Extraction of apigenin from German chamomile *Matricaria Chamomilla*
Research question: How many milligrams of apigenin can be extracted from 150 grams of German Chamomile using the method of the College of Light Industry and Food Science, South China University of Technology[1], to prepare apigenin from *Adinandra nitida*?

Chemistry

[1] Liu, Benguo, et al. "Preparing Apigenin from Leaves of Adinandra Nitida." *Food Technology and Biotechnology*, vol. 46, no. 1, 14 Mar. 2008, pp. 111–115.

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1. Introduction

This work studies the reliability of chamomile products for positive health impacts. The first section of the work covers the origins of the use of chamomile as medical treatment, as well as the extent of scientific evidence to support the belief. The later sections focus on expanding how the active substance in chamomile, apigenin, could be utilized and produced in the medicine industry. Finally, I attempt to extract apigenin from the flower to study how the health effects of domestic chamomile to global literature.

Many of us are familiar with "natural food" products that are claimed to be able to help people relax, reduce stress and anxiety, and provide a cure for numerous health problems. One of the most popular medical herbs is chamomile, which is familiar to many, especially from tea products that are claimed to be the secret to a good night sleep. At least intuitive support based on research evidence can be provided for the claimed health benefits of chamomile. The plant contains a significant number of natural flavones, which are believed to help with, to name a few, inflammatory conditions, menstrual problems, a common cold, and cardiovascular diseases.[2] Flavones are a subgroup of flavonoids, organic compounds that are found in plants, fruits, and vegetables. They are considered to have high nutritional value and are understood to contribute beneficially to one's health when present in a balanced, healthy diet. [3] In chamomile, the flavone responsible for the previously claimed health proprieties is apigenin, which is claimed to have sedative effects if consumed in large amounts. The possible sedative properties of chamomile are often used in advertisements for "natural food" products, such as herbal tea or essential oils, that are claimed to provide solutions to sleep issues and other health problems. However, modern medicine rarely supports the idea of super compounds, that can cure every physical issue. Instead, precisely tailored drug combinations are used to treat specific health issues. Thus, one should not forget to be sceptical of the extent of health benefits that enjoying for example a cup of herbal tea can provide, especially when more serious issues like anxiety or sleep problems are in question. I wanted to discover to what extent the so-claimed health benefits of chamomile consumption are true, and how traditional medical approaches compare to the results achieved with modern medical solutions to treat health problems. Thus, I decided to conduct this research on the quantity of apigenin in chamomile.

2. Background information

2.1. Chamomile

There are two chamomile plants Roman Chamomile *Chamaemelum Nobile* and German Chamomile *Matricaria Chamomilla*. Roman Chamomile is native to Europe, North America and South America and German Chamomile to southern and eastern Europe. When it comes to the therapeutic potential of

[2] Gupta, Sanjay. "Chamomile: A Herbal Medicine of the Past with a Bright Future (Review)." *Molecular Medicine Reports*, vol. 3, no. 6, 28 Sept. 2010, www.ncbi.nlm.nih.gov/pmc/articles/PMC2995283/, https://doi.org/10.3892/mmr.2010.377.

[3] Hostetler, Gregory L, et al. "Flavones: Food Sources, Bioavailability, Metabolism, and Bioactivity12." *Advances in Nutrition*, vol. 8, no. 3, 5 May 2017, pp. 423–435, www.ncbi.nlm.nih.gov/pmc/articles/PMC5421117/, https://doi.org/10.3945/an.116.012948.

the species, their chemical and biological properties are almost identical. Thus, both have been used historically to treat similar medical disorders, as they contain similar contents of the therapeutic compound we are targeting in our study, apigenin. I will be using the German Chamomile in my experiment because that is the chamomile plant found in my country.

2.2. The medical history of chamomile

Perhaps due to the wide geological distribution of chamomile flowers, significant records of the use of the plant in early medicine exist extensively across the world. The medical use of chamomile dates to ancient times. The first appearance of the use of *Chamaemelum Nobile* has been traced to traditional Chinese medicine[4] with records from approximately 5000 years ago. The first detailed medical records of chamomile are found in traditional Uighur medicine, which is known for its progressive understanding of the use of herbal treatment for illnesses. During the wide use of the plant in different times and different countries, the plant has been used as a treatment for almost every bodily issue, including allergies and diarrhoea, although some of these uses lack scientific background in modern medicine. Other records of the use of chamomile treatments can also be found in ancient Egypt and medieval time central Europe. Additionally, the roots of German Chamomile in Finland stem from traditional medicine. The plant was first brought here to be cultivated for medical purposes.[5] However, historically, and up to today only the flowers of the plant have been used as treatments.

