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An Improved Synthesis of Apigenin and Luteolin

A. Rambabu[1], K.J. Kumari[1], M. Baby Ramana[1], M.V. Ramani[2], G.V. Subbaraju[2] and B. Hari Babu[1,*]

[1]Department of Chemistry, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, India
[2]Natsol Laboratories Private Limited, Ramky Commercial Hub, J.N. Pharma City, Visakhapatnam-531 019, India

*Corresponding author: E-mail: dr.b.haribabu@gmail.com

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Apigenin and luteolin are the antioxidant flavonoids found in foods such as parsley, artichoke, basil and celery. Both of these compounds have shown the ability to protect cells against cancer and also to inhibit DNA oxidative damage. These flavonoids are part of many nutraceutical formulations available in the market. There is a need for the development of cost effective methodologies to produce them in large quantities. The synthetic process developed for both these compounds is general and can be applied for other flavonoids also. An industrially applicable high pure product, cost effective synthesis and general synthetic method has been developed and presented.

Keywords: Optimized synthesis, Apigenin, Luteolin.

INTRODUCTION

Apigenin is a flavonoid found in parsley, artichoke, basil, celery and other plants. Recently a number of published studies have demonstrated the anticancer properties of apigenin [1]. In a study conducted on 21 different flavonoids have reported that apigenin has shown to be the most effective antiproliferative [1]. A related study has also reported that flavonoids such as apigenin bind to estrogen receptor sites on cell membranes in order to prevent over-proliferation of these cells in response to estrogen [2]. Apigenin was tested to ascertain its effect on human leukemia cells. Apigenin was shown to induce apoptosis more effectively than quercetin and other flavonoids tested. The researchers attributed a unique mechanism of inducing apoptosis to the cancer preventive activity of apigenin [3].

Luteolin can be found in *Terminalia chebula*. It is most often found in leaves, but it is also seen in rinds, barks, clover blossom and ragweed pollen [4]. It has also been isolated from *Salvia tomentosa* [5]. Dietary sources include celery, green pepper, parsley, thyme, dandelion, perilla, chamomile tea, carrots, olive oil, peppermint, rosemary, navel oranges and oregano [6,7]. Luteolin is a strong good antioxidant and it helps the body to withstand radiation and chemotherapy. In a study from Japan, researchers were looking for the factor in Rooibos tea that was protecting DNA from radiation-induced free radicals. They discovered that the protective factor is luteolin. Mice with pure luteolin gave dramatic protection to the bone marrow and spleen against radiation. It was better than any other plant product tested. Luteolin in conjunction with doxorubicin (adriamycin), alleviated the drug induced cardiac and bone marrow toxicities, but, did not interfere with the therapeutic effects of doxorubicin [8]. Together, the actions of luteolin and other bioflavonoids can help provide powerful cancer-fighting benefits.

Apigenin and luteolin strongly inhibited the growth of human leukemia cells and were shown to be the most potent inhibitors of these cancer cell lines [9]. In another study, apigenin and luteolin were found to be a new class of therapeutic agents in the management of thyroid cancer [10].

In view of the strong antioxidant activity and therapeutical potential, apigenin and luteolin are part of many nutraceutical formulations. Some of them are Apigenin 40 mg by Cosomed, Apigenin by Swanson Ultra, Apigenine by EtatPur.

There are synthetic approaches [11-14] available for the synthesis of many flavonoids, but most of them needs lot of development in the yields by utilizing proper solvents, reagents, workup methods, *etc*. In view of the importance of the flavonoids and their use as nutraceuticals as discussed above and impending need in the development of methodologies we have developed an improved and commercially applicable synthesis for these apigenin and lutolin. The synthetic approach is general and can be applied to other flavonoids also. The overall synthesis is presented in **Schemes I** & **II** and the improvements have been discussed.

EXPERIMENTAL

Phloroglucinol and all other compounds obtained from Merck Specialties Private Limited, Mumbai, India. Reactions were monitored by TLC using silica gel-G (Merck grade) as the adsorbent and the solvent systems are indicated at appropriate places. Silica gel (100-200 mesh, Merck grade) has been used for column chromatography. The column was subjected to gradient elution using *n*-hexane, mixtures of hexane and ethyl acetate. All the melting points of the compounds were determined in open capillaries, using Elico digital melting point apparatus and are uncorrected.

