Phytochemical screening and Anti-oxidant activity of the two plants *Ziziphus oxyphylla* Edgew and *Cedrela serrata* Royle

Rizwan Ahmad, Mansoor Ahmad[*], Mehjabeen and Noor Jahan
[1]Research Institute of Pharmaceutical Sciences, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan
[2]Department of Pharmacology, Federal Urdu University of Arts, Science & Technology, Karachi, Pakistan
[3]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan

Abstract: Phytochemical studies of medicinal plants are a basic and helping tool for the isolation of active secondary metabolites. The isolation of active compounds is made easy by the help of preliminary phytochemical studies, which shows the presence of a specific class or group of compounds present in these medicinal plants. *Ziziphus oxyphylla* and *Cedrela serrata* are medicinal plants with valuable local uses. The present study is for the first Phytochemical investigation of these two medicinal plants which consists of, Quantitative tests showing very good results except *Ziziphus oxyphylla* plants which does not showed the results for Ester value and Peroxide value. Color reactions are studied for all the crude extracts showing the presence of a number of chemical groups belonging to the class of Alkaloids, Phenol compounds, Phenothiazines, Aromatic compounds, Amino acids, Sulfur compounds etc. Brine shrimp activity was performed which showed a LD_{50} value of 45.74 and 53.36 in the case of *Ziziphus oxyphylla* roots and *Cedrela serrata* bark respectively, which is comparable to the standard drug *Cyclophosphamide* results of 16.09. Insecticidal activity did not show any promising result indicating the absence of any insect killing potency. Antioxidant activity was very positive for all the extract particularly, the *Ziziphus oxyphylla* roots, which showed even better results than the standard drug Ascorbic acid used in various dilutions.

Keywords: Color tests, brine shrimp, insecticidal activity, anti-oxidant, LD_{50} value.

INTRODUCTION

Medicinal plants are considered the basic and important source of a number of Active secondary metabolites, new drug leads and chemical entities. Phytochemical screening of these medicinal plants is one of the very important preliminary (pre-clinical) studies, for the isolation of secondary active metabolites, which aids in the identification of a group or class of active constituents present in a particular plant. The isolation of this specific class of active constituent up to great extent necessitates a sound knowledge of the phytochemical studies of these medicinal plants.

Phytochemical studies comprise the study of active constituents of plants, which is important for the finding of active constituents and may be very helpful in revealing new sources of a lot of such chemical classes as tannins, oils, gums and novel drugs for the synthesis of complex secondary metabolites. While adding up, the knowledge of these active constituents of plants would further be priceless in discovering the exact value of folkloric remedies (Mojab *et al.,* 2003). Active constituents may be therapeutically dynamic or dormant. Those, which show any activity are called active chemicals and the ones with no activity are called inactive chemical constituents (Iyengar, 1995).

This study on these two medicinal plants is carried out for the first time. The aim is to know the basic components and chemicals, which can be very helpful for further isolating bioactive compounds of these yet phyto-chemically unexplored plants. Also this preliminary study will act as a basic tool in linking the Folkloric uses as cited in literature on these plants and the chemical constituents which may be responsible for playing a role in these folkloric uses. *Ziziphus oxyphylla* and *Cedrela serrata* are the medicinal plants upon which the study was performed to explore the profile of chemical nature of these medicinal plants in order to facilitate the researcher in future studies of these yet to be explored medicinal plants.

Ziziphus is a genus of about 100 species distributed mostly in tropical America, Africa, and Mediterranean region, Indo-Malaya, Australia, Pakistan and India. Represented by 6 species in Pakistan, consisting of *Ziziphus rugosa, Ziziphus mauritiana, Ziziphus nummularia, Ziziphus spina-christi, Ziziphus jujuba, Ziziphus oxyphylla* (www.tropicos.org).

Aqueous extract of root bark is mixed with extract *of Citrus medica* (lemon) in equal quantity and given twice a day to cure hypertension (Khan *et al.,* 2012). Two cyclopeptide alkaloids have recently been isolated from the *Ziziphus oxyphylla* species, which showed potent α-glucosidase enzyme inhibitory activity and protein glycation (Choudhary *et al.,* 2011).

**Corresponding author:* e-mail: herbalist53@yahoo.com

Folkloric uses of the plant includes its Antidiabetic activity (Sher, 2011), along with its application in the Jaundice and liver disease (Shah *et al.,* 2006).

