	>1000	38.6	+	+	4+
Dioscorea esculenta Dioscoreaceae	-	-	+	>1000	111.8	+	-	4+
Shorea curtisii Dipterocarpaceae	-	-	2+	>1000	946.40	-	-	+
Diospyros frutescens Ebenaceae	-	-	3+	>1000	1000.00	-	-	+
Cephalomappa malloticarpa Euphorbiaceae	-	-	1+	>1000	541.17	-	-	3+
Drypetes pendula Euphorbiaceae	-	-	2+	>1000	512.86	-	-	-
Cyrtandra cf. *anisophyllea* Gesneriaceae	-	-	-	>1000	354.81	+	-	2+
Cinnamomum javanicum Lauraceae	-	-	2+	>1000	>1000	-	-	3+

Table 2. Contd

Cryptocarya costata Lauraceae	-	-	1+	>1000	>1000	+	+	3+
Saraca declinata Leguminosae	-	+	1+	>1000	729	+	+	3+
Magnolia villosa Magnoliaceae	-	-	1+	>1000	363	+	+	3+
Galearia fulva Pandaceae	-	-	1+	>1000	21.540	+	-	2+
Galearia maingayi Pandaceae	-	+	1+	>1000	1091.70	-	-	+
Microdesmis caseariifolia Pandaceae	-	-	1+	>1000	681.00	-	+	2+
Lasianthus densifolius Rubiaceae	-	+	1+	>1000	39	+	+	3+
Rothmannia macrophylla Rubiaceae	-	-	2+	>1000	407.38	+	-	-
Urophyllum blumeanum Rubiaceae	1+	-	2+	>1000	1212.50	-	-	2+
Madhuca kingiana Sapotaceae	-	-	1+	>1000	380.19	-	-	2+
Smilax megacarpa Smilacaceae	-	+	2+	>1000	500.00	-	-	3+
Callicarpa candicans Verbenaceae	-	-	1+	>1000	794.33	+	-	-
Scaphochlamys biloba Zingiberaceae	-	-	1+	>1000	1083.30	+	-	2+
Guanidine hydrochloride (control)	-	-	-	-	141.25	+	+	-

*Score for phytochemical studies
+ = positive (detected), 1+, 2+ = positive (moderate), 3+, 4+ = positive (high), - = negative
*Score of antiviral activity based on the difference in optical density reading between experimental and cells infected with virus (E-V)
- = no activity (<0),
+ = very mild antiviral activity (0 – 0.20)
2+= mild antiviral activity (0.21 – 0.40)
3+ = moderate antiviral activity (0.41- 0.60)
4+ = high antiviral activity (>0.61)
Treatment I = (C+V) + E
Treatment II = (C+E) + V
Treatment III = (E+V) + C
C = Cell, V = Virus (measles) E = Extract.

cycle and increasing women fertility (Ong, 2004). Triterpenoids have been proven to inhibit HIV but the mechanism is still unclear and there is a suggestion that the mechanism is related to membrane damage through lipophilic component on the alkaloid involved (Cowan, 1999).

In vitro cytotoxicity test is an important step in screening an antiviral product before *in vivo* test was conducted. Any material has potential to be commercialized only after it is proved not to be toxic to normal cells. The accuracy of cytotoxicity analysis is very important to avoid mistakes during antiviral screening (Buchnall, 1973). According to Ahmad and Romlah (1993), if the extracts are too toxic, the antiviral results are not valid because it will have a low therapeutic value (treatment value: toxicity level ratio).

The purpose of using several treatments is to screen the antiviral activities during different stages of MV life cycle (Nurul Aini et al., 2006). The extracts might inhibit virus infection by interfering with virus-host recognition, preventing virus release into the cells, intervening with virus life cycle or damaging the virus particle itself. The first treatment demonstrates antiviral activity after viral capsid was released into the cells. The mechanism that might be involved in this post-infection treatment is the inhibition of virus replication or in virus assembly phase and during virus release into other cells (Yip et al., 1991). The inhibition will stop virus spreading to other cells because it is not infectious (Ahmad, 1993).

The second treatment was meant to be a prophylactic therapy which involves interferon activity from the cells (Wachsman et al., 1988). Interferon can be induced by viral infection or metabolite in the extract. It was secreted by infected cells to protect neighbouring cells. Mammalian cells start producing interferon two hours after exposure to the virus and reached maximum level after 24 h (Ahmad, 1993). Marini et al. (1998) reported that extracts might need longer time to increase cells immune system towards MV infection.

