Literature Review and Phytochemical Analysis

On

Stem bark of *Aegel marmelos*

Research Report

By

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Department of Pharmacy

Literature review and phytochemical analysis

On

Stem bark of ***Aegel marmelos***

A Research Submitted to the Department of Pharmacy of
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In Partial Fulfillment of the Requirements for the Degree of
Bachelor of Pharmacy

Under the Supervision of

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EAST WEST UNIVERSITY

DECLARATION

I, Shams-e-ara Afroge, University ID # 2005-2-70-056 have completed the research on topic, "Leterature review and Phytochemical analysis on stem bark of Aegel marmelos", under PHRM-404 course regarding the partial fulfillment of our undergraduate degree of Bachelor in Pharmacy.

I, therefore declare that this project has been published previously neither in whole nor in part of any degree except this publication. I also mentioned work found by other researchers by reference.

27/12/09
Signature of the Supervisor

Signature of Author

Acknowledgement

At first I thank to almighty Allah for giving me his knowledge and wisdom and also enough strength to complete this research work properly. Then I want to express my intense gratefulness to my research instructor A. T. M Jamaluddin; without his earnest help, all sorts of supportive suggestions and co-operation; it would be a day-dream to complete my research in this due time. His continuous support and valuable ideas, feedback with excellent comments to carry out this research work.

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I feel deepest admiration to my department for providing necessary chemicals and apparatus and giving me the honor to perform the research a partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

I would like to thank my friends of East West University for their cordial help and support during my research work.

Finally, I would like to express of special thanks to my parents, whose continuous support of mental, physical and financial during my study at East West University. Without their constant support and blessings this research will never come to an end.

Abstract

Aegle marmelos (Linn) family rutaceae is highly reputed ayurvedic medicinal tree commonly known as the bale fruit tree. All the parts of the tree root, leaf, trunk, fruit, are used in traditional system of medicine. A number of chemical constituents and various therapeutic effects of A. marmelos have been reported by different workers. Extensive investigations have been carried out on different parts of Aegle marmelos and as a consequence, varied classes of compound *viz., alkaloids,* coumarins, terpenoids, fatty acids and amino acids have been isolated from its different parts. Work on methanolic extract of stem bark is rare. Phytochemical constituents of a methanolic extract of stem bark of Aegle marmelos were investigated. The phytochemical screening of the crude extract of methanol are use to find a active compound .Performing TLC and column chromatography using toluene (1), dichloromethane (2) and methanol(22 drops) ratio. Finally, getting a compound having better solubility profile and physical properties.

Keywords*:* ***Aegle marmelos*** (Linn); Literature review, Introduction, Phytochemical evaluation, Result.

Introduction

The effects of plant extract have been studied by a very large number of researches in different parts of the world (Ates and Erdogrul, 2003). Much work has been done on ethnomedicinal plants in India (Negi et al., 1993). Interest in a large number of traditional natural products has increased (Taylor et al., 1996). It has been suggested that aqueous and Ethanolic extract from plants used in allopathic medicine but there is very small number of research have been perform on methanolic extract. Work on stem bark of Aegel marmelos is also absent in previous researches

Botanical Name: Aegle Marmelos (CORREA)

Family: Rutaceae

English names: Bengal quince, golden apple, stone apple

Local Name: Bael, bhel, bilwa, belaphal, Bengal Quince

Parts Used: Fruits & Leaves

Pic: 1 Tree of ***Aegel marmelos***

Indian names: *maredu* (Andhra Pradesh), *bel* (Bengal), *bil* (Gujrat), *bael, bil* (Himachal Pradesh), *bael* (Hindi), *bilpatra, kumbala, malura* (Karnatka)

Aegle marmelos Correa is a sacred tree, dedicated to Lord Shiva. The offering of *bael* leaves is a compulsory ritual of the worship of Lord Shiva in the hills. This importance seems largely due to its medicinal properties. All parts of this tree, viz., root, leaf, trunk, fruit and seed, are used for curing one human ailment or another. *Bael* is a handsome

tree, native to northern India, but is found widely throughout the Indian peninsula and in Ceylon. Burma, Thailand and Indo-China (Bailey, 1963). It grows wild throughout the low hills of Himachal Pradesh, ascending up to 1,000 meters. The fruits of the wild trees are, however considerably smaller than those of the cultivated types grown in the plains.

Scientific classification

Kingdom: Plantae

Order: Sapindales

Family: Rutaceae

Subfamily: Aurantioideae

Tribe: Clauseneae

Genus: Aegel (Correa)

Species: ***Aegel marmelos***

Pic: 2 Fruit of Aegel marmelos

Morphology

A small to medium-sized aromatic tree, deciduous; stem and branches, light brown to green; strong axillary spines present on the branches; the average height of tree, 8.5 meters. Leaves, alternate, pale green, trifoliate; terminal leaflet, 5.7 cm long, 2.8 cm broad, having a long petiole; the two lateral leaflets, almost sessile, 4.1 cm long, 2.2 cm wide, ovate to lanceolate having reticulate pinnate venation; petiole, 3.2 cm long. Flowers, greenish white, sweetly scented, bisexual, actinomorpbic, ebracteate. hypogynous, stalked; stalk, 8 mm long; diameter of a fully open flower, 1.8 cal; flowers, borne in lateral panicles of about 10 flowers, arising from the leaf axil; calyx, gamosepalous, five-lobed, pubescent, light green, very small in comparison with petals;

corolla polypetalous, with 5 petals, imbricate, leathery, pale yellow from above and green from beneath, length 4 mm; androecium, polyandrous, numerous, basifixed, 4 mm long, dehiscing longitudinally; gynoecium, light green, 7 mm long, having capitate stigma and terminal style.

