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Original article

SECONDARY METABOLITES AS WELL AS ANTIOXIDANT AND β-GLUCOSIDASE INHIBITORY POTENTIAL OF *Hopea Scaphula* ROXB.

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Abstract

*Four compounds were isolated from the petroleum ether and ethyl acetate extracts of the stem bark of Hopea scaphula Roxb. The structures of the isolated compounds were elucidated as stigmasterol (**1**), 1,2-dimethoxy-4-allylbenzene (**2**), 3,4-dimethoxycinnamaldehyde (**3**) and β-amyrin (**4**) by extensive spectroscopic studies. The isolated compounds and crude extracts were subjected to antioxidant screening through free radical scavenging activity by DPPH (1, 1-diphenyl-2-picrylhydrazyl), where compounds **1** and **2** showed substantial antioxidant activity with IC_{50} value 18.0 and 63.0 µg/ml, respectively. In case of β-glucosidase inhibition assay, the ethyl acetate extract revealed strong inhibitory activity (94.18%) while the petroleum ether extract showed 79.11% enzyme inhibition. This is the first report of antioxidant and β-glucosidase inhibitory activities of H. scaphula* Roxb.

Key words: *Hopea scaphula* Roxb., *Dipterocarpaceae, Stigmasterol, 1,2-dimethoxy-4-allylbenzene, 3,4-dimethoxycinnamaldehyde, β-amyrin, Antioxidant, β-glucosidase inhibition*

Hopea scaphula Roxb'un Sekonder Metabolitlerinin Yanısıra Antioksidan ve β-glukosidaz Inhibisyonu Potensiyeli

*Hopea scaphula Roxb kabuğunun etil asetat ve petrol eteri ekstraktlarından 4 komponent izole edilmiştir. İzole edilen bu komponentlerin yapıları spektroskopik çalışmalar vasıtası aydınlatılmış ve stigmasterol (**1**), 1,2-dimethoxy-4-alkylbenzene (**2**), 3,4- dimethoxycinnamaldehyde (**3**) ve β-amyrin (**4**) oldukları anlaşılmıştır. İzole edilen komponentler ve ham ekstraktların DPPH (1,1--diphenyl-2-picrylhydrazyl) ile serbest radikal yakalama aktivitesi yoluyla antioksidan etkileri taranmıştır. Bu tarama sonunda 1 ve 2'ci komponentler önemli antioksidan aktivite göstermiştir. Buna gore elde edilen IC_{50} değerleri sırasıyla 18.0 ve 63.0 µg/ml olarak tespit edilmiştir. β-Glukosidaz inhibisyon testi sonuçlarına gore ise, etil asetat ekstresi güçlü bir inhibisyon aktivitesi (% 94.18) gösterirken petrol eteri ekstraktı %71.1 oranında enzim inhibisyonu göstermiştir. Bu çalışma, H. scaphula Roxb'un antioksidan ve β-glukosidaz inhibisyonu etkisi olduğunu gösteren ilk çalışmadır.*

Anahtar kelimeler: *Hopea scaphula Roxb., Dipterocarpaceae, Stigmasterol, 1,2-dimethoxy-4-allylbenzene, 3,4-dimethoxycinnamaldehyde, β-amyrin, Antioksidan, β-glucosidase inhibisyonu*

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INTRODUCTION

Plants are the reservoir of enormous secondary metabolites, which made it possible to remain as essential element for healing arts and to provide diversified lead compounds for the drug development. In recent times, natural products are applied extensively to combat free radical mediated disorders like cancer, aging, neurodegenerative disease, arteriosclerosis and other pathological events (1,2). As a result, a number of plant-derived antioxidants have come into light (3-10). Besides, medicinal plants and herbs are also screened to search the β-glucosidase inhibitor, which are useful as antiviral, antibacterial, antimetastatic or immunostimulatory agents (11-12). On our way to continuous exploration of natural resources of Bangladesh, we investigated the plant *Hopea scaphula* Roxb. for the present study.