In the third treatment, extract and virus were added concurrently to Vero cells to observe extracts capability to prevent virus attachment to the cells. Virus hemagglutinin glycolipid structure may be altered by extract attachment to the virus binding site so it cannot be complement to the cell receptor. Extract may inhibit virus penetration into the cells through receptor modifying or attachment to the virus itself. It also can interrupt virus genome replication by inhibit acid nucleic function and replication enzyme. Infection process can only occur when glycoprotein virus attach to specific cell receptor and penetrate to the susceptible cells (Ahmad, 1993). Other than helping virus to penetrate into the cells, glycoprotein membrane is also a target for antiviral drug making it an important component of the virus (Streissle, 1981).

No extracts showed activity in all three treatments indicating the variation in active compounds in different plants. Active compounds are used to protect plants from microorganism infection and animal threat (Cowan, 1999). Mammalian cells play an important role in screening of active compounds with antiviral property. Virus only infect particular host by attaching it receptors to specific host cells receptors (Schattner, 2005). Cells selection, virus strain and cell culture physiology are the important criteria in screening for antiviral drugs (Pütz et al., 2003; Streissle, 1981).

Further studies should be conducted to identify antiviral compounds in each extracts. Cytopathic effect and virus protein can be determined to calculate virus activity accurately (Erturk et al., 2000). Antiviral activities can be enhanced using chaperon compound to help transport-tation of antiviral drugs to the infected cells. Studies by Grancher et al. (2004) showed ribavirin combined with cyclodextrins have higher antiviral activities against MV than ribavirin itself.

As a conclusion, we managed to survey 30 different plants for their chemical constituent in the methanolic leaf extracts. None of them are lethal to brine shrimp with LC50 values are more than 1000 mg/L. Six plants from Ulu Muda were found to be cytotoxic, that is, *Connarus cochinchinensis, Dioscorea esculenta, Euonymus wrayi, Hodgsonia macrocarpa, Kopsia pauciflora* and *Lasianthus densifolius* and four plant extracts from Gunung Stong (*Gluta elegans, Saurauia roxburghii, Galearia maingayi* and *Tabernaemontana corymbosa)* with CTC_{50} lower than 208 µg/ml. The plant extracts have different antiviral activities. At least 2 extracts showed moderate post-infection antiviral activity and six extracts were effective when added simultaneously during virus infection.

ACKNOWLEDGEMENT

The project is funded by Universiti Kebangsaan Malaysia (Vote 27700) and Ministry of Higher Education (ST-018-2005, UKM-OUP-KPB-32-162/2009). We would like to thank Mr. Ujang Suki for his technical assistance.

REFERENCES

Ahmad IB(1993). *Pengenalan Virologi*. Kuala Lumpur: Dewan Bahasa Dan Pustaka.

Ahmad IB, Normah N, Nor AO (1993). Kesan in vitro ekstrak kasar segar serai wangi dan kecubung terhadap beberapa bakteria, virus penyakit Newcastle dan Measles. Sains Malaysiana 22(3): 27-37.

Ahmad, IB, Romlah YS (1993). Kesan ekstrak *Chaetomium trilaterale* Chivers ke atas kultur sel HeLa dan poliovirus. J. Biosci. 4 (1): 63-68.

Amoros M, Fauconnier B, Girre RL (1987). *In vitro* antiviral activity of a saponin from *Anagallis arvensis*, Primulaceae, against herpes simplex virus and poliovirus. Antivir. Res. 8 (1): 13-25.

Brandis D (1978). Indian trees. India: Bishen Singh Mahendra Pal Singh.

Buchnall RA (1973). The continuing search for antiviral drugs. Adv. Pharmacol. Chemother. 11: 295-318.

Burkill IH (1965). A dictionary of the economic products of the Malay Peninsula. 2nd Edn. Kuala Lumpur: Kementerian Pertanian dan Kooperatif.

Burkilll H, Haniff M (1930). Malay village medicine. Gard. Bull. Str. Settl. 6: 167-332.