The ^{1}H NMR spectra of the compounds were recorded on Bruker AMX 400 MHz, NMR spectrometer using TMS as an internal standard and the values are expressed in δ ppm. The ^{13}C NMR spectra of the compounds were recorded on Bruker AMX 100 MHz NMR spectrometer using TMS as an internal standard and the values are expressed in δ ppm. Mass spectra of the compounds were recorded on API-ES Mass spectrometer and Eligant 1100 series mass spectrometer using positive/negative mode of ionization method. The elemental analysis (C, H) of the compounds were recorded on Elementar-Vario Micro Cube-2008.

General procedure

Phloroacetophenone (1): A mixture of well-dried phloroglucinol (250 g, 1.98 mol), anhydrous acetonitrile (365 mL, 6.95 mol), diisopropyl ether (838 mL, 5.95 mol) and finely powdered fused zinc chloride (49.5 g, 0.036 mol) was cooled in an ice-salt mixture at 0 °C under stirring for 7 h, by passing dry HCl gas. The flask is allowed to cool in an ice-chest in refrigerator for overnight. On decanting the diisopropyl ether a bulky orange-yellow precipitate was separated and washed the precipitate with diisopropyl ether (100 mL). The solid was transferred into a round bottom flask and added 2.5 L of distilled water (2.5 L) and refluxed under stirring for 2 h at 120 °C. The mixture was cooled to room temperature and left overnight. Pale yellow needles were observed and the product was filtered and dried under vacuum oven at 120 °C. The yield was 96.2 % with a purity of 99.9 % obtained.

2-Hydroxy-4,6-dimethoxyacetophenone (2): A mixture of phloroacetophenone (**1**) (300 g, 1.78 mol), acetone (1.5 L) and potassium carbonate (739.2 g, 5.35 mol) was charged under stirring at room temperature. Dimethyl sulphate (494.9 g, 3.92 mol) was charged drop wise with the help of the addition funnel at 10-15 °C for 3 h. TLC (20 % EtOAc in hexane) showed no traces of starting material but observed traces of trimethoxyacetophenone. After completion of the reaction, the reaction mixture was poured into ice cold water and stirred for 3 h. A crude solid material was obtained on filtration and washed with cold water and drained thoroughly. The yield was 84 % with a purity of 95.8 % obtained.

4',5,7-Trimethoxychalcone (3): To a mixture of potassium hydroxide (57.1 g, 1.02 mol) and methanol (300 mL) was added 2-hydroxy-4,6-dimethoxyacetophenone (**2**) (100 g, 0.51 mol) under stirring. To the above 4-methoxy benzaldehyde (anisaldehyde, 83.2 g, 0.61 mol) was added slowly at room temperature and reaction was stirred vigorously at room temperature for 24 h. TLC (20 % EtOAc in hexane) showed complete absence of the starting material. After completion of the reaction the reaction mixture was quenched with 25 % dil. HCl (400 mL) up to pH = 6 and stirred for 1 h. Then the mixture was filtered and washed with water. The crude product was dried under vacuum to get corresponding pure 4',5,7-trimethoxychalcone (**3**). The yield was 91.8 % obtained.

4',5,7-Trimethoxyflavone (5): To a mixture of 4',5,7-trimethoxychalcone (**3**) (100 g, 0.31 mol) in DMSO (300 mL, 3 vol) was added catalytic amount of iodine (5.2 g, 0.04 mol) and reaction mixture was heated at 130-140 °C for 45 min. TLC (60 % EtOAc in hexane) showed complete absence of starting material. But when the reaction was heated for more than 45 min we have observed formation of impurities. After the completion of reaction it was poured into ice cold water and added 15 g of hypo (sodium thiosulphate). Then the solution was stirred for 3 h, filtered and dried to get the pure 4',5,7-trimethoxyflavone (**5**). The yield was 93.1 % with a purity of 99.2 % obtained.