Cedrela is a genus of about 16 species, with a wide distribution in tropical Asia, Mexico to S. America and Australia. Represented in W. Pakistan by 2 species, *Cedrela toona* and *Cedrela serrata* (www.tropicos.org). *Cedrela serrata* leaves and bark are used to treat fever, diabetes, dysentery, blood diseases, skin diseases (Jan *et al.,* 2011). Leaves and root bark are used in joint dislocation, sprain and high blood pressure (Pfoze *et al.,* 2012). Its use in fever, dysentery, diabetes, blood diseases and skin diseases are also found in the literature (Awan *et al*., 2011). Leshmanicidal activity was reported by (Takahashi, 2004). Antioxidant and DNA protection activities of leaves extract and fractions were reported by (Perveen *et al.,* 2012).

MATERIALS AND METHODS

Plant materials

Ziziphus oxyphylla Edgew roots and leaves, and *Cedrela serrata* Royle bark and leaves were collected from local mountains of District Swat Valley of Malakand Division, Pakistan. The collection was done during the season of September and December. All the plants were identified by Dr. Sirajul Haq, professor at the Botany Department, Post Graduate Jehanzeb College Saidu Sharif, District Swat, Pakistan. All collected parts of the plants were washed with tap water and thereafter dried under shade for 12 to 18 days in normal air. The plant materials were cutted to small pieces after drying.

Plant material extraction

For extraction the methods described by Emeruwa AC (1982) and Trease and Evans (1996) were followed. Previously dried and finely cut parts of both the medicinal plants were soaked in methanol. The Maceration process lasted for 12 days with intermittent shaking in a glass bottle, which was covered by an aluminum sheet and cotton wool plug. Now after 12 days the solvent was decanted from the bottles and was filtered through Whatman No. 1 filter paper. All the filtered solvent was evaporated to dryness at 40°C in a rotary evaporator (BUCHI Rota vapor R-200, Switzerland).

Color tests

All the Color tests were performed following the methods described in (Clarke and Williams, 1955; Mukherjee, 2002; Evans, 2005; Kokate *et al.,* 2005; Harborne, 1973; Sofowra, 1993).

Brine Shrimp lethality Test

Bioactive compounds sometimes shows toxicity to *Artemia salina* shrimp larvae. For the test of this activity, eggs of brine shrimp *Artemia salina* were bought from the local market. In order to hatch the eggs artificial seawater was prepared at a concentration of Sea salt (38g/L in water and maintained the pH at 7.4). Then 50 mg of Brine shrimp eggs were sprinkled in the artificial seawater and were incubated at 37^{o}C. The Shrimp eggs hatched in 24 to 48 hours, giving a large number of shrimp larvae. After 2 days the mature nauplii were taken as 10 larvae/vial with the help of a micropipette. Then different concentrations of 100, 10 and 5ug/ml of the crude extract were added to these larvae in different vials. The volume was made 5 ml with the help of seawater. All the vials were incubated under illumination. After 24 hours the results were taken by counting the larvae that survived. The data was analyzed with the help of Finney computer programme to find the LD_{50} value (Alves *et al.,* 2000).

The standard run was *Cyclophosphamide* with LD_{50} value of 16.09 (Atta-ur-Rahman and Choudhary, 2001).

Insecticidal activity

The insects *Tribolium castaneum,* and *Rhyzopertha dominica,* were tested in for this activity. The method consisted of cutting a filter paper exactly the same size as that of Petri plate, about 9 cm or 90 mm and put it in the plate. The crude extract samples were dissolved in a volatile solvent (Isman *et al.,* 1987) and were loaded over the whole filter paper with the help of micropipette. Plates were kept for 24 hours in order to evaporate the solvent completely.

Next day after drying of the plates, 10 insects of each species were put in each plate including the test and control also. Results were observed after 24 hours by assessing the survival of insects in each plate. The positive control *Permethrin* and negative control volatile solvent Methanol was also run in parallel (Atta-ur-Rahman and Choudhary, 2001; Collins, 1998).
The % inhibition or Mortality was found by the following formula.

$$\text{Percentage Mortality} = 100 - \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

DPPH test for free radical scavenging activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl; molecular formula $C_{18}H_{12}N_5O_6$) was used in this test to evaluate the free radical scavenging or antioxidant potential of natural products (Takao and Watanabe, 1984).