Figure 1. *Chamomile Matricaria* [6]

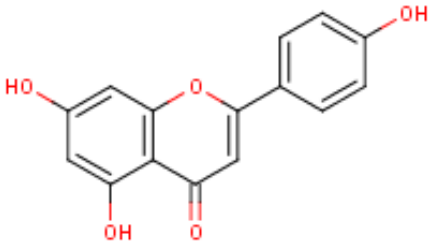
[4] Dai, Yun-Lei, et al. "Chamomile: A Review of Its Traditional Uses, Chemical Constituents, Pharmacological Activities and Quality Control Studies." *Molecules*, vol. 28, no. 1, 23 Dec. 2022, p. 133, https://doi.org/10.3390/molecules28010133

[5] "Kamomillasaunio, Matricaria Chamomilla - Kukkakasvit - LuontoPortti." *Luontoportti.com*, luontoportti.com/t/1350/kamomillasaunio

[6] Rignanese, Luigi. "Matricaria Chamomilla L.," *Forum Acta Plantarum*, Feb. 2008.

2.3. Apigenin

Figure 2. apigenin [7]



Apigenin, by its IUPAC name *5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one*, is a natural flavone, that can be found in chamomile, and many other plant species[8].

Due to its historical significance in traditional medicine, apigenin is one of the most studied flavones. Its therapeutic potential has been analysed for skin conditions, cancer prevention, anxiety, Alzheimer's prevention, anti-inflammatory properties and many more[9]. However, the physiological impact of the flavone on the human body is not fully understood in modern medicine. Thus, if apigenin is used as a treatment, it is not often used solemnly alone, but instead, it is combined with other drugs to reach the maximal potential.

However, chamomile does not contain excessive quantities of apigenin. According to the research published by Sanjeev Shukla[10] infusions and other extracts of *Matricaria Chamomila,* such as herbal tea, contain only from 0.8% to 1.2% of apigenin. Despite the reputation of the flower, some plants have higher apigenin contents than chamomile. Dried parsley hosts 45 000µg of apigenin per gram of the herb, whereas the apigenin content of the chamomile plant is around 3 000µg to 5 000µg per gram of the plant[11].

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[7] "Apigenin (CHEBI:18388)." *Www.ebi.ac.uk*, www.ebi.ac.uk/chebi/searchId.do?chebiId=CHEBI:18388.

[8] PubChem. "Apigenin." *Pubchem.ncbi.nlm.nih.gov*, 4 Sept. 2004, pubchem.ncbi.nlm.nih.gov/compound/apigenin#section=Structures.

[9] Salehi, Bahare, et al. "The Therapeutic Potential of Apigenin." *International Journal of Molecular Sciences*, vol. 20, no. 6, 15 Mar. 2019, p. 1305, www.ncbi.nlm.nih.gov/pmc/articles/PMC6472148/, https://doi.org/10.3390/ijms20061305. Accessed 14 June 2023.

[10] Shukla, Sanjeev, and Sanjay Gupta. "Apigenin: A Promising Molecule for Cancer Prevention." *Pharmaceutical Research*, vol. 27, no. 6, 20 Mar. 2010, pp. 962–978, https://doi.org/10.1007/s11095-010-0089-7.

[11] Sung, Bokyung, et al. "Role of Apigenin in Cancer Prevention via the Induction of Apoptosis and Autophagy." *Journal of Cancer Prevention*, vol. 21, no. 4, 30 Dec. 2016, pp. 216–226, https://doi.org/10.15430/jcp.2016.21.4.216.

