International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.1, pp 478-481, Jan-Mar 2013

Isolation and identification of major chemical components found in the leaves of Lagerstroemia indica plant grown in the city of Tehran, Iran.

Ashnagar A[1]*, Ghanad AR[2], and Motakefpour M[2]

**[1]School of Pharmacy, Isfahan University of Medical Sciences,
[2]Pasteur Institute of Iran, Nanobiotechnology department, Pasteur Avenue, SQ.NO. 69, Post Code No. 1316943551, Tehran, Iran.**

***Corres. author: alamdarashnagar@yahoo.com
Tel. No. 00982166953311, Fax No. 00982166465132***

Abstract: Corosolic acid has attracted much attention for its beneficial health effects, particularly with respect to its potential anti diabetic activity. Multicomponent mixtures are usually difficult to separate in a single chromatographic run. In this research, we tried the combination of column chromatography (LC), thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) which is more or less an inexpensive method for the separation of the multicomponent mixture of the extract. Distillation of the ground powdered leaves of the plant in water followed by successive refluxing of the residues in ethanol afforded a crude extract which its spectra and HPLC chromatogram showed that it should be a multicomponent mixture. Refluxing of the ground leaves in methanol showed also two major peaks at t_R = 2.433, 85.81%, and t_R = 4.017, 8.53%. These two methods showed similar results but obtaining a 100% pure corosolic acid was not materiliazed. The results showed that Lagerstroemia indica plant has much lower corosolic acid than Lagerstroemia speciosa plant reported in literature. Soxhlet extraction in various solvent systems was not a satisfactory method as well.
Keywords: Lagerstroemia indica, corosolic acid, banaba leaves.

Introduction

Lagerstroemia speciosa (Giant Crape-myrtle, Queen's Crape-myrtle, Banabá Plant for Philippines, or Pride of India) is a species of Lagerstroemia native to tropical southern Asia. It is also widely cultivated as an ornamental plant in tropical and subtropical areas. It is a small to medium-sized tree growing to 20 m tall, with smooth, flaky bark. The leaves are deciduous, oval to elliptic, 8-15 cm long and 3-7 cm broad, with an acute apex. The flowers are produced in erect panicles 20-40 cm long, each flower with six white to purple petals 2-3.5 cm long. [Scientific classification (Kingdom: Plantae; Division:Magnoliophyta ; Class: Magnoliopsida ; Order: Myrtales; Family: Lythraceae; Genus: Lagerstroemia; Species: L. speciosa). Binomial name : (Lagerstroemia speciosa, (L.) Pers.), Synonyms: Lagerstroemia macrocarpa Wall].[1] Banaba has a long history of folkloric medical applications that include blood pressure control, urinary dysfunctions (helps ease urination), controls the cholesterol levels, treatment of diarrhea, facilitates bowel movement, Diabetes and as analgesic. The primary active chemical ingredient of the extract is corosolic acid, and there are also numerous possible synergists including lagerstroemin, flosin B and reginin A. The leaves of the Banaba and other parts are used widely by the Philippines, Taiwan, and Japan as a tea preparation. Banaba herb is one of the ten herbal plants approved by the Philippine Department of Health (DOH)

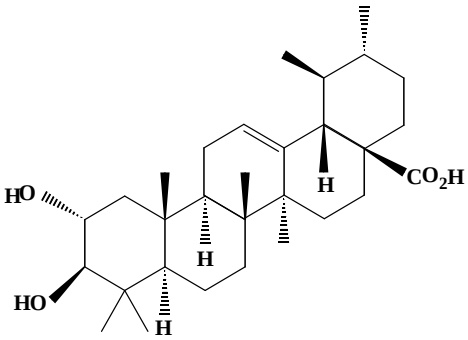
through its "Traditional Health Program". Corosolic acid is a known potent glucophage, helpful in decreasing blood sugar levels.
Lagerstroemia speciosa, also known as Queens Crape Myrtle, has been used for many years for a variety of ailments, including treatment for diabetes and hyperglycemia. Banaba has been shown to have the following properties:

1. Blood sugar regulating properties to balance blood sugar.
2. Helpful in reducing blood glucose and insulin levels
3. Promote healthy insulin levels
4. Support for appetite control
5. Helps to reduce food cravings
6. Beneficial in maintaining normal weight management
7. Antioxidant activity for protection against oxidative stress and free radicals