For preparation of the DPPH solution 4 mg was dissolved in 50 ml of Methanol in order to achieve a concentration of 80 µg/ml. This activity was carried only quantitatively using a UV-Vis spectrometer. For the quantitative assay, the stock solution of crude extracts was prepared using Methanol to achieve a concentration of 10 mg/ml (Sarker and Eynon, 2003), whereas the positive standard was prepared at a concentration of 0.5 mg/ml. Dilutions of the stock solutions of the crude extracts were prepared to achieve concentrations of 5×10^{-2}, 5×10^{-3}, 5×10^{-4}, 5×10^{-5}, 5×10^{-6}, 5×10^{-7}, 5×10^{-8}, 5×10^{-9}, 5×10^{-10} mg/ml. From these

dilutions 1.0 ml from each was mixed with 1.0 ml of DPPH. Wait for 30 min, so that the reaction takes place. The absorbance of these solutions was measured at 517 nm. Triplicate for each result was carried out and the mean of absorbance's was recorded for every concentration. The standard drug Ascorbic Acid was also treated by the same method.

The decrease in absorbance was then converted into percentage Antioxidant activity by the following formula.
AA% = 100 – [{Abs sample-Abs blank} x 100 / Abs control]

RESULTS

Color tests performed showed the presence of a large number of active and therapeutic constituents as shown in table 1, i.e. Alkaloids, Phenol compounds, Amino acids and Nitrogenous substances. Aromatic compounds, Nitrates, Nitrites, Xanthine like compounds, Phenothaizines and other chemical groups and moieties were also found in some parts of the plants tested for color reactions as given in table 1.

Quantitative tests were also carried out for the presence of free acid (Acid value), active oxygen/peroxide content (Peroxide value) and esters present (Ester value), along with the quantity of free acids and to saponify the esters present (Saponification value) for different parts of these medicinal plants as given in table 2. Both medicinal plants showed different types of results as shown in table 2, except for the *Ziziphus oxyphylla* plant. Although both, roots and leaves of *Ziziphus oxyphylla* do not show any peroxide value, the peroxide value of *Cedrela serrata* plant was also not in the range as mentioned in the European pharmacopeia tables, which is 20 to 30 for 1 g of the extract used. While keeping aside the peroxide value, if we compare the results of other quantitative tests, the *Ziziphus oxyphylla* plant showed very good results for Saponification value test. The range of this test by British pharmacopeia is from 300 to 400 as shown for 1 g of the substance.

Cytotoxic studies were carried out with a little modification in dose used. The idea behind this concept was to check the lowest concentration at which these crude extracts of the plants, like Roots of *Ziziphus oxyphylla* and bark of *Cedrela serrata* can be effective, because these two parts of the plants were highly cytotoxic at normal doses generally tested.

As shown in table 3, the cytotoxic activity of all the parts of these two medicinal plants, *Ziziphus oxyphylla* roots and *Cedrela serrata* bark are still very effective even at the lowest dose concentration of 5ug/ml.

The Insecticidal study was also performed for these two medicinal plants but no mortality was recorded at any of the doses tested against the selected insects as mentioned above. So it shows no insecticidal activity at the doses tested.

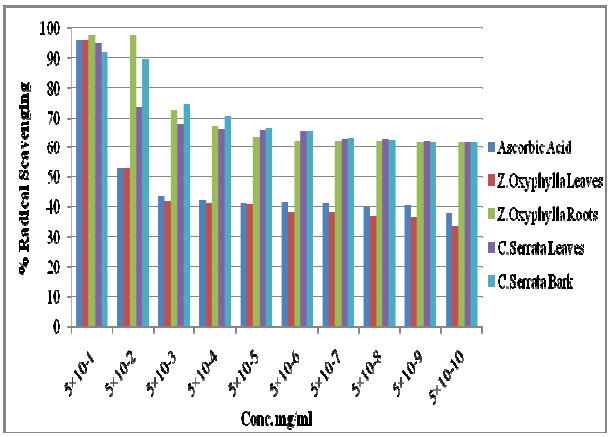

Fig. 1: Radical scavenging (%) activity comparison for *Ziziphus oxyphylla* and *Cedrela serrata*

