International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 11, p. 74-80, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Chemical investigations of stem bark's extracts of *Aegle mermelos*: (An initiative exploration pro medicinally important plant)

Mohammad Mamunur Rahman[1], Md. Raihan Chowdhury[2*], Partha Saha[2], Mahedy Hasan Chowdhury[3], S. M. Estiar Haque[2], Md. Saddam Nawaz[2]

[1]*Department of Applied Chemistry and Chemical Engineering, University of Dhaka, Bangladesh*

[2]*ACI Pharmaceuticals Limited, Narayanganj-1400, Bangladesh*

[3]*Sunbeams School, Plot# 6-12, Road#13/A, Sector#10, Uttara, Dhaka, Bangladesh*

Received: 24 october 2012
Revised: 04 November 2012
Accepted: 05 November 2012

Key words: *Aegle marmelos,* Stem bark, Lupeol, stigmasterol, 5-Methoxy psoralen, nuclear magnetic resonance.

Abstract

This study describes the chemical investigations of *Aegle marmelos (A.marmelos)*, a plant belonging to the family rutaceae. Although some investigations mainly on leaves and fruits have previously been reported with this plant, but very little investigations on bark of *A.marmelos* have been reported to date. The stem bark of *A. marmelos* was extracted with organic solvent and the extracts were fractionated by using standard chromatographic techniques. A total of three compounds were isolated of which two (AM-1 & AM-5) have been isolated from different plant, but this is the first report of their occurrences in the bark of *A. marmelos*. The isolated pure compounds were identified by extensive analyses of their high resolution Nuclear Magnetic Resonance (^{1}H-NMR) spectral data. The powdered stem bark of *A. marmelos* was extracted with methanol. Chromatographic (vacuum liquid chromatography) fractionation and purification of the crude methanolic extract yielded compound (AM-1), one coumarin (AM-5), and one sterol (AM-2). The purified compound (AM-1) was identified as Lupeol, compound (AM-5) was identified as 5-Methoxy psoralen and compound (AM-2) was identified as stigmasterol or 24-ethyl-cholesta-5- 22-dien-3beta-ol.

*Corresponding Author: Md. Raihan Chowdhury ✉ mcraihan@gmail.com

Introduction

Herbs are nutritional foundation nutrients and good alternative medicines to nourish the body's deepest and most basic elements. It has been accepted that herbal medicines are main stream in modern civilization. Developing a phytomedicine is definitely a way of benefit of many more years for the humanity with a look to the future with a great deal of anticipation. In developing countries, about 80% of the world population relies on traditional medicine for their primary health care (Suleyman *et al.,* 2009). Herbal medicine is a common element in ayurvedic, homeopathic, naturopathic, traditional, oriental, native American & Indian medicine (Philomena *et al.,* 2011). According to WHO, from 119 plant-derived pharmaceutical medicines, about 74% are used in modern medicine (Mishra SB *et al.,* 2010). Chemical investigations of the stem bark of *A. marmelos* was conducted in this study because of significant traditional medicinal uses in treatments, such as for intermittent fever, intestinal ailments, fertility control, and treatment after childbirth and fish poison (Basu *et al.,* 1974).The tree *A. marmelos* has been used for millennia in the Indian subcontinent and Indochina as a traditional medicinal herb. Historic mention of Bael fruit (*A. marmelos*) has been traced to Vedic times (2000-800 BC) (Kumar DS *et al.,* 1987). A large number (more than 110) of individual chemical constituents have been identified in various parts of *A. marmelos* (Badam *et al.,* 2002, Takase *et al.,* 1974, Jagetia GC *et al.,* 2005, Misra *et al.,* 1991, & Goel RK *et al.,* 1997). Skimmianine, aegelin, lupeol, cineole, citral, citronellal, cuminaldehyde, eugenol and marmesinin have been purified from bael leaves (Rastogi RP *et al.,* 1993, Rastogi RP *et al.,* 1995, Geetha *et al.,* 2001, Jagetia GC *et al.,* 2003, Katayama *et al.,* 1960 & Parasakthy *et al.,* 1993). In various experimental animal models, skimmianine has shown sedative, hypnotic, analgesic, anticonvulsive, antipyretic, hypothermic and antidiuretic effects (Rastogi RP *et al.,*1995), aegelin & lupeol are potent cardio active compound (Arul *et al.* 1999 & Vimal *et al.,* 2004), cineole exhibits antioxidant property (Jagetia GC *et al.,*2003) and so on.