H. scaphula Roxb. (Syn: *Anisoptera scaphula, Vatica scaphula* and *Scaphula glabra*; Bengali name-Boilsur, Boilam or Sada Boilam; Family- Dipterocarpaceae) is a tall tree that grows in Chittagong and Chittagong Hill-Tracts of Bangladesh (13). The plant is reported to have significant antimicrobial activity and cytotoxicity (14). Various species of this genus are also reported to have anititumor (15), anti-HIV (16), astringent, CNS depressant, hypotensive and antifungal (17) properties. Previous phytochemical investigations of *Hopea* species led to the isolation of hopeanolin (17), (-)-hopeaphenol (18), balanocaprol, dibalanocaprol (16), cycloartane triterpenoid (19), hopeaphenol A, isohopeaphenol A (20) and vaticanol C (15). We, herein, report the isolation of stigmasterol (**1**), 1,2-dimethoxy-4-allylbenzene (**2**), 3,4-dimethoxycinnamaldehyde (**3**) and β-amyrin (**4**) as well as antioxidant and β-glucosidase inhibitory potential of *H. scaphula* Roxb. for the first time.

EXPERIMENTAL

General experimental procedures

The ^{1}H NMR spectra were recorded using a Bruker AMX-400 (400 MHz) instrument. For NMR studies deuterated chloroform was used and the NMR instrument was locked at the solvent peak and the δ values for ^{1}H are reported relative to the residual nondeuterated solvent signal. Column chromatography (CC) was carried on Merck Si gel 60 H. TLC and PTLC were conducted on glass plate coated with Merck Si gel 60 PF_{254} at a thickness of 0.5 mm. Spots on TLC and PTLC plates were visualized under UV light (254 and 366 nm) and by spraying with 1% vanillin in sulfuric acid, followed by heating.

Plant Material

Stem bark of *Hopea scaphula* Roxb. was collected from Dhaka, Bangladesh in the month of August 2006. A voucher specimen (DACB, AN-32064) has been deposited in Bangladesh National Herbarium, Dhaka for this collection.

Extraction and Isolation

The air-dried and powdered plant material (200.5 g) was extracted with petroleum ether followed by ethyl acetate at room temperature with occasional shaking and stirring for 7 days for each successive extraction. It was then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The filtrates were then evaporated using a Buchii rotavapor at low temperature and pressure to afford petroleum ether (1.7 g) and ethyl acetate (1.5 g) extracts.

The petroleum ether extract was subjected to column chromatography over silica gel (Kiesel gel 60H, mesh 70-230) and the column was eluted with petroleum ether followed by mixtures of petroleum ether and ethyl acetate in order of increasing polarities. Compound **1** was isolated as colorless needles from the column fraction of the petroleum ether fraction eluted with 30% ethyl acetate in petroleum ether.

1.5 g of ethyl acetate extract was subjected to gel permeation chromatography over Sephadex LH-20 (Lipophilic) and the column was eluted with hexane-dichloromethane-methanol (2:5:1) mixture (300 ml) to give a total of 30 fractions (each 10 ml). Fractions 11 and 12 provided compounds **2**, **3** and **4** in pure forms upon rechromatographed over silica gel using 12% ethyl acetate in toluene, as the developing solvents.

Compounds isolated

Stigmasterol (**1**) (5 mg, 0.003% yield): White needles; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.34 (1H, br. s, H-6), 5.14 (1H, dd, *J*=15.0, 6.5 Hz H-22), 5.04 (1H, dd, *J* = 15.0, 9.0 Hz, H-23), 3.51 (1H, m, H-3), 1.00 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.0 Hz, Me-21), 0.84 (3H, d, *J* = 6.0 Hz, Me-26), 0.82 (3H, t, *J* = 6.5 Hz, Me-29), 0.80 (3H, d, *J* = 6.0 Hz, Me-27), 0.67 (3H, s, Me-18).