Apigenin (7) (4',5,7-trihydroxyflavone): A mixture of 4',5,7-trimethoxyflavone (**5**) (50 g, 0.16 mol) and toluene (500 mL, 10 vol.) were added to aluminium chloride (191.8 g, 1.44 mol) at 80-100 °C. After completion of the addition the temperature was increased slowly to 130-140 °C and stirred for 1.5 h. TLC (50 % EtOAc in hexane) showed complete absence of starting material. After completion of the reaction the flask is cooled in an ice-salt mixture at 0 °C. Charged dilute HCl 25 % solution (1000 mL) dropwise for 2 h. The solution was filtered, dried and then washed with hot toluene. The crude product was dissolved in methanol (600 mL) at reflux condition for 1 h and filtered to obtain the pure 4',5,7-trihydroxy flavones (apigenin) (**7**). The yield was 61.5 % with a purity of 98.21 % obtained.

3',4',5,7-tetramethoxychalcone (4): To a mixture of potassium hydroxide (57.1 g, 1.02 mol) and N,N-dimethyl foramide (300 mL) was added 2-hydroxy-4,6-dimethoxyacetophenone (**2**) (100 g, 0.51 mol) under stirring. To the above 3,4-dimethoxy benzaldehyde (veratraldehyde, 101.6 g, 0.61 mol) was added slowly at room temperature and reaction was stirred vigorously for 4 h. TLC (20 % EtOAc in hexane) showed complete absence of starting material. After completion of the reaction the reaction mixture was quenched with 25 % dil. HCl (400 mL) up to pH = 6 and stirred for 1 h. Then the reaction mixture was filtered and washed with water. The crude product was dried under vacuum to get corresponding pure 3',4',5,7-tetramethoxychalcone (**4**). The yield was 91.1 % obtained.

3',4',5,7-Tetramethoxyflavone (6): To the mixture of 3',4',5,7-tetramethoxy chalcone (**4**) (100 g, 0.29 mol) suspended in DMSO (300 mL), was added catalytic amount of iodine (4.8 g, 0.03 mol). The reaction mixture was heated at 130-140 °C for 45 min. TLC (60 % EtOAc in hexane) showed complete absence of starting material. If the reaction mixture exceeds 45 min observed formation of impurities. After the completion of reaction was cooled and poured into ice cold water and added 15 g of hypo (sodium thiosulphate). Then the solution was stirred for 3 h, filtered and dried to get pure 3',4',5,7-tetramethoxyflavone (**6**). The yield was 91.5 % with a purity of 94.3 % obtained.

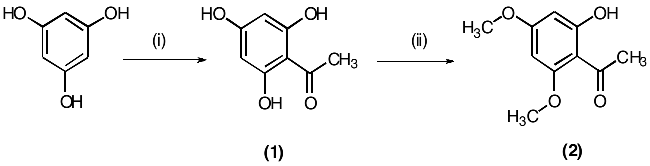
Luteolin (8) (3',4',5,7-tetrahydroxyflavone): A well-dried aluminium chloride (233.2 g, 1.7 mol) was heated at 80-100 °C and added 3',4',5,7-tetramethoxyflavone (**6**) (50 g, 0.14 mol) and toluene (500 mL). Slowly increased temperature to

130-140 °C and stirred the solution for 1.5 h. TLC (50 % EtOAc in hexane). After completion of the reaction the flask is cooled in an ice-salt mixture at 0 °C and shaken occasionally while adding 25 % dil. HCl solution (1000 mL) drop by drop with the help of additional funnel. Quenched the solution for 2 h. Then the solution was filtered and dried and then washed with hot toluene. A pure 3',4',5,7-tetrahydroxy flavone (luteolin) (**8**). The yield was 70.2 % and purity 97.0 % obtained.