Although studies of the sedative effects of apigenin have not been conducted with humans, rat experiments show that 25mg/kg[12] was the minimum dosage for tranquillizing effects to be achieved on rats. To understand the stated information on human physiology, *The Finnish Institute for Health and Welfare* (THL) published the results of the *FinTerveys2017* study, which reported that the average weight of a Finnish adult is 78.8kg[13]. This would mean, that an average Finnish adult would need to enjoy 1.97g of apigenin to obtain the sedative effects of the substance. This 1.97g could be obtained from 390g to 650g of chamomile. For reference common tranquilising drugs like Alprazolam should be enjoyed only at a dosage of approximately three milligrams for similar effects.[14] Thus, the use of chamomile as a source of apigenin in a modern context does not seem reasonable for treating stress or anxiety. Moreover, the effects of tranquillizing drugs of common use, including apigenin, are not strong enough to be the sole reason behind an individual falling asleep. Instead, tranquillizing drugs might make it easier for one to fall asleep due to the bodily relaxation felt. Additionally, the medical effects of a substance on rats do not necessarily correlate with the effects for humans due to the biological differences of the species. Thus, the masses required for the positive effects of apigenin consumption are only a rough estimation of the mass of apigenin required for the sedative results in the human body.

For the practicality of my experiment, I need to scale my experiment to a level that is feasible in the school environment, without producing too low a yield of apigenin. Accordingly, I am doing my experiment with 150g of apigenin. Thus, I would expect the yield of apigenin obtained from the *Matricaria Chamomilla* in my experiment to be between 450 and 750 milligrams.

3. Medical profile of apigenin and its physiological reactions in the human body

Although studies show that apigenin has sedative effects on test rats[15], the reason why this physiological effect occurs is yet unclear. The sedative effects have been claimed to result from the interaction between apigenin and the gamma-aminobutyric acid (GABA) receptors. The GABA receptors are a part of the nervous system, that control the chemical messages of the nervous system via the transmission of ions. The GABA receptor antagonists can bind to the receptors, increasing or decreasing the transmission of ions. This affects the quantity of messages sent by the nervous system, which can decrease stress and anxiety levels.

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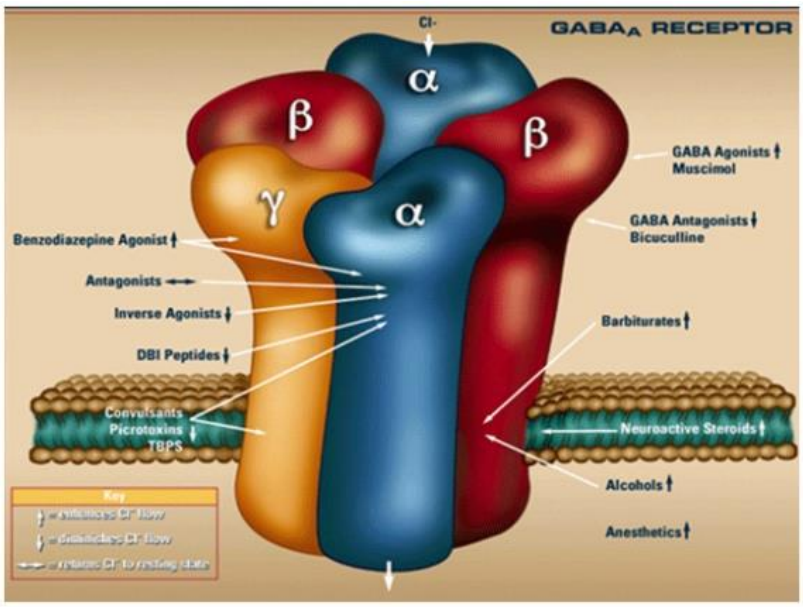
[12] Zanoli, P, et al. "Behavioral Characterisation of the Flavonoids Apigenin and Chrysin." *Fitoterapia*, vol. 71, no. 1, Aug. 2000, pp. S117–S123, https://doi.org/10.1016/s0367-326x(00)00186-6.

[13] Jääskeläinen, Tuija, et al. "Nuorten Aikuisten Terveys Ja Elintavat Suomessa : FinTerveys 2017 -Tutkimuksen Tuloksia." *Www.julkari.fi*, 2019, urn.fi/URN:ISBN:978-952-343-319-9.

[14] "Alprazolam IR Oral Tablet: Side Effects, Uses, Dosage, and More." *Healthline*, 22 July 2021, www.healthline.com/health/drugs/alprazolam-ir-oral-tablet#what-it-is. Accessed 16 July 2023.