Clinical studies conducted at the Southeastern Institute of Biomedical Research in Florida, using a 1% corosolic acid extract of Banaba leaf, reportedly reduced serum glucose in people with Type II diabetes, but did not reduce serum glucose in healthy individuals. For some people, fluctuations in blood sugar and insulin are related to appetite, hunger and various food cravings. Because of the way Banaba helps the body to handle glucose, it is also used effectively in weight-loss products as a safe, natural component for reducing weight. A tighter control of blood sugar and insulin levels has shown to have a significant tendency to promote weight loss.[2]

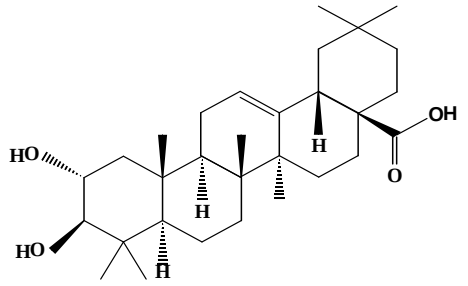
Corosolic acid **(I)** is useful as an ingredient in medicines, cosmetics and health foods, is known to be contained in Eriobotrya japonica (loquat), Lagerstroemia spesiosa (banaba), Rhabdosia japonicas (Isodonis Herba), Epilobium angustifolium (fireweed), Elliottia paniculata (Hotsutswuji) and many other plants and it has been found to have pharmaceutical actions such as an anti-diabetic action and a blood glucose level lowering action. However, a problem exists in that corosolic acid commonly occurs in a plant together with maslinic acid **(II)**, its position isomer with respect to methyl group being as follows, which makes it difficult to obtain corosolic acid in pure form especially on an industrial scale, thus limiting its medical usability. In small scale in the laboratory, corosolic acid is prepared in low yield by chromatographic isolation from plant extracts.[3]

Corosolic acid ($C_{30}H_{48}O_4$, MW 472.70 g/mol, IUPACname:1S,2R,4aS,6aR,6aS,6bR,8aR,10R,11R ,12aR,14bS)-10,11-dihydroxy-1,2,6a,6b,9,9, 12a-heptamethyl-2,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydro-1H-picene-4a-carboxylic acid), is a pentacyclic triterpene acid, found in Lagerstroemia speciosa, used in dietary supplements as a glucose-lowering agent and insulin mimetic. It is similar in structure to ursolic acid, differing only in the fact that it has a 2-alpha-hydroxy attachment.[4]



Corosolic acid (I)

Maslinic acid is a compound derived from dry olive-pomace oil (an olive skin wax) which is a byproduct of olive oil extraction. It is a member of the group of triterpenes known as oleananes.[5]



Maslinic acid (II)

Maslinic acid $C_{30}H_{48}O_4$, 472.70g/mol;IUPAC Name; (4aS,6aR,6aS,6bR,8aR,10R,11R,12aR,14bS)-10,11-dihydroxy-2,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,7,8,8a,10,11,12,13,14b-tetradecahydropicene-4a-carboxylic acid.

Experiment

NMR spectra were recorded on a Bruker NMR spectrophotometer Avance III 400 MHz (Germany) using TMS as internal standard.Mass spectra were recorded on Mass spectrometer Agilent Technology (HP) with Quadrupole Analyzer, China. Infrared spectra were recorded using Rayleigh WQF-510/520 FT-IR spectrometer, China. UV-Visible spectra were measured by using Optima 3000S, Japan. HPLC Knauer Chromatograph (LPG, manager 5000, pump 1000, RI detector 2300, PDA detector 2800 and a column oven) with Chromgate Software version 3.1.7 and a reverse phase C-18 column (250 × 4.6 mm, packing: Euroshper 100-5) were used for recording the chromatograms. All the chemicals were purchased from Merck.

1. **Collecting the plant:** The plant was collected in June-July around in the city of Tehran, the capital city of Ian and identified as

   Lagerstroemia indica by the research centre of Tehran, Azad University, Iran. Sufficient amount of the plant were collected and air dried in the shade then ground by using an electrical mill.