The potential benefits of herbal medicines could lie in their high acceptance by patients, efficacy, relative safety, and relatively of low costs. Thus documentation of indigenous knowledge on the use of plants and providing an inventory of useful plants from local flora can be a great help for accurate use of traditional medicines. Identification and isolation of the active constituents from traditionally used phyto-therapy can ensure the health care. Thus the rationality of the ongoing study lies in meeting the challenge of developing herbal medicines for our own survival, which needs a systematic research on indigenous medicinal plants with scientific approach. And also to strengthen the existing health care system and chemical analyses of an indigenous plant *A. marmelos* (Family: Rutaceae) is the primary objective of the present study.

Methods and materials

Chemicals used

The chemicals and reagents used for the purpose were obtained from E Merck (Germany) and BDH (England). All the chemicals and reagents used were of analytical grade.

Collection and preparation of plant material

The stem bark of the plant *A. marmelos* was collected from healthy trees (from Dhaka University Campus) and was collected in fresh condition. It was sun-dried and then dried in an oven at reduced temperature (not more than 50 ^{o}C) to make it suitable for grinding purpose. The coarse powder was then stored in airtight container with marking for identification and kept in cool, dark and dry place for future use.

Extraction of the plant material

About 700 g of the powdered material was taken in a clean, round bottomed flask (5 liters) and soaked in 2.5 liter of methanol. The container with its content was sealed by foil and kept for a period of 15 days accompanying occasional shaking and stirring.

Table 1. Different solvent systems used for VLC analysis of crude extract.

Fraction no	Solvent systems	Volume collected (ml)	Fraction no	Solvent systems	Volume collected (ml)
1	*n*-hexane 100%	100	13	*n*-hexane + ethyl acetate (55 : 45)	100
2	*n*-hexane + ethyl acetate (95 : 5)	100	14	*n*-hexane + ethyl acetate (50 : 50)	100
3	*n*-hexane + ethyl acetate (92.5 : 7.5)	100	15	*n*-hexane + ethyl acetate (30 : 70)	100
4	*n*-hexane + ethyl acetate (90 : 10)	100	16	*n*-hexane + ethyl acetate (20 : 80)	100
5	*n*-hexane + ethyl acetate (88.5 : 12.5)	100	17	ethyl acetate 100 %	100
6	*n*-hexane + ethyl acetate (85 : 15)	100	18	ethyl acetate + methanol (97 : 3)	100
7	*n*-hexane + ethyl acetate (82.5 : 17.5)	100	19	ethyl acetate + methanol (95 : 5)	100
8	*n*-hexane + ethyl acetate (80 : 20)	100	20	ethyl acetate + methanol (92 : 8)	100
9	*n*-hexane + ethyl acetate (75 : 25)	100	21	ethyl acetate + methanol (90 : 10)	100
10	*n*-hexane + ethyl acetate (70 : 30)	100	22	ethyl acetate + methanol (85 : 15)	100
11	*n*-hexane + ethyl acetate (65 : 35)	100	23	ethyl acetate + methanol (70 : 30)	100
12	*n*-hexane + ethyl acetate (60 : 40)	100	24	ethyl acetate + methanol (50 : 50)	100
			25	Methanol 100%	100

Table 2. Comparison between ^{1}H NMR spectral data of AM-1 and Lupeol (400 MHz in $CDCl_3$).