The last activity performed was for antioxidant/free radical scavenging ability of these medicinal plants. Various dilutions were made and tested for this activity. All the parts of these two medicinal plants showed a very good and potent antioxidant activity, but *Ziziphus oxyphylla* roots were very potent amongst all the dilutions tested for these plants. The roots of *Ziziphus oxyphylla* free radical scavenging capability was as the same as that of standard drug Ascorbic acid used. But it does not exclude the other parts of these plants and their dilutions from the scope of antioxidant potent plants as shown in

DISCUSSION

These plants, after a thorough phytochemical and biological investigation, showed very interesting and potential results.

Color tests carried for both the plants were of prime importance because prior to go in detail for a phytochemical studies of a medicinal plant, it is very necessary to find the worth of the plant for being active and to be selected for further phytochemical screening, biological or pharmacological activities. All the color reactions carried out were interpreted for results by matching the color of the crude extracts after each color reaction with the table provided along the procedures for color reaction. It is very lengthy to discuss all the tables and results here but a complete view of these medicinal plants crude extracts for the presence of various chemical groups present, as confirmed by color tests.

Quantitative tests show the presence of free acids and esters in *Ziziphus oxyphylla* plant. *Cedrela serrata* plant does not comply with Ester value and Peroxide value results according to the prescribed results of the European pharmacopoeia. Acid value and Ester value calculated comply the results of the British pharmacopeia. All the quantitative tests results are given in table 2.

Table 1: Results for color tests

Color Test	Plant Extract			
	Cedrela serrata Bark	*Cedrela serrata* Leaves	*Ziziphus oxyphylla* Root	*Ziziphus oxyphylla* Leaves
Amalic acid Test	(-)	(+)	(-)	(-)
Ammonical Ag-Nitrate	(+)	(-)	(+)	(-)
Aromaticity	(+)	(+)	(+)	(+)
Benedicts	(+)	(-)	(+)	(-)
Chromo Tropic Acid	(-)	(-)	(-)	(-)
$CuSO_4$ test	(+)	(+)	(+)	(+)
Diazotization	(-)	(-)	(-)	(-)
$FeSO_4$ (A)	(+)	(-)	(+)	(-)
Forrest Reagent	(+)	(+)	(+)	(+)
FPN Reagent	(+)	(+)	(+)	(+)
$FeSO_4$ (B)	(-)	(-)	(-)	(-)
$FeCl_3$ (Ferric Chloride)	(+)	(+)	(+)	(+)
Froehde Reagent	(+)	(+)	(+)	(+)
Methanolic KOH	(+)	(-)	(+)	(-)
Mercurous nitrates	(-)	(-)	(-)	(-)
Dragendorff reagent	(-)	(-)	(+)	(+)
Liebermann's Reagent	(+)	(+)	(+)	(+)
McNally test	(+)	(-)	(+)	(-)
Formaldehyde-H_2SO_4	(+)	(+)	(+)	(+)
Marquis test	(+)	(+)	(+)	(+)
Millon's Reagent	(-)	(-)	(-)	(-)
Napthol – Sulfuric Acid	(+)	(+)	(-)	(+)
Nitrous Acid	(+)	(+)	(+)	(+)
Ninhydrin	(+)	(-)	(-)	(-)
Potassium dichromate	(+)	(+)	(+)	(+)
Sodium Nitroprusside	(-)	(-)	(-)	(-)
Vanillin Reagent	(+)	(+)	(+)	(+)
Nesslers Reagent	(+)	(-)	(+)	(-)
Phosphorus Test	(+)	(+)	(+)	(+)

Table 2: Quantitative tests and values observed for the crude extracts

Crude Extract	Acid Value	Saponification Value	Ester Value	Peroxide Value
ZOR	100.98	336.6	235.62	No Results.
ZOL	106.59	490.875	384.285	No Results
CSB	92.565	98.175	5.61	157
CSL	86.955	112.2	25.245	160

Cytotoxicity test shows a very positive indication of these plants to be studied and explored for phytochemical studies as having the potency for cell killing activity. Roots of *Ziziphus oxyphylla* were the most potent of all parts of the two plants tested, followed by *Cedrela serrata* bark, showing results which are almost comparable with the standard drug *cyclophosphamide* used. Leaves of both the plants were not so much toxic to be considered as cytotoxic active.