   Distillation of an aqueous suspension of the powdered plant: 50 g of the ground plant leaves was placed in a 1 liter round bottomed flask and around 650 mL water was added, then distilled. The distillate was filtered and discarded. The residual content of the round bottomed flask was filtered and the solid residue transferred to another round bottomed flask. 200 mL of ethanol was added and refluxed for 6-8 hours. Filtration was carried out and filtrate was collected. The new residue transferred to another round bottomed flask and 400 mL of ethanol was added to it and refluxed for further 6-8 hours. Filtration was carried out and the filtrate was collected**.** The filtrates were added together and the solvent was removed on a rotary evaporator. FT-IR spectrum was taken. The residues were also added together and dried. FT-IR spectrum was taken, too. FT-IR spectra of the distillate and residue confirmed the presence of corosolic acid. Its FT-IR spectrum of the residue had (KBr pellet)ν $^{-}$(cm^{-1}): 3390 – 2400 (OH_{acidic} , s), 2941 ($C\text{-}H_{str}$, s), 1734 (C=O,s), 1616 (C=C, m-w), 1452 (CH_2 bending, m), 1361 (CH_3 bending, m), 1184, 1043 (C-O, s); its MS (EI) showed m/z: 472 [$M^{\cdot+}$, 4.7 %], 470 (7.8%), 457 [$(M - CH_3)^+$, 47%], 455 [$(M - OH)^+$, 10.2%], 431 (89%), 427[$(M - CO_2H)^+$, 17.2%], 410[$(427 - OH)^+$, 80.4%], 395[$(410 - CH_3)^+$, 75%], 381[$(410 - CHO)^+$, 78.9%], 368{[M –(CO_2H +CH_3 2×$OH)^+$, 100%]}. UV $^{a}_{max}$ (MeOH): 216 nm. Its HNMR spectrum was taken in d-chloroform and in d_5-pyridine. The sample was only partially soluble in d-chloroform. Therefore, its HNMR in d_5-pyridine was taken and showed (400 MHz, Bruker) δ (ppm). 1H NMR (400 MHz, d_5-pyridine) δ/ppm: 1.5 – 2.8 (m, several saturated H atoms), 3.4-3.6 (m, several saturated H atoms), 5.5-6.7 (m, several H-atoms), 10.3 (s, OH).

   Based upon these results and comparison of the spectra with the corresponding standard spectra of **(I)**, it was concluded that corosolic acid is extracted from the leaves of the plant, but not as a pure component.

2. **Soxhlet extraction of the ground plant:** 55 g of the dried ground leaves of the plant was Soxhlet extracted with 650 mL methanol for about 8 hours. The solvent was removed on a rotary evaporator. The residue was HPLC chromatographed by using several different mobile phases:

(i) **Pure acetonitrile:** It showed two major peaks with t_R = 2.783 (26.70%) and t_R = 3.017 (52.37%).

(ii) **Acetonitrile – water (4:1 v/v):** It showed five major peaks with t_R = 3.00 (14.51%), t_R = 3.167 (8.25 %), t_R = 3.317 (53.75%), t_R = 7.917 (6.80%), and t_R = 10.00 (6.30%).

(iii) **Pure methanol:** It showed one major peak with t_R = 2.417 (84.10%).

(iv) **Methanol-water (4:1 v/v):** It showed two major peaks with t_R = 1.667 (7.84%), t_R = 2.417 (81.94%).

3. **Refluxing of the ground leaves in methanol:** 40 g of the ground leaves and 250 mL of methanol were placed in a 500 mL round-bottomed flask and refluxed for 150 minutes. After filtration, the residue was washed with further 100 mL methanol. The solvent was removed on a rotary evaporator and the extract was collected and dried. 3.98 g of a pale green solid material was obtained. A small amount of the extract was dissolved in solvent mixture of methanol:diethyl ether:acetonitrile (1:1:1 v/v/v) and HPLC chromatogram was taken by using methanol : water (4:1 v/v, respectively) as mobile phase. Essentially, two major peaks were observed at with t_R = 2.433, 85.81%, and t_R = 4.017, 8.53%.

4. **Column chromatography of the extract:** 0.5 g of the crude extract was washed with chloroform (38 mL) and filtered. The solid residue on the filter paper was column chromatographed on silica gel as the stationary phase, and acetone:n-hexane (4:1 v/v, respectively) as the mobile phase. The eluates were collected in 12 (25 mL) erlenmyers. The separation was monitored by tlc. Finally, two fractions were collected, the content of erlenmyers 1-10, as the first fraction (I), and the content of erlenmyers 11-12, as the second fraction (II). The solvent of each fraction was removed on a rotary evaporator. Fraction (I) was further column chormatographed by using pure acetonitrile as the mobile phase. HPLC showed two major peaks **(**t_R = 2.983, 79.81%), and t_R = 4.017, 14.11%)**.** Fraction (II) was also column chormatographed by using pure acetonitrile as the mobile phase. HPLC showed one major peak **(**t_R =3.0, 98.05%). The whole separation process was repeated several times with almost identical results.