RESULTS AND DISCUSSION

The total synthesis of apigenin and luteolin was developed as shown in successive **Schemes I** and **II**. In the first step of **Scheme-I** the synthesis of phloroacetophenone (**1**) was reported earlier using diethyl ether as a solvent, but diethyl ether is an expensive and highly volatile [15,16] solvent, further, the recovery of the solvent would be very low. Moreover, the yields in reported synthesis are in the range of 82 to 87 %. We have found the use of di-isopropyl ether instead of diethyl ether which resulted in the increase of the yield upto 96.2 %. Further, simple work up gave highly pure compound (99.9 % purity by HPLC without any further purification) (Fig. 1). Later, the selective methylation at 4,6-positions was achieved in high yield (84 % yield) (**Scheme-I**) by carrying out the reaction at 10-15 °C. The purity of the compound obtained was 95.8 % (Fig. 2). The reported conditions of using reflux temperature gave, intact, lower yields (71.4 %) [17] because of the formation of trimethyl ether. An industrially applicable high pure product, cost effective synthesis and general synthetic method has been developed for phloroacetophenone (**1**) and 2-hydroxy-4,6-dimethoxyacetophenone (**2**) (key intermediate), in this approach (**Scheme-I**).

The condensation of 2-hydroxy-4,6-dimethoxyacetophenone (**2**) with anisaldehyde in aqueous methanol in presence of KOH under the conditions reported gave the chalcone (**3**) (68.9 %) only [17]. However, the use of pure methanol and KOH, improved the yields further, in the range of 91.8 % (**Scheme-II**). Further, the cyclization of 4',5,7-trimethoxychalcone (**3**) in DMSO was carried out by the use



Scheme-I: Chemical equation for the synthesis of phloroacetophenone (**1**) and 2-hydroxy-4,6-dimethoxyacetophenone (**2**) (key intermediate); **Reagents and conditions:** i) Dry HCl, CH_3CN, DIPE, $ZnCl_2$; ii) K_2CO_3, DMS, acetone

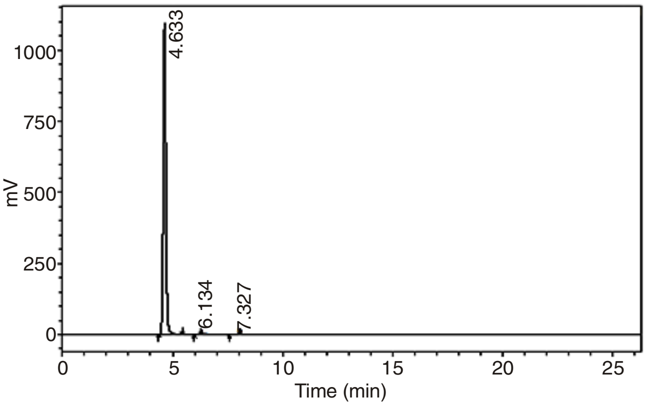


Fig. 1. HPLC chromatogram of phloroacetophenone (**1**)

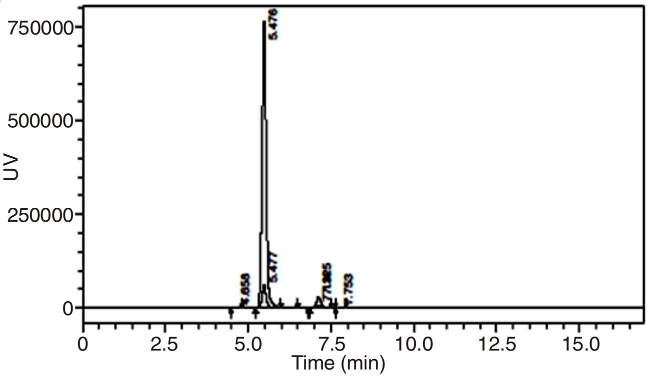
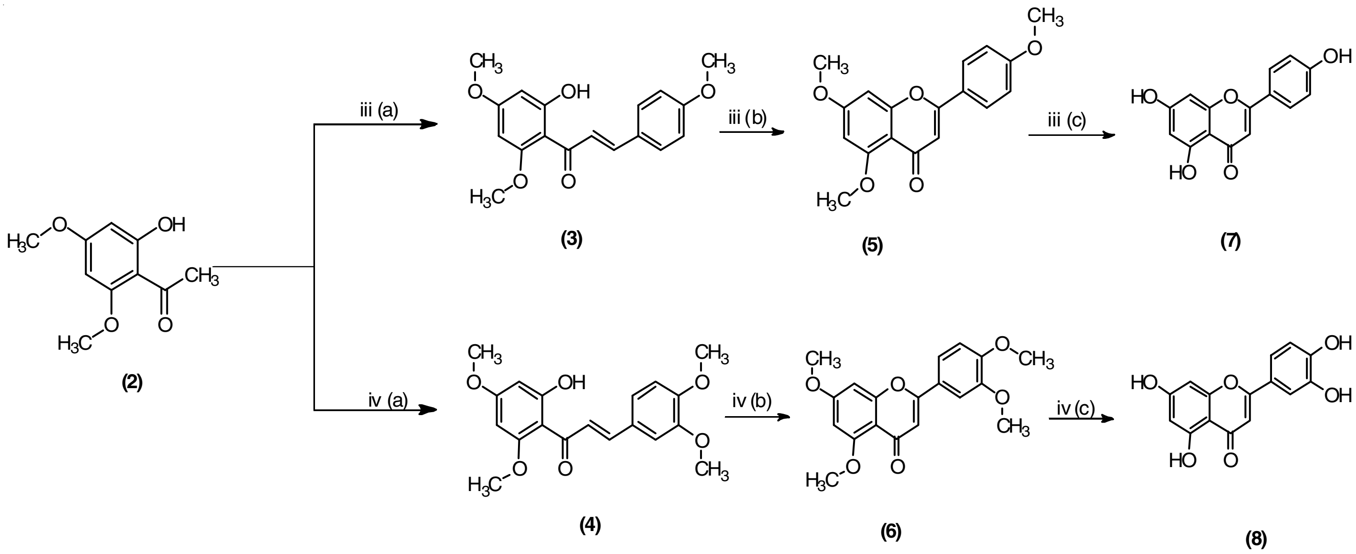