Fruits, yellowish green, with small dots on the outer surface, oblong to globose, 5.3 cm to 7 2 cm in diameter; weight, 77.2 g; volume, 73.7 ml; pulp, yellow and mucilaginous, the pulp of dried fruits retains its yellow, and also remains intact; rind woody, 4 to 5 mm thick. Seeds, numerous, embedded in the pulp, oblong, compressed, white, having cotton-like hairs on their outer surface.

The plant has been used in the Indian traditional medicines from time immemorial. It is associated with various important medicinal properties. Chemical investigation on the different parts of the plant has resulted in the isolation of a large number of novel and interesting metabolites. Some of the compounds have been screened for bioactivity.

Varieties

One esteemed large cultivar with thin rind and few seeds are known as 'Kaghzi'. Dr. L.B. Singh and co-workers at the Horticultural Research Institute, Saharanpur, India, surveyed bael fruit trees in Uttar Padesh, screened about 100 seedlings, selected as the most promising for commercial planting: 'Mitzapuri', 'Darogaji', 'Ojha', 'Rampuri', 'Azamati', 'Khamaria'. Rated the best was 'Mitzapuri', with very thin rind, breakable with slight pressure of the thumb, pulp of fine texture, free of gum, of excellent flavor, and containing few seeds. S.K. Roy, in 1975, reported on the extreme variability of 24 cultivars collected in Agra, Calcutta, Delhi and Varanasi. He decided that selections should be made for high sugar content and low levels of mucilage, tannin and other

phenolics. Only the small, hard-shelled type is known in Florida and this has to be sawed open, cracked with a hammer, or flung forcefully against a rock. Fruits of this type are standard for medicinal uses rather than for consuming as normal food.

Chemical composition of the fruit

The chief constituents appear to be mucilage and pectin contained in the pulp of the unripe fruit; the ripe fruit differs in yielding a tannin reaction and possessing a distinct aroma.

Table 1: Food Value per 100 g of Edible Portion

Ascorbic acid	8.60 mg
Tartaric acid	1.19 mg
Ash	1.04 g
Potassium	0.746
Magnesium	0.127
Phosphorus	0.137
Iron	0.007
Total sugar	8.36 percent
Reducing sugar	6.21 percent
Non reducing sugar	2.04 percent
Acidity	0.46 percent
Niacin	1.1 mg
Riboflavin	1.19 mg
Pectin	2.62 percent
Thiamine	0.13 mg
Tannin	0.21 percent

Toxicity

The leaves are said to cause abortion and sterility in women. The bark is used as a fish poison in the Celebes. Tannin, ingested frequently and in quantity over a long period of time, is antinutrient and carcinogenic.

Other Uses

Fruit: The fruit pulp has detergent action and has been used for washing clothes. Quisumbing says that bael fruit is employed to eliminate scum in vinegar-making. The gum enveloping the seeds is most abundant in wild fruits and especially when they are unripe. It is commonly used as household glue and is employed as an adhesive by jewelers. Sometimes it is resorted to as a soap-substitute. It is mixed with lime plaster for waterproofing wells and is added to cement when building walls. Artists add it to their watercolors, and it may be applied as a protective coating on paintings.
The limonene-rich oil has been distilled from the rind for scenting hair oil. The shell of hard fruits has been fashioned into pill- and snuff boxes, sometimes decorated with gold and silver. The rind of the unripe fruit is employed in tanning and also yields a yellow dye for calico and silk fabrics.

Leaves: In the Hindu culture, the leaves are indispensable offerings to the 'Lord Shiva'. The leaves and twigs are lopped for fodder.

Flowers: Cologne is obtained by distillation from the flowers.

Wood: The wood is strongly aromatic when freshly cut. It is gray-white, hard, but not durable; has been used for carts and construction, though it is inclined to warp and

crack during curing. It is best utilized for carving, small-scale turnery, tool and knife handles, pestles and combs, taking a fine polish.

Medicinal properties

Watt (1889) reported the unripe dried fruit to be astringent, digestive and stomachic. According to him, they are prescribed to cure diarrhoea and dysentery. The ripe fruit is a good and simple cure for dyspepsia. The roots and the bark of the tree are used in the treatment of fever by making a decoction of them. The leaves are made into a poultice and used in the treatments of ophthalmia. According to Dastur (1962), the rind of the ripe fruit is also sometimes used as a medicine.

The roots are sweet, cure the fevers caused by *tridosho*, stop pain in the abdomen, the palpitation of the heart, and allay urinary troubles. They are also useful in the disordes of *vata, pitta* and *kapha* (Kirtikar and Basu, 1935).

The fruits are very useful in chronic diarrhoea and dysentery, particularly in the case of patients having diarrhoea, alternating with the spells of constipation. Sweet drink (sherbet) prepared from the pulp of fruits produce a soothing effect on the patients who have just recovered from bacillary dysentery, are taken for their mild laxative, tonic and digestive effects. The unripe and half-ripe fruits improve appetite and digestion (Jain, 1968; Jauhari, 1969).

The pulp from the unripe fruits is soaked in gingelly oil for a week and this oil is smeared over the body before bathing. This oil is said to be useful in removing the peculiar burning sensation in the soles and also prescribed in cases of hemorrhoids. It has been surmised that the psoralen in the pulp increases tolerance of sunlight and aids in the maintaining of normal skin color. It is employed in the treatment of leucoderma.

Marmelosin derived from the pulp is given as a laxative and diuretic. In large doses, it lowers the rate of respiration, depresses heart action and causes sleepiness.