5. **Column chromatography of the extract:** the experiment (4) was repeated by using acetone:n-hexane (1:9 v/v, respectively) as the mobile phase. After work up as described in experiment (4), not a satisfactory result was obtained. The solvent system of the mobile phase was changed to acetone:n-hexane (1:4 v/v, respectively), again no satisfactory separation was achieved. The separation was repeated by using acetone:n-hexane (1:5 v/v, respectively) as the mobile phase. The separation was monitored by tlc. The eluates were collected in 40 tubes. Tlc revealed 3 fractions. The three fractions were collected (the content of tubes 1-12) as fraction (I), the content of tubes 13-27 as fraction (II), and content of tubes 28-40 as fraction (III). The solvent of each fraction was removed on a rotary evaporator. Then, each fraction was dissolved in methanol and analyzed twice by HPLC. The first fraction (I) showed almost a major single peak (t_R = 5.900, 86.37%), The second fraction (II) showed a major peak (t_R = 6.083, 59.00%), and the third fraction (III) showed a major peak (t_R = 6.167, 65.39%). It was concluded that fractions (II) and (III) were the same. Therefore, with this mobile phase system, two fractions, as in experiment (4) were obtained. The FT-IR spectrum of each fraction was taken. In each, the presence of corosolic acid could be confirmed and was similar as the FT-IR spectrum described above in detail.

Discussion

Corosolic acid has attracted much attention for its beneficial health effects, particularly with respect to its potential anti diabetic activity. In view of the therapeutic importance of corosolic acid, development of analytical methods for the determination of corosolic acid in plant materials is required. A high performance liquid chromatography (HPLC) was reported for estimation of corosolic acid.[4]Complex multicomponent mixtures are usually difficult to separate in a single chromatographic run. Therefore, we studied the possibility to separate natural multicomponent plant extracts by combining different chromatographic systems. In this research, we tried the combination of column chromatography (LC), thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) which is more or less an inexpensive method. It can be used for the preliminary purification of plant extracts prior to HPLC analysis. Distillation of the ground powdered leaves of the plant in water followed by successive reflux of the residues in ethanol afforded a crude extract which its spectra and HPLC chromatogram showed that it should be a multicomponent mixture.

Refluxing of the ground leaves in methanol was also carried out and the extract was collected and dried. A pale green solid material was obtained. A small amount of the extract was dissolved in solvent mixture of methanol:diethyl ether:acetonitrile (1:1:1 v/v/v) and HPLC chromatogram was taken by using methanol : water (4:1 v/v, respectively) as mobile phase. Essentially, two major peaks were observed at with t_R = 2.433, 85.81%, and t_R = 4.017, 8.53%. These two methods showed similar results but obtaining a 100% pure corosolic acid was not materiliazed. It needs more painstaking successive HPLC separation technique. The result also showed that the plant which is used in this research (Lagerstroemia indica) has totally different percentage (much lower) of corosolic acid than Lagerstroemia speciosa plant reported in literature. Soxhlet extraction in various solvent systems was not satisfactory as well.

References:

1. http://en. Wikipedia.org/wiki/Lagerstroemia; retrieved on 11/23/2010. Article
2. Takayama H, Kitajima M, Ishizuka T, and Seo S; "US patent application publication", Pub. No.: US 2005/0020681 A1; Pub. Date: Jan. 27, 2005.
3. Vijaykumar K, Murthy PB, Kannababu S, Syamasundar B, and Subbaraju GV; "Quantitative Determination of Corosolic acid in Lagerstroemia Speciosa Leaves, Extracts and Dosage Forms", International Journal of Applied Science and Engineering, 2006, **4**, 2:103-114.
4. Retrieved from: "http://en.wikipedia.org/w/index.php?title=Corosolic_acid&oldid=504837030"
5. Retrieved from;"http://en.wikipedia.org/w/index.php?title=Maslinic_acid&oldid=488461862."

*****