Fig. 2. HPLC chromatogram of 2-hydroxy-4,6-dimethoxyacetophenone (**2**) (key intermediate)



Scheme-II: Chemical equation for the synthesis of apigenin (**7**) and luteolin (**8**); **Reagents and conditions:** iii(a) $C_8H_8O_2$, KOH, methanol; iii(b) DMSO in iodine; iii(c) $AlCl_3$ in toluene; iv(a) $C_9H_{10}O_3$, KOH, DMF; iv(b) DMSO in Iodine; iv(c) $AlCl_3$ in toluene of catalytic amount of iodine at 130-140 °C for 45 min to obtain the product 4',5,7-trimethyl apigenin (**5**). The yield was 93.1 % with a purity of 99.2 % [18].

The condensation of 2-hydroxy-4,6-dimethoxyacetophenone (**2**) with vertraldehyde and methanol in presence of KOH under the conditions reported gave 68.9 % of the chalcone (**4**) only [17]. But it was found that the reaction in DMF solvent improved the yields upto 91.1 %. Further, the use of DMF has reduced the reaction time from 24 to 4 h (**Scheme-II**). Later, cyclization of 3',4',5,7-tetramethoxychalcone (**4**) in DMSO and use of catalytic amount of iodine at 130-140 °C for 45 min provided the product 3',4',5,7-tetramethyl luteolin (**6**). The yield was 91.5 % and with a purity of 94.3 % [19] (**Scheme-II**).

Finally to obtain the desired compounds apigenin and luteolin, several reagent systems were tried for improving the demethylation processes for **5** and **7** as shown in Tables 1 and 2.

However, the tested reagent and reaction conditions clearly indicated that the reaction conducted with aluminium chloride in toluene (120 °C, 1.5 h) and aluminium chloride in chlorbenzene (120 °C, 1.5 h) gave apigenin exclusively. But the use of chlorobenzene was not considered further, as it is a halogenated solvent to use it in the commercial scale synthesis. Based on the above we have found that, demethylation of 4',5,7-trimethylapigenin (**5**), with aluminium chloride in toluene at 110-120 °C for 90 min provided the product apigenin (**7**). The yield was 61.5 % with a purity of 97.6 %. Further, treatment of apigenin in methanol under reflux conditions improved the purity upto 98.21 % [20] (Fig. 3).