Bitter, light-yellow oil extracted from the seeds is given in 1.5 g doses as a purgative. It contains 15.6% palmitic acid, 8.3% stearic acid, 28.7% linoleic and 7.6% linolenic acid. The seed residue contains 70% protein.

The bitter, pungent leaf juice, mixed with honey, is given to allay catarrh and fever. With black pepper added, it is taken to relieve jaundice and constipation accompanied by edema. The leaf decoction is said to alleviate asthma. A hot poultice of the leaves is considered an effective treatment for ophthahnia and various inflammations, also febrile delirium and acute bronchitis.

A decoction of the flowers is used as eye lotion and given as an antiemetic. The bark contains tannin and the cournarin, aegelinol; According to Dixit and Dutt (1932), the fruits of *Aegle marmelos* Correa contain a furocoumarin marmalosin, which is responsible for its medicinal properties. The bark contains umbelliferone and other hydroxy coumarins and the alkaloids, fagarine and skimmianine. The bark decoction is administered in cases of malaria. Decoctions of the root are taken to relieve palpitations of the heart, indigestion, and bowel inflammations; also to overcome vomiting.

The fruit, roots and leaves have antibiotic activity. The root, leaves and bark are used in treating snakebite. Chemical studies have revealed the following properties in the roots: psoralen, xanthotoxin, *O*-methylscopoletin, scopoletin, tembamide, and skimmin; also decursinol, haplopine and aegelinol, in the root bark.

Aegle Marmelos Extract

Active Ingredient: Tannins 5%, Mucilage10% & Mucilage 15%

Common Name: Bael Tree, Holy Fruit Tree, Quince

Chemical Constituents and Components

Main chemical components are marmelosin, alloimperatorin, marmelide, tannic acid, marmin, umbelliferone, isoimperatorin, isopimpinellin, skimmin, marmesin, marmesinin, fatty acids, and beta-sitosterol

Action

Mucilage:

1. It increases the glucose level and glycosylated hemoglobin in diabetic patients.
2. It decreases plasma insulin and liver glycogen in diabetic patients.
3. It decreases the lipid peroxidation.
4. It stimulates macrophage functioning.
5. It causes significant elevation in the GSH (glutathione) concentration in liver, kidney, stomach, and intestine.

Tannins:

1. It shows potent anti-viral activity.
2. It causes significant decrease in lipid peroxidation, conjugated diene and hydroperoxide levels in serum.
3. It significantly reduces the blood sugar level.
4. It reduces the significant oxidative stress.

Curing Diseases

1. Sweet drink (sherbet) prepared from the pulp of fruit produce soothing and cooling effect.
2. The unripe and half-ripe fruits improve appetite and digestion.

3. The ripe fruit is a good and simple cure for dysentery and dyspepsia.

4. The roots and bark of the tree are used in the treatment of fever and malaria.

5. The roots are used to cure pain and palpitation of the heart.

6. Possible Combinations: ***Aegle marmelos*** + cynodon dactylon (prevents constipation)

Research Information

Extract of ***Aegle marmelos*** fruits shows hypoglycaemic activities. It significantly reduces the blood glucose, plasma thiobarbituric acid reactive substances, hydroperoxides, ceruloplasmin and alpha-tocopherol. It also shows significant elevation in glutathione and Vitamin C. (Ref. Kamalakkanna, N and Stanely Mainzen Prince, P, Hypoglycemic effect of water extracts of Aegle marmelos fruits, J. Ethanopharmacology, 2003, Aug;87 (2-3) 207-210)

Literature Review Of ***Aegel marmelos***

Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents. There is an ever increasing need to limit toxic clinical drugs (Lown, 1993). In modern times, the active ingredients and curative actions of medicinal plants were first investigated through the use of European Scientific methods (Herborn, 1998). The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steriods, phenols glycosides and tannins (Abayomi, 1993). *Aegle marmelos* (Linn) family rutaceae is highly reputed ayurvedic medicinal tree commonly known as the bale fruit tree, is medium sized tress growing throughout the deciduas forest of India. It is found whole over India, from sub-Himalayan forest, Bengal, central and south India .All the parts of the tree viz, root, leaf, trunk, fruit, are used in traditional system of medicine. Various phytochemical and biological evaluations have been reported in this literature for the importance of the *Aegle marmelos*.

In 2 April 2004, A new insecticidal protolimonoid Preared from Aegle marmelos. Bioassay-directed fractionation of the ethyl acetate extracts of the stem bark of *A. marmelos*, afforded a new compound, named skimmiarepin C, along with skimmiarepin A. The latter is a known compound but its isolation from *A. marmelos* is new. The new compound is a senecioate ester analogue of the latter. Full identification of the new compound was achieved using spectroscopic methods on the separated mono-acetate derivatives. Skimmiarepins A and C exhibited moderate insecticidal activity against *Phaedon cochleariae* and *Musca domestica* in comparison with natural pyrethrum

extract. The two epimeric acetates of skimmiarepin C were both less active. (Samarasekera., Hemalal, 2004)

In April 2004 , A new 7-geranyloxycoumarin [7-(2,6-dihydroxy-7-methoxy-7-methyl-3-octaenyloxy) coumarin] named marmenol (1) has been isolated from the leaves of methanolic extract of*Aegle marmelos*. In addition to marmenol, several known compounds have also been obtained for the first time from the same source. They include: praealtin D, *trans*-cinnamic acid, valencic acid, 4-methoxy benzoic acid, betulinic acid, N-*p-cis*- and *trans*-coumaroyltyramine, montanine, and rutaretin.(M. S. Ali ; M. K. Pervez)

In September 2004, the serial extracts of the leaves of *Aegle marmelos* Corr. were investigated for anti-inflammatory property. The analgesic and antipyretic properties were also evaluated. The most of the extracts derived from the plant *Aegle marmelos* caused a significant inhibition of the carrageenan-induced paw oedema and cotton-pellet granuloma in rats. The extracts also produced marked analgesic activity by reduction the early and late phases of paw licking in mice. A significant reduction in hyperpyrexia in rats was also produced by the most of the extracts. This study was established anti-inflammatory, antinociceptive and antipyretic activities of the leaves of *Aegle marmelos*. (V. Arul , S. Miyazaki and R. Dhananjayan ,2004.)