Positions	δ_H in ppm in $CDCl_3$		Positions	δ_H in ppm in $CDCl_3$	
	AM-1	Lupeol		AM-1	Lupeol
1	1.64	1.65	18	1.38	1.37
2	1.59	1.59	19	2.34	2.38
3	3.18	3.2	21	1.38	1.37
5	0.75	0.68	22	1.38	1.37
6	1.38	1.4	23	0.96	0.97
7	1.35	1.32	24	0.75	0.76
9	1.32	1.29	25	0.82	0.83
11	1.24	1.2	26	1.02	1.03
12	1.02	1.07	27	0.93	0.94
13	1.67	1.68	28	0.78	0.79
15	1.02	1	29	4.55,4.67	4.54,4.67
16	1.38	1.37	30	1.67	1.68

The whole mixture was then filtered through filter paper and the filtrate was concentrated at 50 °C with a rotary evaporator. The concentrated extract thus obtained is termed as crude extract as well as estimated about 28 g extract was obtained.

Fractionation and isolation of compounds

23 g crude extract was subjected to TLC screening to see the type of compounds present in the extract. The whole portion of the methanolic extract was subjected to Vacuum Liquid Chromatography (VLC) for rapid fractionation. The VLC fractions were screened by TLC to find out interesting fractions. The sample for VLC was prepared by absorbing methanolic extract onto silica gel (Kiesel gel 60, mesh 70-230) allowed to dry and subsequently applied on top of the adsorbent layer. The column was then eluted with n-hexane followed by mixture of n-hexane and ethyl acetate and then ethyl acetate and methanol. The polarity was gradually increased by adding increasing proportions of ethyl acetate and methanol. A total of 25 fractions were collected each in 100 ml beakers (Table 1). All the VLC fractions were screened by TLC under UV light and by spraying with vanillin-sulfuric acid reagent followed by heating at 110 °C.

Instrumentation for isolation and characterization of compounds

The ^{1}H NMR (400 MHz and 300 MHz) spectra was acquired in $CDCl_3$ on an Ultra Shield Bruker DPX 400 spectrometer and the chemical shifts are reported in parts per million relative to the residual nondeuterated solvent signals. Checking the homogeneity of the compounds were made by TLC on Kieselgel gel 60 F254 pre-coated sheets (E. Merck) and the spots were detected by exposure to UV-lamp at 254 nm. Column chromatography was done on silica gel (60-120 mesh ASTM).

Results and discussion

Characterization of AM-1 as Lupeol

Compound AM-1 was isolated from the methanol extract of *A. marmelos* (fraction 6) as white crystals. Spraying the developed plate with vanillin-sulfuric acid followed by heating at 110 °C for several minutes gave a dark colored spot. The R_f value of the compound was 0.48 in n-hexane-ethyl acetate (85:15) on silica gel 60 F_{254} plate. The compound AM-1 was found to be soluble in chloroform. Its melting point was 215 °C .The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of table 2 showed one triplet of one proton intensity at δ 3.17 typical for H-3. The spectrum displayed two singlet at δ 4.67 and δ 4.55 (1H each) assignable to protons at C-29. Doublet of double doublet at δ 2.36 assignable to proton at C-19, The spectrum displayed seven singlets at δ 0.95, 0.78, 0.84, 1.02, 0.93, 0.82 and 1.67 (3H each) assignable to protons of methyl groups at C-4 (H3-23, H3-24), C-10 (H3-25), C-8 (H3-26), C-14 (H3-27), C-17 (H3-28) and C-20 (H3-30) respectively. By comparing the ^{1}H NMR data of AM-1shown in table 2 & fig. 1, with that of previously published data (Aratanchemuge *et al.,* 2004), it was confirmed as Lupeol.

Characterization of AM-2 as Stigmasterol

Compound AM-2 was isolated from the methanol extract of *A. marmelos* (fraction 4) as needle shaped crystals. It was evident as a purple spot on TLC (Silica gel PF_{254}) when the developed plate was sprayed with vanillin-sulfuric acid followed by heating at 110 °C for 5-10 minutes. The R_f value of the compound was 0.33 in n-hexane-ethyl acetate (90:10) on silica gel 60 F_{254} plate. It was found to be soluble in petroleum ether, hexane, ethyl acetate and chloroform. Its melting point was 160-164 °C which is identical to that observed for stigmasterol (Khan RI., 1991).