1,2-Dimethoxy-4-allylbenzene (**2**) (4 mg, 0.002% yield): Pale yellow oily liquid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.78 (1H, d, *J*=8.0 Hz, H-6), 6.72 (1H, br. d, *J*=8.0 Hz, H-5), 6.70 (1H, br. s, H-3), 5.94 (1H, m, H-8), 5.06 (1H, s, H_a-9), 5.04 (1H, m, H_b-9), 3.86 (3H, s, OMe-2), 3.85 (3H, s, OMe-1), 3.32 (2H, d, *J*=6.5 Hz, H_2-7).

3,4-Dimethoxycinnamaldehyde (**3**) (4 mg, 0.002% yield): White amorphous powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.80 (1H, s, CHO-9), 7.41 (1H, d, *J* = 16.0 Hz, H-7), 7.15 (1H, dd, *J* = 8.0, 1.5 Hz, H-6), 7.00 (1H, br.s, H-2), 6.89 (1H, d, *J* = 8.0 Hz, H-5), 6.60 (1H, dd, *J* = 16.0 Hz, H-8), 3.93 (3H, s, OMe-3), 3.92 (3H, s, OMe-4).

β-Amyrin (**4**) (4 mg, 0.002% yield): Colorless powder; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.24 (1H, m, H-12), 3.21 (1H, dd, *J*=10.5, 4.7 Hz H-3), 1.07 (3H, s, H_3-27), 0.98 (3H, s, H_3-25), 0.98 (3H, s, H_3-26), 0.93 (3H, s, H_3-23), 0.92 (3H, s, H_3-24), 0.88 (3H, s, H_3-29), 0.88 (3H, s, H_3-30), 0.84 (3H, s, H_3-28).

Antioxidant Activity

The antioxidant activity (free radical scavenging activity) of the purified compounds on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method developed by Brand-Williams *et al.*, 1995 (20). In the experiment, 2 mg of each of the compounds **1-4** and extracts were dissolved in methanol. Solution of varying concentrations such as 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.8125 µg/ml, 3.91 µg/ml, 1.95 µg/ml and 0.98 µg/ml were obtained by serial dilution technique. 2 ml of a methanol solution of the extract of each concentration was mixed with 3 ml of a DPPH-methanol solution (20 µg/ml) and was allowed to stand for 20 minutes for the reaction to occur. Then the absorbance was determined at 517 nm and from these values the corresponding percentage of inhibitions were calculated by using the following equation: % inhibition = [1- (ABS_{sample} / $ABS_{control}$)] x 100.

Then % inhibitions were plotted against respective concentrations used and from the graph the IC_{50} was calculated. Here, butylated hydroxytoluene (BHT), a potential antioxidant, was used as positive control.

β-glucosidase inhibitory activity

β-glucosidase inhibitory activity was measured sepctrophotometrically using *p*-nitrophenyl-*β*-D-glucopyranoside as substrate as reported earlier (21,22). Due to the scarcity of the purified compounds, we evaluate the activity of extracts only. Here, 0.4 ml of substrate (*p*-nitrophenyl-*β*-D-glucopyranoside, 2 mg/ml), 1 mg of extract, 0.4 ml of pH 5 phosphate buffer (0.1M potassium hydrogen phthalate - NaOH) were placed in a tube and incubated at 37°C for 10 min. Then, 0.2 ml of enzyme (*β*-glucosidase, 20 mg/ml) solution was added and the mixture was incubated for another 30 minutes at 37°C. After this time, the reaction was terminated by adding 2.6 ml of pH 10 buffer (2M glycine–NaOH). In the positive control, a mixture of solvents was added instead of the extract, while in the negative control, pH 10 buffer was added at the beginning of the test in order to block enzyme activity. Absorbance of the test samples was measured at 410 nm and the activity was calculated using the following formula:

% Enzymatic inhibition = 100 - [(ABS_{sample} – $ABS_{negative\ control}$) / $ABS_{positive\ control}$] x 100