Discussing the Antioxidant potential of the plants, looking in detail to the table 4, it is quite clear that all other parts of these plants tested showed very good antioxidant activity, as can be proved by the comparison of dilutions used for the standard drug Ascorbic acid and other parts of the plants tested. Except for the *Ziziphus oxyphylla* leaves, *Ziziphus oxyphylla* roots and *Cedrela serrata* bark and leaves are still very much potent as free radical scavenger as compared to the standard drug ascorbic acid used.

It is clear from the above screening that these medicinal plants contain very potent chemical classes of drugs and this is the reason why these plants are used widely in folkloric uses and have a very wide application. The need is to explore these plants for pure chemical compounds, which are responsible for the use of these plants in emerging and challenging diseases i.e. Hepatitis, other Liver diseases and Diabetes etc.

Table 3: LD_{50} Value calculated for *Ziziphus oxyphylla* and *Cedrela serrata*

S. No.	Concentration (µg/ml)	No. of test Vials	No. of Shrimp test	Average mortality (24 hrs)	Percent average mortality	LD50 (µg/ml)
Control	Without drug	1	10	00	00	00
ZOR	05	A	10	00	00	45.74
		B	10			
		C	10			
	10	A	10	04	40	
		B	10			
		C	10			
	100	A	10	08	80	
		B	10			
		C	10			
ZOL	05	A	10	00	00	217.25
		B	10			
		C	10			
	10	A	10	01	10	
		B	10			
		C	10			
	100	A	10	02	20	
		B	10			
		C	10			
CSL	05	A	10	00	00	169.67
		B	10			
		C	10			
	10	A	10	00	00	
		B	10			
		C	10			
	100	A	10	02	20	
		B	10			
		C	10			
CSB	05	A	10	00	00	53.36
		B	10			
		C	10			
	10	A	10	03	30	
		B	10			
		C	10			
	100	A	10	08	80	
		B	10			
		C	10			

Table 4: Antioxidant results calculated for *Ziziphus oxyphylla* and *Cedrela serrata*

Concentration	*Ascorbic Acid*	*Z. Oxyphylla Leaves*	*Z. Oxyphylla Roots*	*C. Serrata Leaves*	*C. Serrata Bark*
5×10^{-1}	96.09	96.10	97.86	95.10	92.10
5×10^{-2}	53.08	53.08	97.70	73.56	89.74
5×10^{-3}	43.43	41.82	72.75	67.80	74.52
5×10^{-4}	42.20	41.25	67.26	66.26	70.65
5×10^{-5}	41.30	40.86	63.73	66.11	66.54
5×10^{-6}	41.66	38.48	62.53	65.70	65.54
5×10^{-7}	41.10	38.24	62.34	63.10	63.44
5×10^{-8}	40.10	37.10	62.30	63.11	62.68
5×10^{-9}	40.53	36.76	62.11	62.50	62.25
5×10^{-10}	38.20	33.42	61.87	61.87	61.82

REFERENCES

Alves TMA, Silva AF, Brandao M, Grandi TSM, Smania EFA, Smania JA and Zani CL (2000). Biological screening of Brazilian medicinal plants. *Mem. Inst. Oswaldo Cruz*, **95**: 367-373.

Atta-ur-Rahman, Choudhary MI and William JT (2001). Bioassay Techniques for Drug Development. Harvard Academic Publisher, Amsterdam, Netherland pp. 67-68.

Awan MR, Iqbal Z, Shah SM, Jamal Z, Jan G, Afzal M, Majidand A and Gul A (2011). Studies on traditional knowledge of economically important plants of Kaghan Valley, Mansehra District, Pakistan. *J. Med. Plants Res.*, **5**: 3958-3967.

Choudhary MI, Adhikari A, Rasheed S, Marasini BP, Hussain N, Ahmad W, Kaleem and Atta-ur-Rahman (2011). Cyclopeptide alkaloids of *Ziziphus oxyphylla* Edgew as novel inhibitors of α-glucosidase enzyme and protein glycation. *Phytochem. Letters*, **4**: 404-406.

Clarke EGC and Todd RG (1955). Isolation and Identification of drugs in pharmaceuticals, body fluids and post-mortem material. The Pharmaceutical Press, Londres, Inglaterra, pp.123-134.