The ^{1}H NMR spectrum (400 MHz, CDCl3) of AM-2 (Fig.2) revealed one proton multiplet at δ 3.51, the position and multiplicity of which was indicative of H-3 of the steroidal nucleus. The typical signal for the olefinic H-6 of the steroidal skeleton was evident from a multiplet at δ 5.33 integrating one proton. The olefinic protons (H-22 and H-23) appeared as characteristic downfield signals at δ 5.16 and δ 5.03 respectively in the ^{1}H NMR spectrum. Each of the signal was observed as

double-doublets (J = 15.0 Hz, 8.3 Hz) which indicated couplings with the neighbouring olefinic and methine protons. The spectrum further revealed signals at δ 0.67 and δ 1.00 (3H each) assignable to two tertiary methyl groups at C-13 and C-10, respectively.

The ^{1}H NMR spectrum showed two doublets centered at δ 0.83 (J = 6.0 Hz) and 0.85 (J = 6.0 Hz) which could be attributed to the methyl groups at C-25. The doublet at δ 0.91 (J = 6.4 Hz) was demonstrative of a methyl group at C-20. On the other hand, the triplet (J = 6.5 Hz) of three-proton intensity at δ 0.82 could be assigned to the primary methyl group attached to C-28. The spectral features shown in table 2 & fig.2 are in close agreement to those observed for stigmasterol. On this basis, the identity of AM-2 was confirmed as stigmasterol.

Table 3. Comparison between the ^{1}H NMR spectral data of AM-2 (400 MHz) and stigmasterol in $CDCl_3$ [20].

Protons	AM-2 δ_H in ppm	Stigmasterol δ_H in ppm
H-3	δ 3.51 m	δ 3.52 m
H-6	δ 5.33 m	δ 5.32 m
Me-13	δ 0.67 s	δ 0.65 s
Me-10	δ 1.01 s	δ 1.00 s
H-20	δ 0.91 s	δ 0.90 s
Me-20	δ 0.90 d (*J* = 6.5 Hz)	δ 0.90 d (*J* = 6.5 Hz)
H-22	δ 5.13 dd (*J* = 15.0, 6.5 Hz)	δ 5.15 dd (*J* = 15.0, 6.5 Hz)
H-23	δ 5.01 dd (*J* = 15.0, 9.0 Hz)	δ 5.03 dd (*J* = 15.0, 9.0 Hz)
Me_2-25	δ 0.83 d (*J* = 6.0 Hz), δ 0.81 d (*J* = 6.0 Hz)	δ 0.83 d (*J* = 6.0 Hz), δ 0.83 d (*J* = 6.0 Hz)
Me-28	δ 0.83 t (*J* = 6.5 Hz)	δ 0.81 t (*J* = 6.5 Hz)

Characterization of AM-5 as 5-Methoxy psoralen

Compound AM-5 was isolated from the methanol extract of *A. marmelos* (fraction 14) as light yellow compound. Spraying the developed plate with vanillin-sulfuric acid followed by heating at 110 °C for several minutes gave a yellow colored spot. Its melting point was 188-191 °C. The compound AM-5 was found to be soluble in, ethyl acetate, chloroform. The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) displayed doublet at δ 7.76 (1H), doublet at δ 7.69 (1H). Singlet at δ 7.33 (1H). The spectrum also showed doublets at δ 6.81 and δ 6.36 for (H). By comparing the ^{1}H NMR data (David *et al.,* 1984, Guranowski *et al.,* 1981 & Chiang PK *et al.,* 1979) of AM-5 with that of previously published data which was confirmed as 5-Methoxy psoralen.

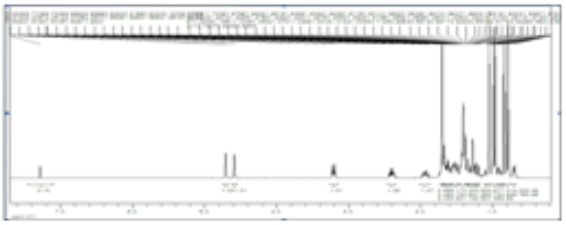

Fig. 1: ^{1}H NMR spectrum of AM-1 in $CDCl_3$.