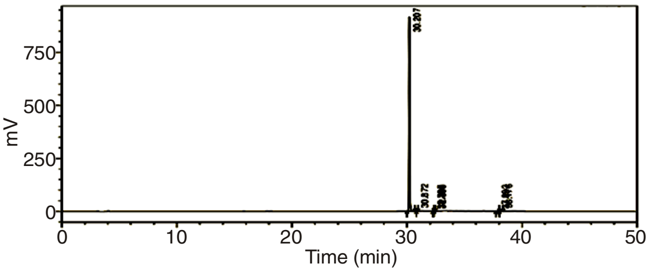


Fig. 3. HPLC chromatogram of apigenin (**7**)

Demethylation of 3',4',5,7-tetramethyl luteolin (**6**), with aluminium chloride in toluene at 110-120 °C for 90 min provided the pure product luteolin (**8**) (**Scheme-II**). The yield was 70 % with a purity of 97 % [20] (Fig. 4).

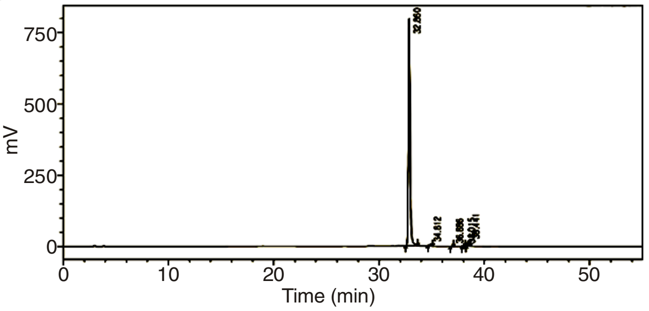


Fig. 4. HPLC chromatogram of luteolin (**8**)

Conclusion

The process reported is economical and can be used for the synthesis of apigenin or luteolin in a commercial scale.

TABLE-1
DEVELOPMENTS IN DEMETHYLATION REACTIONS FOR APIENIN

S. No.	Reagent	Solvent	Temperature (°C)	Time (h)	Yields
1	$AlCl_3$/N,N-Dimethyl aniline	Dichloromethane	Room temperature	1.0	Only di was formed
2	$AlCl_3$/N,N-Di methyl aniline	Chloro benzene	100	3.0	Mono, di, tri are formed
3	$AlCl_3$/N,N-Di methyl aniline	Chloro benzene	150-160	1.0	80 % tri was formed
4	Pyridinium chloride	Dichloromethane	Room temperature	1-48	Di and starting material
5	$AlCl_3$/Triethyl amine	Dichloromethane	Room temperature	1.0	Only di was formed
6	HBr/CH_3COOH	–	120-140	3.0	Mixture was formed
7	HBr/H_2O	–	120-140	3.0	Mixture was formed
8	$AlCl_3$	Toluene	120	1.5	Single spot (tri) was formed
9	$AlCl_3$	Chloro benzene	150-160	1.5	Single spot tri was formed

TABLE-2
DEVELOPMENTS IN DEMETHYLATION REACTIONS FOR LUTEOLIN

S. No	Reagent	Solvent	Temperature (°C)	Time (h)	Yields
1	$AlCl_3$/N,N-Dimethyl aniline	Toluene	120-140	1.0	Tri and tetra are formed
2	$AlCl_3$/N,N-Dimethyl aniline	Without solvent	160	3.0	Tri and tetra are formed
3	$AlCl_3$/N,N-Dimethyl aniline	Without solvent	170	1.0	Tri and tetra are formed
4	$AlCl_3$/N,N-Dimethyl aniline	Without solvent	140	1.0	Tri and tetra are formed
5	$AlCl_3$/N,N-Dimethyl aniline	Without solvent	120	1.0	Tri and tetra are formed
6	$AlCl_3$/Tetra methyl ethylene diamine	Without solvent	170	1.0	Tri and tetra are formed
7	$AlCl_3$/Pyridine/KI	DCE	70-80	1.0	Di was formed
8	$AlCl_3$/N,N-Dimethyl aniline/KI	Without solvent	140	1.0	Tri and tetra are formed
9	HBr/CH_3COOH	–	120-140	3.0	Mixture was formed
10	HBr/H_2O	–	120-140	3.0	Mixture was formed
11	$AlCl_3$	Toluene	120	1.5	Single spot (tetra) was formed
12	$AlCl_3$	Chloro benzene	150-160	1.5	Single spot (tetra) was formed

The process can be utilized for the synthesis of other flavonoids also.

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