Statistics

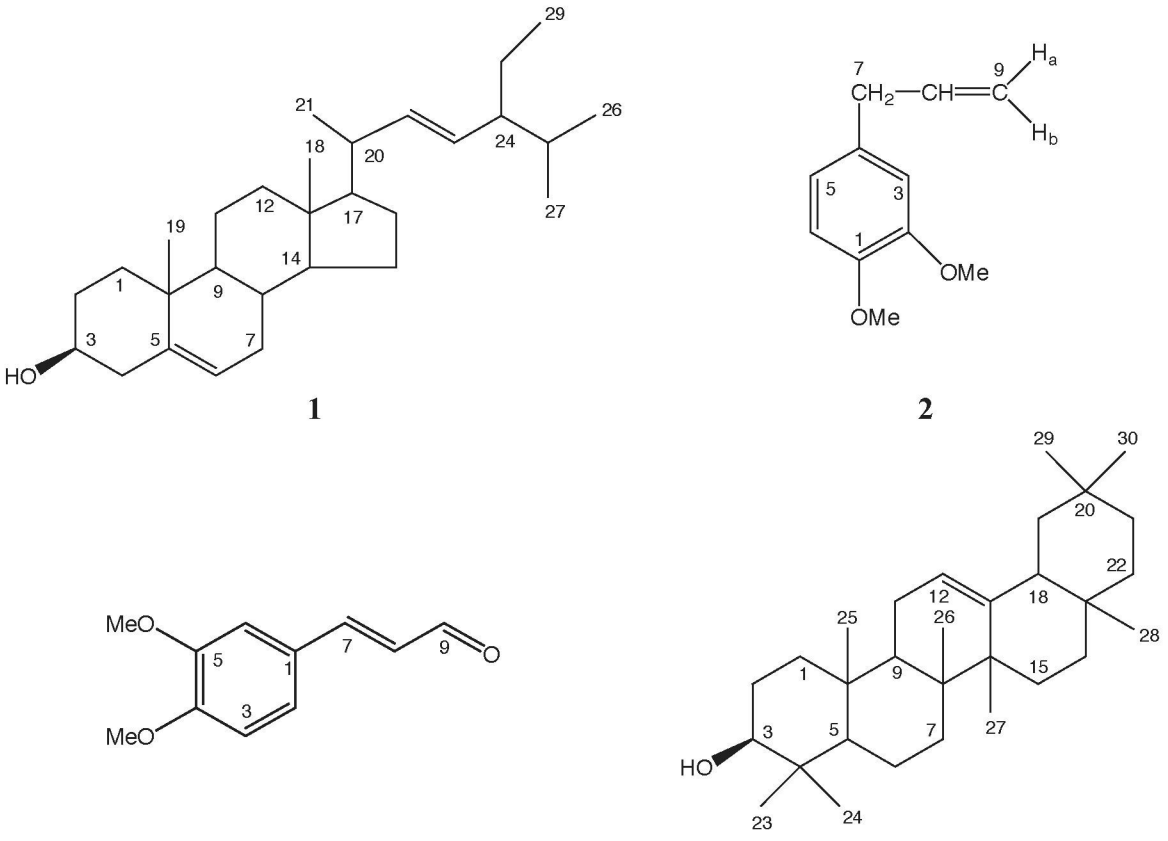
All the assays were carried out in triplicate and the results were expressed as mean ± SD.

RESULTS AND DISCUSSION

A total of four compounds were isolated (Figure 1) from petroleum ether and ethyl acetate soluble extracts of stem bark of *H. scaphula* by repeated chromatographic separation and purification over silica gel. The structures of the isolated compounds were solved by extensive NMR data analyses, comparison of their spectral data with literature and by co-TLC with authentic sample.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of the compound **1** revealed a one-proton multiplet at δ 3.51, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. Analysis of the remaining signals and by comparison of the spectrum data with published values allowed us to characterize compound **1** as stigmasterol (23).