In December 2006, The plant *Aegle marmelos*. From the leaves of *A. marmelos* an alkaloidal-amide, Aegeline **2**, was isolated and found to have antihyperglycemic activity as evidenced by lowering the blood glucose levels, in sucrose challenged streptozotocin induced diabetic rats (STZ-S) model at the dose of 100 mg/kg body weight. Aegeline **2** has also significantly decreased the plasma triglyceride (Tg) levels by 55% ($P < 0.001$), total cholesterol (TC) by 24% ($P < 0.05$), and free fatty acids (FFA) by 24%,

accompanied with increase in HDL-C by 28% and HDL-C/TC ratio by 66% in dyslipidemic hamster model at the dose of 50 mg/kg body weight. The reasonable mapping of compound **2** to validated pharmacophoric hypothesis and 3D QSAR model with an estimated activity (283 nM) suggest that the compound **2** might be a β_3-AR agonist. (T. Narender, S. Shweta, P. Tiwari, K. Papi Reddy, T. Khaliq, P. Prathipati, A. Puri, A.K. Srivastava, R. Chander, S.C. Agarwal and K. Raj)

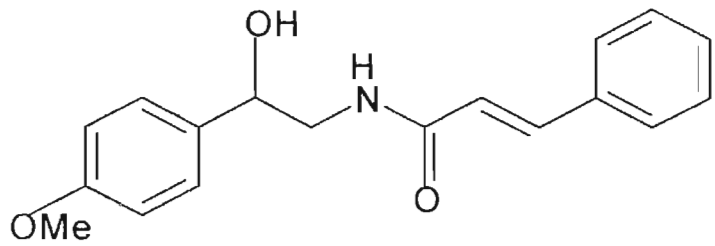

Fig 1: Aegeline 2 (Alkaloidal- amide)

In year 2007, the test for alkaloids (Harbone, 1973, Trease and Evans, 1980 and Tewari et al., 1992), presence of flavanoides was determined. The extract was also tested for anthrocyanin , presence of carboxylic acid, of coumarins , phenols, test for steroids, phytosterols was also carried out .Xanthoproteins was also detected . Chloroform extract of *Aegle marmelos*, shows the presence of alkaloids. Chloroform extract of *Aegle marmelos* indicate the presence of carboxylic acids. Phenols are present in all the extracts of plants. Flavanoids is present in the petroleum ether extract of *Aegle marmelos*. Petroleum ether extract of *Aegle marmelos*, ethanol extract of *Aegle marmelos*, indicate the presence of anthocyanin. Saponins present in the chloroform extract of *Aegle marmelos*. Sterols are present only in the petroleum ether extract of the plants. Chloroform extract and ethanol extract of *Aegle marmelos*, indicate the presence of xanthoproteins (K. Sudharameshwari and J. Radhika,)

In November 2007, A brassinosteroid was isolated from *Aegle marmelos* Correa, which was characterized to be 24-epibrassinolide (EBL). It was evaluated for the antigenotoxicity against maleic hydrazide (MH) induced genotoxicity in *Allium cepa* chromosomal aberration assay. It was shown that the percentage of chromosomal aberrations induced by maleic hydrazide (0.01%) declined significantly with 24-epibrassinolide treatment. EBL (10^{-7} M) proved to be the most effective concentration with 91.8% inhibition. This is the first report on the isolation of 24-epibrassinolide from *Aegle marmelos* and its antigenotoxic effects against MH employing *Allium cepa* chromosomal aberration assay. (Nishi Sondhi, Renu Bhardwaj , Satwinderjeet Kaur, Veeraj Kumar and Bikram Singh)

In November 2008, A rare alkaloid, shahidine , having an unstable oxazoline core has been isolated as a major constituent from the fresh leaves of *Aegle marmelos*. It is moisture-sensitive, and found to be the parent compound of aegeline and other amides, however, it is stable in dimethyl sulfoxide. Shahidine showed activity against a few Gram-positive bacteria.(S. Faizi , F. Farooqi, S. Zikr-Ur-Rehman, A. Naz, F.Noor, F. Ansari, A. Ahmad and S. A. Khan)