Characterization of AM-7 as lupeol and minor compound

Compound AM-7 was isolated from the methanol extract of *A. marmelos* (fraction 16) as white crystals. Spraying the developed plate with vanillin-sulfuric acid followed by heating at 110 °C for several minutes gave a dark colored spot. The compound AM-7 was found to be soluble in, chloroform. The ^{1}H

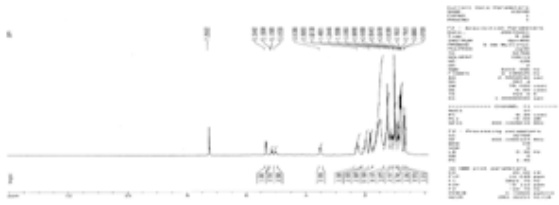

Fig. 2: ^{1}H NMR spectrum (400 MHz, $CDCl_3$) of AM-2.

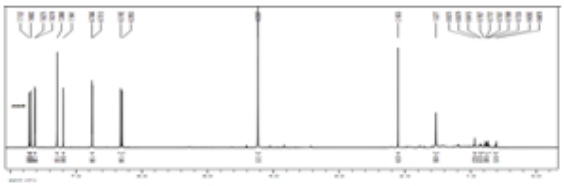

Fig. 3: ^{1}H NMR spectrum of AM-5 in $CDCl_3$.

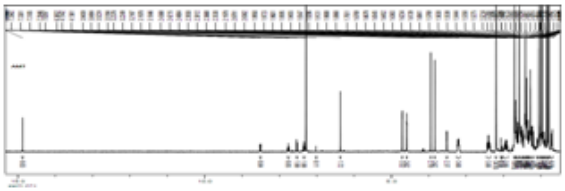

Fig. 4: ^{1}H NMR spectrum of AM-7 in $CDCl_3$.

NMR spectrum (400 MHz, $CDCl_3$) of fig. 4 showed one triplet of one proton intensity at δ 3.17 typical for H-3. The spectrum displayed two singlet at δ 4.67 and δ 4.55 (1H each) assignable to protons at C-29. Doublet of double doublet at δ 2.36 assignable to proton at C-19. The spectrum displayed seven singlets at δ 0.95, 0.78, 0.84, 1.02, 0.93, 0.82 and 1.67 (3H each) assignable to protons of methyl groups at C-4 (H3-23, H3-24), C-10 (H3-25), C-8 (H3-26), C-14 (H3-27), C-17 (H3-28) and C-20 (H3-30) respectively. By comparing the ^{1}H NMR data of AM-7 with that of previously published data (Aratanchemuge *et al.,* 2004) was confirmed as Lupeol.

Conclusion

Successive chromatographic separation and purification of the methanolic fraction of bark of *A. marmelos* yielded a total of three compounds. The structures of these compounds were elucidated as (1) Lupeol (2) stigmasterol 24-ethyl-cholesta-5, 22-dien-3beta-ol and (3) 5-Methoxy psoralen. Medicinal plants are rich sources of bioactive compounds and thus serve as important raw materials for drug production. They constitute a precious natural wealth of a country and contribute a great deal to its health care programmes. Judicious and scientific exploitation of this wealth can significantly improve the general health of the people.

Acknowledgement:

The authors are grateful to Department of Applied Chemistry & Chemical Engineering, University of Dhaka and ACI Pharmaceuticals, Narayanganj-1400, Bangladesh for providing support and collaborating assistance for the entire study.

References:

Aratanchemuge, Hiroshige H, Koh S, H, Kunio I, Takashi K. 2004. Induction of apoptosis by Lupeol isolated from mokumen (*Gossampinus malabarica L. Merr*) in human promyelotic leukemia HL-60 cells. Journal of Oncology reports. **11,**289-292.

Arul V, Kumaraguru S, Dhananjayan R.1999. Effects of aegeline and lupeol-the two cardioactive principles isolated from the leaves of Aegle marmelos Corr. J Pharm Pharmacol. **51,** S252.

Basu D, Sen R. 1974. Alkaloids and coumarins from root-bark of Aegle marmelos, Phytochemistry. **13,** 2329-2330.