Collins PJ (1998). Resistance to grain protectants and fumigants in insect pests of stored products in Australia. *In*: Banks HJ, Wright EJ and Dameevski KA (Eds.). CSIRO stored grain research laboratory, presented in Australian Post Harvest Technical Conference, pp.55-57.

Emeruwa AC (1982). Anti-bacterial substances from *Carica papaya* fruit extract. *J. Nat. Products*, **45**: 123-127.

European Pharmacopoeia (2011). Supplement 7.3-7.5. 7th Edition. European Pharmacopoeia Commission, Council of Europe European Directorate for the Quality of Medicines. Council of Europe.

Evans WC (2005). Trease and Evan's Pharmacognosy, 15th Edition, Elsevier, New Delhi, p.193.

Harborne JB (1973). Phytochemicals Methods. Chapman and Hall Ltd., London, pp.49-188.

Isman MB, Proksch P and Yan J (1987). Insecticidal chromenes from the Asteraceae: structure-activity relations. *Entomol. Exp. Appl.*, **43**: 87-93.

Iyengar MA (1995). Study of Crude Drugs. 8th ed., Manipal Power Press, Manipal, India, p.2.

Jan G, Khan MA, Khan A, Jan FG, Khan R, Ahmad M, Atta-Ur-Rehman, Danish M, Asif M, Khan M, Zafar M and Jan M (2011). An ethnobotanical survey on fuel wood and timber plant species of Kaghan Valley, Khyber Pakhtoonkhwa Province, Pakistan. *African J. Biotechnol.*, **10**: 19075-19083.

Khan MA, Khan MA and Hussain M (2012). Medicinal plants used in folk recipes by the inhabitants of Himalayan region Poonch Valley Azad Kashmir (Pakistan). *J. Basic Applied Sci.*, **8**: 35-45.

Kokate CK, Purohit AP and Gokhale SB (2005). Pharmacognosy, 33rd Edition, Nirali Prakashan, Pune, pp 108-109.

Meyer NB, Ferrigni RN, Putnam EJ, Jacobsen BL, Nichols ED and McLaughlin IJ (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *J. Planta Medica.*, **45**: 31-35.

Mojab F, Kamalinejad M, Ghaderi N and Vahidipour H (2003). Phytochemical screening of some Iranian plants. *Iranian J. Pharm. Res.*, **2**: 77-82.

Mukherjee PK (2002). Quality Control of Herbal Drugs, 1st edition. Published by Business Horizons, p.303.

Perveen F, Zaib S, Irshad S and Hassan M (2012). Anti-oxidant and DNA protection activities of the hill toon, *Cedrela serrata* (Royle) leaves extract and its fractions. *J. Natural Products*, **5**: 207-213.

Pfoze NL, Kumar Y and Myrboh B (2012). Survey and assessment of ethno medicinal plants used in Senapati District of Manipur State, Northeast India. *Phytopharmacology*, **2**: 285-311.

Sarker SD, Eynon E and Fok K (2003). Screening the extracts of the seeds of *Achillea millefolium, Angelica sylvestris and Phleum pratense* for antibacterial, antioxidant activities and general toxicity. *Orient. Pharm. Exp. Med.*, **33**: 157-162.

Shah SR, Hasan G, Rehman A and Ahmed I (2006). Ethnobotanical studies of the flora of district Musakhel and Barkhan in Balochistan, Pakistan. *Pak. J. Weed Sci.*, **12**: 199-211.

Sher H (2011). Ethnoecological evaluation of some medicinal and aromatic plants of Kot Malakand Agency. *Pakistan Sci. Res. Essays*, **6**: 2164-2173.

Sofowra A (1993). Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp.191-289.

Takahashi M, Fuchino H, Satake M, Agatsuma Y and Sekita S (2004). *In vitro* screening of Leishmanicidal activity in Myanmar timber extracts. *Biol. Pharm. Bull.*, **27**: 921-925.

Takao T, Watanabe N, Yagi I and Sakata K (1994). A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. *Biosci. Biotechnol. Biochem.*, **58**: 1780-1783.

Trease and Evans WC (1996). Pharmacognosy, 11th Edition, Braillar Tiriden Company. MacMillan Publishers, pp.56-109.