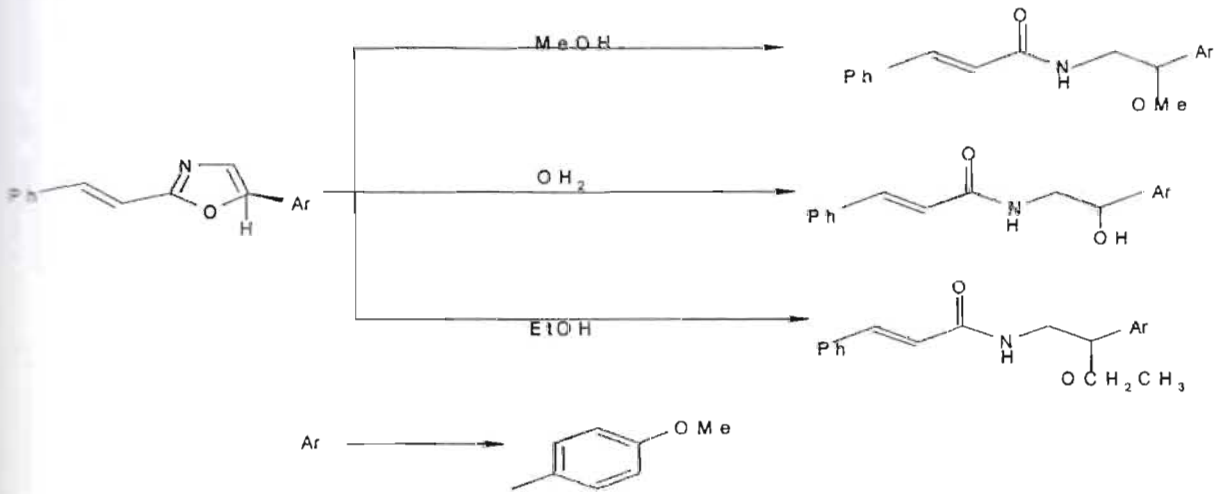

Fig 2: Shahidine (1)

In year 2008, isolated few compound from *A. marmelos* bark from petroleum ether extract. The crude extract was subjected to column chromatography. Two triterpenes, lupenone and lupeol were obtained in the form of white crystal.Khangan *et al*, studied the binding of copper ions with modified *Aegle marmelos* bark substrate. The effect of pH, contact time, temperature, anion, light metal ion, concentration and effect of amount of substrate on the uptake of Cu^{2+} were studied. Substrate indicated that Cu was removed to <0.02 mg/L from solutions.Ohashi *et al* isolated four isomeric lignan-glucosides from the bark of *Aegle marmelos* .Two new lignan – glucoside, (-) – lyoniresinol 2α-O-β-D glucopyranoside and (-) 4 - epi-lyoniresinol, 3α -O -β -D-glucopyranoside, have been isolated together with two known lignan - glucosides, (+)-lyoniresinol. Ohashi *et al* isolated two new 7-geranyloxycoumarins from the bark of the *Aegle marmelos*. Two new 7-gerayloxycoumarins and aeglin, were isolated from the bark of *Aegle marmelos,* and there structures were assigned on the basis of the NMR data .The absolute configuration was confirmed by chemical synthesis.

Nema *et al* isolated new pigment from stem bark of the *Aegle marmelos*. The isolation and structure elucidation of new compound is marmesin – 1"- α -L - rhamnopyranoside and 1,5 -dihydroxy - 6 - methoxy -2 -methyl anthraquinone, which occur together with lupeol and β-sitosterol in the stem bark of *Aegle marmelos* were describes

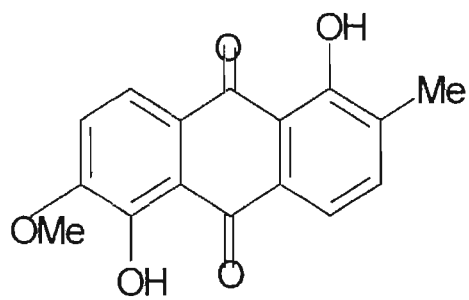

Fig 3: 1, 5-dihydroxy-6-methoxy-2-methylanthraquinone

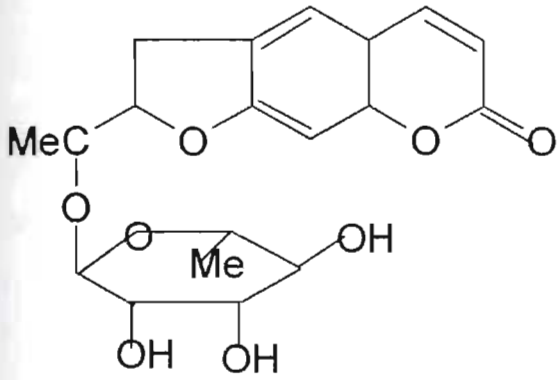

Fig 4: Marmesin-1-alpha-L-rhamnopyranoside

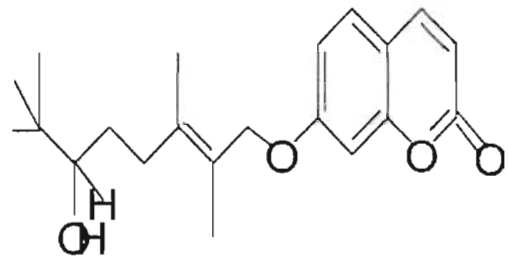

Fig 5: R-(+)-MARMIN

V.K. Gupta *et al* studied the sample coumarin compound R- (+) – marmin from the trunk bark of the *Aegle marmelos* by methanol extract. Then extracted compound concentrated and chromatographed over the silica gel and the chemical structure were assigned on the basis of the H^+ NMR and mass spectra . Chatterjee *et al* studied the isolation and constitution of marmin, a new coumarin from *Aegle marmelos* umbelliferone (i), skimmianine (ii) and a sitosterol (iv), were isolated from the immature bark of *Aegle marmelos*. The constitution of (iii) was established as 7- (3, 7 dihydroxy-3, 7- dimethyloctyloxy) coumarin.