Badam L, Bedekar SS, Sonawane KB, Joshi SP.2002. In vitro antiviral activity of bael (Aegle marmelos Corr) upon human coxsackieviruses B1-B6. J Commun Dis. **34,** 88.

Chiang PK, Cantoni GL.1979. Biochem., Pharmacol. **28,** 1897.

Dr. Philomena G, Nimmi OS. 2011. Cent Percent Safe Centum Plants for Antiobesity,International. Journal of Innovative Technology & Creative Eng.**3**, 2045-8711.

David K, Henry R, John E.1984. Hearst, 8-Methoxypsoralen-Nucleic Acid Photoreaction. Effect of Furan Photoaddition. J. Med. Chem. **27,** 531-534.

Geetha T, Varalakshmi P.2001. Anti-inflammatory activity of lupeol and lupeol linoleate in rats. J Ethnopharmacol. **76,** 77.

Goel RK, Maiti RN, Manickam M, Ray AB. 1997. Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins and their effect on prostanoid synthesis using human colonic mucosa. Indian J Exp Biol. **35,** 1080.

Guranowski A, Montgonery JA, Cantoni GL, Chiang PK. 1981**.** Biochemistry **20,** 110.

Jagetia GC, Venkatesh P, Baliga MS. 2005. Aegle marmelos (L.) Correa inhibits the proliferation of transplanted Ehrlich ascites carcinoma in mice. Biol Pharm Bull. **28,** 58.

Jagetia GC, Venkatesh P, Baliga MS. 2003. Evaluation of the radioprotective effect of Aegle marmelos (L.) Correa in cultured human peripheral blood lymphocytes exposed to different doses of gamma-radiation: a micronucleus study, Mutagenesis. **18,** 387.

Katayama T, Nagai I. 1960.Chemical significance of the volatile components of spices from the food preservative view point, IV-structure and antibacterial activity of some terpenes. Nippon Suisan Gakkaishi. **26,** 29.

Khan RI. 1991**.** Natural Product: A Laboratory Guide, 2nd Ed. Academic Press, N.Y., USA.

Kumar DS, Prabhakar YS. 1987. On the ethnomedical significance of the arjun tree, Terminalia arjuna (Roxb.), Wight & Arnot. Journal of Ethnopharmacology. **20(2),**173-90.

Mishra SB, Rao CV, Ojha SK, Vijayakumar M, Verma A. 2010. An Analytical Review of Plants for Anti Diabetic Activity with their Phytoconstituent & Mechanism of Action. Internatinal Journal of Pharmaceutical Sciences & Research. **1(1).**

Misra P, Pal NL, Guru PY, Katiyar JC, Tandon JS.1991. Antimalarial activity of traditional plants against erythrocytic stages of Plasmodium berghei. Int J Pharmacog, **29,** 19.

Parasakthy K, Deepalakshmi PD, Shanthy S, Niranjali SD.1993. The hepatoprotective action of eugenol. Med Sci Res. **21,** 611.

Rastogi RP, Mehrotra BN.1993. In Compendium of Indian medicinal plants, Volume **3,** edited by Rastogi RP (C.D.R.I. Lucknow & Pubications & Information Directorate, New Delhi).

Rastogi RP, Mehrotra BN. 1995. In Compendium of Indian medicinal plants, Volume **4,** edited by Rastogi R P (C.D.R.I., Lucknow & Pubications & Information Directorate, New Delhi).

Suleyman A, Sema A. 2009. Investigation of In vitro Antimicrobial Activity of *Aloe vera* Juice. Journal of Animal and Veterinary Advances. **8,** 99-102.

Takase H, Yamamoto K, Hirano H, Saito Y ,Yamashita A. 1994. Pharmacological profile of gastric mucosal protection by marmin and nobiletin from a traditional herbal medicine. J Pharmacol. **66,** 139.

Vimal V, Devaki T. 2004. Linear furano coumarin protects rat myocardium against lipidperoxidation and membrane damage during experimental myocardial injury. Biomed Pharmacother. **58,** 393.