The ^{1}H NMR spectrum of compound **2** exhibited a doublet centered at δ 3.32 (J = 6.5 Hz), a one proton multiplet at δ 5.94 and two multiplets at δ 5.04 and δ 5.06. These signals were characteristic of an allylic side chain attached to a benzene ring. The aromatic protons at C-3, C-5 and C-6 in the ring were assigned by the presence of ^{1}H NMR signals at δ 6.70 (1H br. s), 6.72 (br. d, J = 8.0 Hz) and 6.78 (1H, d, J = 8.0 Hz). Two singlets (at δ 3.85 and δ 3.86) indicated the presence of two methoxy groups. From this spectral data compound **2** was indicated as 1, 2-dimethoxy-4-allylbenzene, which is a methyl derivative of eugenol (24). This is the first time isolation of this compound from this genus.



1 **2**

3 **4**

Figure-1. Investigated compounds

The ^{1}H NMR spectrum of compound **3** exhibited a doublet centered at δ 6.89 (1H, *J* = 8.0 Hz) and a double doublet δ 7.15 (1H, *J* = 8.0, 1.5 Hz) and a broad singlet was found at δ 7.0 (1H). All of these spectral features demonstrated the presence of 1, 3, 4-trisubstituted benzene ring. It also revealed two singlets at δ 3.92 and δ 3.93, which could be assigned to the methyl groups. A downfield double doublet centered at δ 6.60 (*J* = 16.0, 8.0 Hz) and another doublet δ 7.41 (*J* = 16.0 Hz) suggested the presence of two *trans* coupled olefinic protons at H-7 and H-8, respectively. The ^{1}H NMR spectrum also exhibited a singlet of one proton intensity at δ 9.80, appropriate for an aldehydic proton. The spectral features are in close agreement to those observed for 3,4-dimethoxy cinnamaldehyde (25). On this basis, compound **3** was characterized as 3, 4-dimethoxycinnamaldehyde.

The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of compound **4** revealed eight three proton singlets at δ 0.84 (H_3-28), 0.88 (H_3-29, H_3-30), 0.92 (H_3-24), 0.93 (H_3-23), 0.98 (H_3-25, H_3-26) and 1.07 (H_3-27), which suggested the presence of eight methyl groups in the molecule. It also revealed a broad singlet centered at δ 5.24, which could be assigned for an olefinic proton (H-12). The double doublet (J = 10.5, 4.7 Hz) centered at δ 3.21 can be ascribed to the oxymethine proton at C-3. The above spectral features are in close agreement to those observed for β-amyrin (26). Thus the identity of compound **4** was confirmed as β-amyrin.

The isolated compounds and extracts of the plant were assessed for free radical scavenging activity and β-glucosidase inhibitory effect and results are presented in table-1 and 2, respectively. The antioxidants act either by scavenging various types of free radicals derived from oxidative processes, by preventing free radical formation through reduction precursors or by chelating metals (27-29). The reduction of DPPH assay has been used to detect products with antioxidant activity as free radical scavengers (30-31). In this study, compound **3** showed the highest antioxidant activity with IC_{50} value of 18.0 µg/ml. At the same time, compound **2** exhibited moderate antioxidant activity (IC_{50}=63.0 µg/ml), whereas compounds **1** and **4** displayed no free radical scavenging activity. However, the ethyl acetate and petroleum ether extract showed the IC_{50} = 77.19 and 103.58 µg/ml, respectively.

Table 1. Antioxidant activity of the extracts and compounds **1-4** of *H. scaphula.*

Samples	IC_{50} (µg/ml)*
BHT	19.0 ± 0.33
Petroleum ether extract	103.58 ± 1.71
Ethyl acetate extract	77.19 ± 1.33
Stigmasterol (**1**)	--
1, 2-Dimethoxy-4-allylbenzene (**2**)	63.0 ± 1.11
3, 4-Dimethoxycinnamaldehyde (**3**)	18.0 ± 0.98
β-Amyrin (**4**)	--

*The values of IC_{50} are expressed as mean ± SD (n=3); BHT: Butylated hydroxytoluene (Standard compound)

The enzyme β-glucosidase is responsible for many glycoprotein processing related disorders and so the inhibition of this enzyme is regarded as a preliminary tool for screening antiviral, antibacterial, antimetastatic or immunostimulatory agents (9-10). It has been reported that in screening plant crude extracts for β-glucosidase inhibitory activity, an inhibitory activity 50% can be considered significant (27). In this study, ethyl acetate extract was very potent showing 94.18% β-glucosidase inhibition, where as petroleum ether extract showed 79.11% inhibition.