Samarasekera *et al* isolated various coumarin present in the various part of the *Aegle marmelos*. These are Umbeliferone, Skimmin, Impertonin. The structures of these coumarins are given below .(R. Chanda, A. Ghosh, T. Mitra, J. P. Mohanty, N. Bhuyan & G. Pawankar)

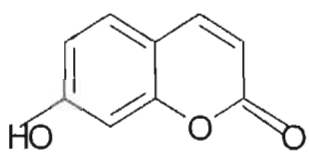

Fig 6: Umbeliferone

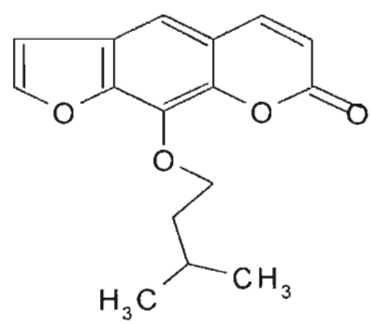

Fig 7: Impertonin

In August 2008; a series of phenylethyl cinnamides, which included new compounds named anhydromarmeline (**1**), aegelinosides a (**7**) and B (**8**), were isolated from *Aegle marmelos* leaves as α-glucosidase inhibitors. The structures of new compounds were characterized by spectroscopic data and chemical degradation. Of compounds isolated, anhydroaegeline (**2**) revealed the most potent inhibitory effect against α-glucosidase with IC_{50} value of 35.8 μM. The present result also supports ethnopharmacological use of *A. marmelos* as a remedy for diabetes mellitus.(P. Phuwapraisirisan[a], T. Puksasook[a, b], J. Jong-aramruang[c] and U. Kokpol[a])

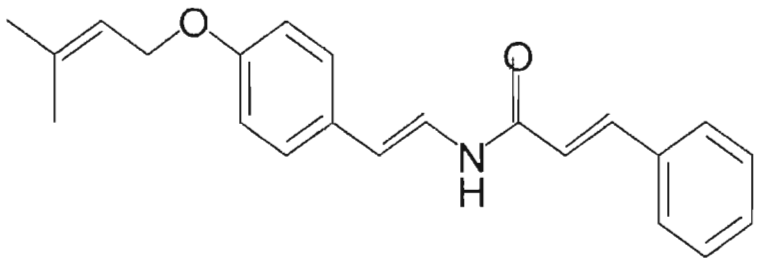

Fig 8: Anhydromarmeline

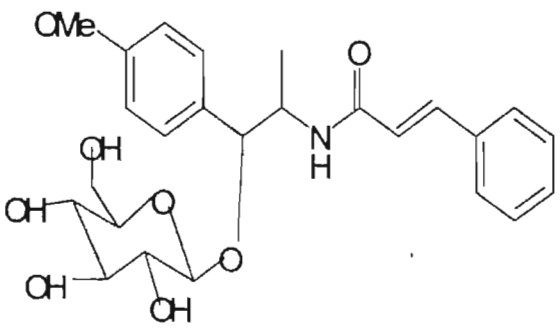

Fig 9: Aegelinoside A

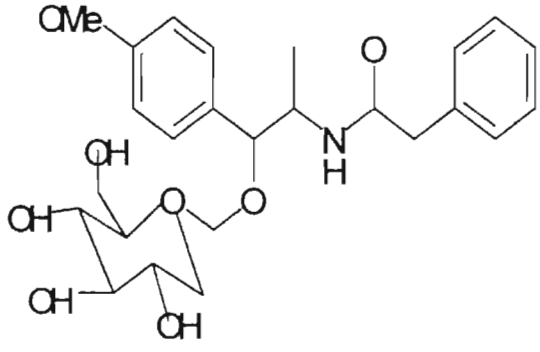

Fig 10: Aogelinoaide B

In December 2008, a study was carried out to screen and evaluate the antimicrobial activity of leaf extracts from *Aegle marmelos* (L.) Corr. Petroleum ether, Dichloromethane, Chloroform, Ethanol and Aqueous extract of the leaves were tested against selected Gram positive and Gram negative bacteria as Eight strains of Gram-positive bacteria - *Micrococcus glutamicus, Lactobacillus bulgaris, Streptococcus faecalis, Staphylococcus aureus, Bacillus stearothermophilus, Staphylococcus pyogenes, Micrococcus luteus, Bacillus cereus* and two strains of Gram negative bacteria - *Escherichia coli* and *Pseudomonas aeruginosa* were used to evaluate the antibacterial activity. Results depict that phytochemical extracts of *A. marmelos* exhibited significant anti-bacterial activity. However, the inhibitory activity was found to be both organism and solvent dependent. Ethanol and chloroform leaf extracts of *Aegel marmelos* were found to be more active towards the bacterial species tested. The leaf extracts inhibited the growth of both Gram-positive and Gram-negative bacterial species. Further, the aqueous leaf extract was moderately active followed by dichloromethane extract. However, petroleum ether extract was not effective against any of the organisms tested. Growth of *Lactobacillus bulgaris* and *Bacillus cereus* was not inhibited by any of the tested leaf extracts of *A. marmelos.* The study shows that ethanol and chloroform leaf extracts of *A. marmelos* can be used as a potential source of antimicrobial agents.(C. Rajasekaran, E. Meignanam, N. Premkumar, T. Kalaivani, R. Siva, V. Vijayakumar, S. Ramya and R. Jayakumararaj)

Phytochemical experiment

Phytochemical work included from the collection of plant material to the laboratory analysis where the plant material collected from different sources and perform different chemical analysis to get the desired result. The purpose of our phytochemical research was to isolate the active chemical constituents present in the stem bark of Aegel marmelos. For this we have used 100gm of stem bark of our sample.

Plant collection

Plant: *Aegel marmelos* (Bale)

Used part: stem bark

Color: light brown to green

Pic: 3 Stem bark part

The selectable plant was collective from munshigong, a district of Bangladesh. Collected plant was the selectable one was identified by a taxonomist from National Herbarium, Mirpur, Dhaka. The identification number which is given by the National Herbarium is:

DACB, Acceffion No. of ***Aegel marmelos*** – 34358

The stem bark portion was separated from the plant and dried under sun-light for 6 days. After drying, bark part was grinded by using Grinding machine. Total grinded part was about 3kg in weight which was preserved in a air tight and light protected container.