Chua S (1996). Kajian etnobotani ke atas komuniti Temuan di

Semenyih, Selangor. Tesis B.Sc., Institut Sains Biologi, Universiti Malaya, Kuala Lumpur.
Cowan MM (1999). Plants products as antimicrobial agents. Clin. Microbiol. Rev. 12(4): 564-582.
Erturk O, Emirbag Z, Belduz AO (2000). Antiviral activity of some plant extracts on the replication of *Autographa californica* nuclear polyhedrosis virus. Turk. J. Biol. 24: 833-844.
Faridah HI, van de Maesen LJG (1997). PROSEA: Plant resources of south-east Asia 11, auxiliary plants. Jakarta: LIPI Press. 391p.
Fasihuddin BA (1993). Medicinal plants used by Kadazan/Dusun community in Sabah. Sabah Museum J. 1: 15-30.
Gurib-FA, Subratty, H, Narod, F, Govinden-Soulange J (2005). Biological activity from indigenous medicinal plants of Mauritius. Pure Appl. Chem. 77(1): 41-51.
Grancher N, Venard V, Kedzierewicz F, Ammerlaan W, Finance C, Muller CP, Le Faou A (2004). Improved antiviral activity *in vitro* of ribavirin against measles virus after complexation with cyclodextrins. Antivir. Res. 62(3): 135-137.
Harborne JB (1993). Phytochemical method. 3rd Edn. London: Chapman and Hall. pp. 135-203.
Horikami SM, Moyer SA (1995). Structure, transcription, and replication of measles virus. Current Topics in Microbiol. Immunol. 191: 35-50.
Jaganath IB, Ng LT (2000). Herbs: The Green Pharmacy of Malaysia. Kuala Lumpur: Vinpress Sdn. Bhd.
Klein M, Hadaschik D, Zimmermann H, Eggers HJ, Nelsen-SB (2000). The picornavirus replication inhibitors HBB and guanidine in the echovirus-9 system: the significance of viral protein 2C. J. Gen. Virol. 81(4): 895-901.
Kott, V, Barbini L, Cruañes M, Muñoz JD, Vivot E, Cruañes J, Martino V, Ferraro G, Cavallaro, L, Campos, R (1999). Antiviral activity in Argentine medicinal plants. J. Ethnopharmacol. 64 (1): 79-84.
Marini AM, Ahmad IB, Yahya MD, Yamin BM 1998. Optimization of cytotoxicity assay for estimating CD_{50} values of plant extracts and organometallic compounds. Malays. Appl. Biol. 27: 63-67.
Mat-Salleh K, Latiff A (2002). Tumbuhan ubatan Malaysia. Pusat Pengurusan Penyelidikan dan Inovasi, Universiti Kebangsaan Malaysia. p. 797.
Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45 (1): 31–34.
Micol V, Caturla N, Perez-Fons L, Mas V, Perez L, Estepa A (2005). The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). Antivir. Res. 66 (2-3): 129-136.
Mohamad T, Hussin K, Surif S (2001). Tumbuhan beracun di sekeliling kita. Kuala Lumpur: Dewan Bahasa dan Pustaka. pp. 1-12.
Nazlina I, Norha S, Noor ZAW, Ahmad IB (2008). Cytotoxicity and antiviral activity of *Melastoma malabathricum* extracts. Malays. Appl. Biol. 37(2): 53-55.
Nurul Aini MN, Said MI, Nazlina I, Hanina, MN, Ahmad IB (2006). Screening for antiviral activity of sweet lemon grass (*Cymbopogon nardus* (L.) Rendle) fractions. J. Biol. Sci. 6 (3): 507-510.
Ong HC. 2004. *Tumbuhan liar. Khasiat ubatan dan kegunaan lain.* Kuala Lumpur: Utusan Publication and Distributor. pp. 6-9.
Pütz MM, Bouche FB, de Swart RL, Muller CP. 2003. Experimental vaccines against measles in a world of changing epidemiology. Int. J. Parasitol. 33(5-6): 525-545.
Rager-Zisman B, Bazarsky E, Skibin A, Chamney S, Belmaker I, Shai I, Kordysh E, Griffin DE (2003). The effect of measles–mumps–rubella (MMR) immunization on the immune responses of previously immunized primary school children. Vaccine 21(19-20): 2580-2588.
Schattner A (2005). Consequence or coincidence? The occurrence, pathogenesis and significance of autoimmune manifestations after viral vaccines. Vaccine 23 (30): 3876-3886.
Sofowora A (1984). Medicinal plants and traditional medicine in Africa. New York: Published in association with Spectrum Books Ltd. Ibadan by John Wiley and Sons. pp. 142-143.
Streissle G. 1981. Persistent viral infections as models for research in virus chemotherapy. Adv. Virus Res. 26:37-64.
Wachsman MB, Coto E, Martino V (1988). Search for antiviral activity in higher plant extracts. Fitoterapia. 5: 422–424.
World Health Organization (2008). Traditional medicine. http://www.who.int/mediacentre/ factsheets/fs134/en/
Wiart C (2002). Medicinal plants of Southeast Asia. 2nd Edn. Malaysia: Prentice Hall.
Yip L, Pei S, Hudson JB, Towers GHN (1991). Screening of medicinal plants from Yunan Province in Southwest China for antiviral activity. J. Ethnopharmacol. 34 (1): 1-6.