Table 2: β-glucosidase inhibitory activity of the extracts of *H. scaphula*.

Test samples	% enzyme inhibition*
Petroleum ether extract	79.11 ± 1.11
Ethyl acetate extract	94.18 ± 1.30

*The values of IC_{50} are expressed as mean ± SD (n=3)

CONCLUSION

Our present study demonstrated a secondary metabolite profile of *H. scaphula* bark. Besides, the potentiality of *H. scaphula* for counteracting many pathological events, at least as antioxidant and β-glucosidase inhibitor was also revealed. To the best of our knowledge, this is the first report of antioxidant and β-glucosidase inhibitory potential. Further comprehensive investigations are required to obtain more bioactive principles from *H. scaphula* and to elucidate their mechanism of action.

REFERENCES

1. Erdemoglu N, Turan NN, Cakıcı I, Sener B, Aydın A, Antioxidant Activities of Some Lamiaceae Plant Extracts, Phytother Res 20, 9–13, 2006.
2. Gutteridge JMC, Biological origin of free radicals and mechanisms of antioxidant protection, Chem Biol Interact 91, 133–140, 1994.
3. Steinmetz KA, Potter JD, Vegetables, fruit, and cancer prevention, A review, J Am Diet Assoc 96 1027–1039, 1996.
4. Wang H, Nair MG, Strasburg GM, Chang Y, Booren AM, Gray JI, DeWitt DL, Antioxidant and antiinflamatory activities of anthocyanins and their aglycon, cyanidin, from tart cherries, J Nat Prod 62, 294-296, 1999.
5. Bandoniene D, Pukalskas A, Venskutonis PR, Gruzdiene D, Preliminary screening of antioxidant activity of some plant extracts in rapeseed oil, Food Res Int 33, 785–791, 2000.
6. Aruoma OI, Free radicals, oxidative stress, and antioxidants in human health and disease, J Am Oil Chem Soc 75, 199–212, 1998.
7. Pieroni A, Janiak V, Dürr CM, Lüdeke S, Trachsel E, Heincrich M, In vitro antioxidant activity of non-cultivated vegetables of ethnic Albanians in Southern Italy, Phytother. Res 16, 467–473, 2002.
8. Couladis M, Tzakou O, Verykokidou E, Harvala C, Screening of some Greek aromatic plants for antioxidant activity, Phytother Res 17, 194–195, 2003.
9. Shahidi F, Janitha PK, Wanasundara PD, Phenolic antioxidants, Crit Rev Food Sci Nutr 32, 67–103, 1992.
10. Pietta P, Sionetti P, Mauri P, Antioxidant activity of selected medicinal plants, J Agric Food Chem 46, 4487–4490, 1998.
11. Montefiori DC, Robinson WEJ, Mitchell WM, Role of protein N-glycosilation in pathogenesis of human immunodeficiency virus type 1, Proc Nat Acad Sci USA 85, 9248–9252, 1984.
12. Wong C, Provencher L, Porco JAJ, Jung S, Wang Y, Chen L, Wang R, Steensma DH, Synthesis and evaluation of homoazasugar as glycosidase inhibitors, J Org Chem 60, 1592–1501, 1995.
13. Khan MS, In: Flora of Bangladesh, Dipterocarpaceae, (Salar, K.M., Eds.), Bangladesh National Herbarium 25, pp. 2-3, 1984.
14. Gazi HR, Rahman MS, Begum B, Rashid MA, Antimicrobial and cytotoxic activities of the crude extracts of Hopea scaphula, Dhaka Univ J Pharm Sci 6, 131-133, 2007.