Laboratory work

Used instruments

1. Grinding machine
2. Rotary evaporator (IKA®RV05 basic,Biometra)
3. Hot plate
4.Electric balance
5. TLC
6. Column chromatography
7. UV lamp (Biometra TI1)
8. Round bottom flask
9. Buckner funnel
10.Seperating funnel
11. Beaker
12. Capillary tube
13. Screw cape tube
14. Conical flask
15. Pipette
16. Pipette pumper
17. Glass rod

Used solvent

1. Methanol
2. Dichloro methane
3. Hexane
4. Acetone
5. Chloroform
6. Silica

Procedure

Extraction

We have taken 100gm dust of stem bark of *Aegel marmelos into 1000ml beaker and* soaked it with approximately double amount of methanol. We kept it for 4 days and every day it was stirred with a clean glass rod so those maximum amounts of constituents that are soluble in methanol with come out from the dust. The Beaker was covered by aluminum foil so that no loss of solvent is ensured. After 4 days, we have filtered the mixture.For filtration, we used Buchner funnel and obtained two parts,

- The residue portion over the filter
- The filtered part

The filtered part, which contains the substance soluble in methanol, is put in a glass flask and placed it in the Rotatory evaporator. The evaporation was done under 40c temperature. When the evaporation seemed to be satisfactory, we collected the sample which is methanolic extract. This part was taken and done thin layer chromatography.

Solvent selection

We have done TLC (Thin layer chromatography) by choosing several solvents or combination of solvent as the mobile phase and performed normal phase TLC. In TLC, used methanol, hexane, chloroform and some other solvent in different ratio. Some of the combinations of solvent we have used are:

Table 2: TLC of methanolic extract in different solvent combination

Acetone: Cyclobenzene	1 : 1 1 : 3	Sample did not run
Toluene:dichloromethane:methanol	1 : 4 : 1 drop	One spot with streaking
Ethyl acetate : Acetone	1 : 2	Streaking
Methanol : Ethyl acetate	1 : 2	Streaking
Acetone : methanol	1 : 2	Streaking
Chloroform : Acetone	2 : 1	Streaking

No combination mentioned above should a better result. So, we again tried by diluting the evaporated sample with methanol (Approximately 30ml) and done TLC with some other combination.

Table 3: TLC of extracted sample in different solvent combination

Solvent	Ratio	Result
Dichloromethane : Methanol	1 : 2	Streaking
Toluene : Dichloromethane	1 : 1	3 spot shown
	1 : 4	5 spot shown
	1 : 6	Sample did not run
Toluene:Dichloromethan:Methanol	1 : 4 : 1 drop	11 spot spots shown

The results of these combinations showed better result so we have gone for column chromatography with the mobile phase containing toluene, dichloromethane and methanol.

Column chromatography

Selected solvent ratio from TLC was used to perform column for separation. Silica gel column was used where the dried sample was applied. Firstly we have inserted some cotton and poured some sand over the glass column, so that a sand layer is formed. Then we have taken dried silica, which was dried over the hot air oven so that no presence of air was there. The silica was placed over the sand layer in the column. Then we run our solvent containing toluene and dichloromethane (1:4) through the column and thus prepared our stationary phase. Then we again made a sand layer over it. Sample was dried along with the silica and was placed over the sand layer. Then 100ml mobile phase containing solvent combination having 20 ml Toluene, 80 ml Dichloromethane and 4 ml Methanol. We found several layer of samples and collected the portion of the samples time to time. At the end of the column chromatography, we got 13 different parts which were slightly different in color.

Table 4: Information of collected column fraction

Column no	Volume	Color
Column 1	3ml	Light yellow
Column 2	8ml	Colorless
Column 3	4ml	Greenish yellow
Column 4	5ml	Yellow
Column 5	3ml	Yellow
Column 6	4ml	Light yellow
Column 7	6ml	Colorless
Column 8	6.5ml	Yellow
Column 9	12ml	Light orange
Column 10	8ml	Light orange

Column 11	10ml	Brownish yellow
Column 12	62ml	Orange
Column 13	60ml	Deep yellow

We have done TLC test with all the column parts, under the detection of ultraviolet radiation, and the solvent combination as mobile phase in those TLC was 1:4:1 drop.

Table 5: TLC result of first three column fraction

Column part	Toluene: dichloromethane: methanol	Result
Column part 1	1:4:1 drop	11 spots were shown
Column part 2	1:4:1 drop	11 spots were shown
Column part 3	1:4:1 drop	3 spots were shown

Then we have done TLC with all the column parts with toluene, dichloromethane and a varying concentration of methanol but no good result was obtained. After that we have used 1.5 ml methanol in addition to the toluene and dichloromethane containing mobile phase and we got different results.

Table 6: TLC result of all column fraction

Column part	Toluene: dichloromethane: methanol	Result
Column 1	1: 4: 1.5	1 spot with stacking
Column 2	1: 4: 1.5	1 spot with stacking
Column 3	1: 4: 1.5	1 spot with stacking
Column 4	1: 4: 1.5	1 spot with stacking
Column 5	1: 4: 1.5	Full stacking

Column 6	1: 4: 1.5	1 spot
Column 7	1: 4: 1.5	Stacking
Column 8	1: 4: 1.5	Stacking
Column 9	1: 4: 1.5	Stacking
Column 10	1: 4: 1.5	Stacking
Column 11	1: 4: 1.5	Stacking
Column 12	1: 4: 1.5	Stacking
Column 13	1: 4: 1.5	Stacking

All column parts were poured into small conical flask and were preserved by packing those with aluminum foil paper.

Since, column 6 showed a satisfactory result, it was poured into a screw cap tube. The tube weight about 14.4 gm. After some days, the solvent portion of the column 6 part had been evaporated and crystal structures were formed. We took some of the crystals and diluted that with Toluene and dichloromethane (1:4) without methanol and performed TLC with some solvent combination.

Table 7: TLC result of column fraction 6

Column part	Toluene: dichloromethane: methanol	Result
Column part 6	1:2: 0.5	No
	1: 2: 0	2 spot irregular
	1: 2: 3	Many spot

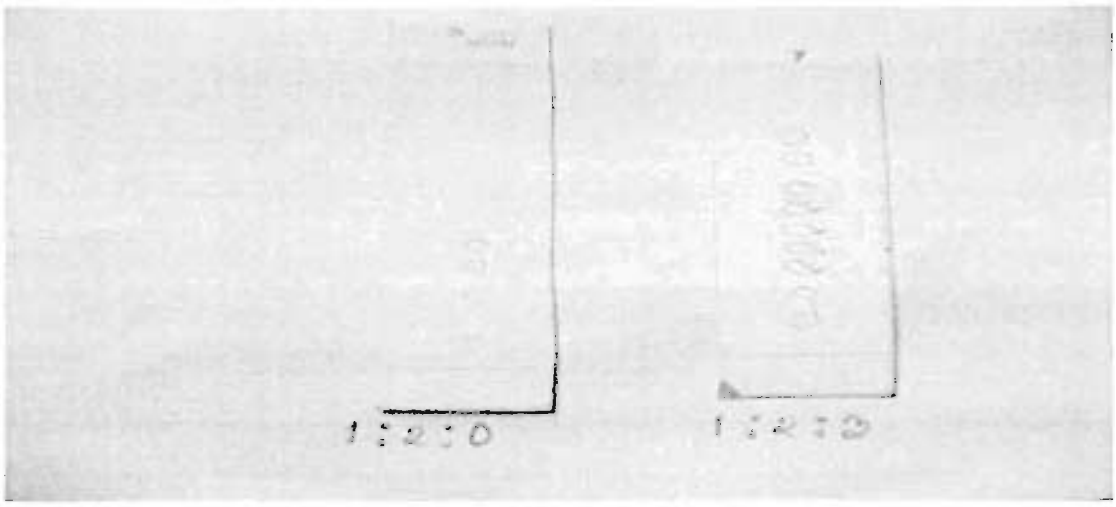

Fig 4: TLC result of column 6 in toluene: DCM: MeOH-1:2:0 & 1:2:3

Another column part 4 had also become crystals and we did TLC with that too.

Table 8: TLC result of column fraction 4

Column part	Toluene:dichloromethane:methanol	Result
Column part 4	1 : 2 : .5	Streaking
	1 : 2 : 0	2 spots with
	1 : 2 : 3	streaking
		many irregular
		spots

From the above test we did not get better result so again we diluted the crystal of column part 6 in previous way and done TLC with following ratio,

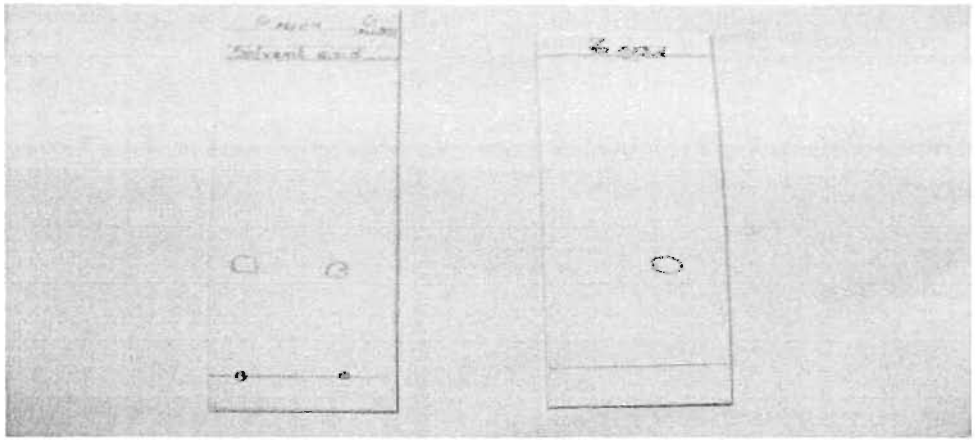

Fig 5: TLC result of column 6 in toluene: DCM: MeOH-1:2:22

Table 9: TLC result of column 6 using high methanol ratio

Column part	Toluene: dichloromethane: methanol	Result
Column part 6	1: 2: 22 drops	1 round spot

Since column 6 alone showed only one perfect spot, it is hope to be a single compound and we will do further tests to isolate any pure compound from this part.

Result

Physical properties

The collected extract is light cream in color. After drying, it is power in form and it preserved in a screw cap tube.

For further analysis, we have to require the NMR facility. so we decided to send the sample abroad for NMR test . NMR test in abroad require enough time so we determine those properties which can easily tested under our laboratory facility.

Solubility profile

Collected column 6 parts are the experimental sample which use further in the research work. We determine the solubility profile of this part as:

- Toluene-Slightly soluble
- Acetone-soluble
- Ethylacetate-soluble
- Dichloromethane-soluble
- Chlorobenzene-Insoluble
- Methanol-soluble
- Water-soluble

Conclusion

From the over all work we find a better result showing extracted part from the methanolic extract of Aegel marmelos bark. We perform column chromatography and get expected result containing fraction which show good result in TLC. So to get the nature and possible structure of the compound which is present in the collected fraction, we send the fraction in abroad for UV, IR, Mass and NMR study.

Reference

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