15. Ito T, Akao Y, Yi H, Ohguchi K, Matsumoto M, Tanaka T, Iinuma M, Nozawa Y, Antitumor effect of resveratrol oligomers against human cancer cell lines and the molecular mechanism of apoptosis induced by vaticanol C, Carcinogenesis 24, 1489-1497, 2003.
16. Dai JR, Hallock YF, Cardellina JH, Boyd MR, HIV-inhibitory and cytotoxic oligostilbenes from the leaves of Hopea malibato. J Nat Prod 61, 351-353, 1998.
17. Ge HM, Huang B, Tan SH, Shi DH, Song YC, Tan RX, Bioactive oligostilbenoids from the stembark of Hopea exalata, J Nat Prod 69, 1800-1802, 2006.
18. Sahidin Hakim EH, Juliawatym LD, Syah YM, Din L, Ghisalberti EL, Latip J, Said IM, Achmad SA, Cytotoxic properties of oligostilbenoids from the tree barks of Hopea dryobalanoides, Z Naturforsch 60, 723-727, 2005.
19. Zhang HJ, Tan GT, Hoang VD, Hung NV, Cuong NM, Soejarto DD, Pezzuto JM, Fong HH, Natural anti-HIV agents part IV. Anti-HIV constituents from Vatica cinerea, J Nat Prod 66, 263-268, 2003.
20. Brand-Williams W, Cuvelier ME, Berset C, Use of a free radical method to evaluate antioxidant activity, Lebensm Wiss Technol 28, 25-30, 1995.
21. Sánchez-Medina A, García-Sosal K, May-Pat F, Peña-Rodríguez LM, Evaluation of biological activity of crude extracts from plants used in Yucatecan Traditional Medicine Part I, Antioxidant, antimicrobial and β-glucosidase inhibition activities, Phytomedicine 8, 144–151, 2001.
22. Rahman MZ, Rahman MS, Kaisar A, Hossain A, Rashid MA, Bioactive isoflavones from Erythrina variegata L, Turk J Pharm Sci 7, 21-28, 2010.
23. Ikan RI, Natural Product: A Laboratory Guide (2nd edition), Academic Press, N.Y., USA, 1991.
24. Sarker SD, Armstrong JA, Waterman PG, (-)-1,12-oxaguai-10(15)-ene: a sesquiterpene from Eriostemon fitzgeraldii, Phytochemistry 40, 1159-1162, 1995.
25. Koeduka T, Fridman F, Gang DR, Vassão DG, Jackson BL, Kish CM, Orlova I, Spassova SM, Lewis NG, Noel JP, Baiga TJ, Dudareva N, Pichersky E, From the Cover: Eugenol and isoeugenol, characteristic aromatic constituents of spices, are biosynthesized via reduction of a coniferyl alcohol ester, Proc Natl Acad Sci 103, 10128-10133, 2006.
26. Zou JH, Dai J, Chen X, Yuan JQ, Pentacyclic triterpenoids from leaves of Excoecaria agallocha, Chem Pharm Bull 54, 920-921, 2006.
27. Antoun MD, Ríos YR, Mendoza NT, Proctor G, Glucosidase inihibiton assay as prescreen for natural products, Puerto Rico Health Sci J 13, 13–15, 1994.
28. Burton GW, Ingold KU, β-Carotene: An unusual type of lipid antioxidant, Science 224, 569–573, 1984.
29. Bors W, Michael C, Saran M, Inhibition of the bleaching of the carotenoid crocin. A rapid test for quantifying antioxidant activity, Biochim Biophys Acta 796, 312–319, 1984.
30. Cavin A, Hostettmann K, Dyatmyko W, Potterat O, Antioxidant and lipophilic contituents of Tinospora crispa, Planta Med 64, 393–396, 1998.
31. Gamez EJC, Luyengi L, Lee S, Zhu L, Zhou B, Fong H, Pezzuto J, Kinghorn AD, Antioxidant flavonoid glycosides from Daphniphyllum calcynum, J Nat Prod 61, 706–708, 1998.

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