[15] Yan, Jun, et al. "Apigenin Accumulation and Expression Analysis of Apigenin Biosynthesis Relative Genes in Celery." *Scientia Horticulturae*, vol. 165, Jan. 2014, pp. 218–224, https://doi.org/10.1016/j.scienta.2013.11.018.

Figure 3. A simplified representation of $GABA_A$ receptor[16]



The most common $GABA_A$ receptor inhibitors are benzodiazepines, which bind to the alpha side of the receptor (see Figure 3.). Although apigenin is not a benzodiazepine, it is speculated to be able to tie to the same protein structure of the receptor, to which larger molecules like benzodiazepines attach themselves. This might be due to the similarities between the general benzodiazepine structure and the molecular structure of apigenin. (see Figures 4. And 5.) Apigenin and benzodiazepines have two benzene rings and an additional ring structure. In addition, the larger or polar functional groups might help the binding of the molecule.

Figure 4. The general structure of benzodiazepines [17]

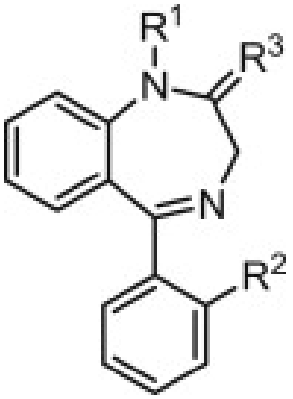
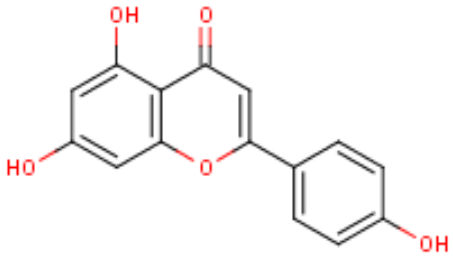


Figure 5. Apigenin



[16] Bourin, Michel . "The GABAA Receptor and Benzodiazepine Acceptor Site." *SOJ Pharmacy & Pharmaceutical Sciences*, vol. 5, no. 2, 16 Mar. 2018, pp. 1–5, https://doi.org/10.15226/2374-6866/5/2/00176.

[17] Pérez-Mayoral, Elena, et al. "Porous Catalytic Systems in the Synthesis of Bioactive Heterocycles and Related Compounds." *Green Synthetic Approaches for Biologically Relevant Heterocycles*, by Goutam Brahmachari, Elsevier, 2015, pp. 377–408.

The assumption that apigenin would bind to $GABA_A$ receptors was based on their similar tranquillizing effects. However, these similarities can only be to a narrow extent to further explain the behaviour of apigenin. Studies on rats have shown, that although apigenin has been found to have similar effects to benzodiazepines, the sedative effects of apigenin could not be further linked to $GABA_A$ receptors.[18] [19] Thus, the chemical remains to be studied to be fully understood. Due to the availability of better-understood sedatives, apigenin technology remains undeveloped. However, apigenin could provide a safer option for the treatment of minor issues, that are currently treated with strongly addictive benzodiazepines. [20]

4. The production of apigenin

Despite the large interest in the use and research of apigenin products in medicine, the chemical is rarely extracted from plants for commercial purposes. The reason for this is, that yields of apigenin reached in the direct extraction of the flavone from plants are so low, that the cultivation and extraction process is not profitable.

Consequently, many alternative routes to produce apigenin in profitable yields are discovered and tested constantly. These applications vary in the use of starting reactants and chemical mechanisms. Thus, I will be introducing a few of these approaches in my essay.

4.1. The flavone conversion of apigenin

Because the direct extraction of apigenin provides low yields, it is synthesized from other compounds, that are easier to be extracted from plants. Due to the large-scale similarities of the structures of all flavones (see Figure 6.) the flavones can be synthesized from other flavones with only a few steps.

[18] Avallone, Rossella, et al. "Pharmacological Profile of Apigenin, a Flavonoid Isolated from Matricaria Chamomilla." *Biochemical Pharmacology*, vol. 59, no. 11, June 2000, pp. 1387–1394, https://doi.org/10.1016/s0006-2952(00)00264-1.

[19] Viola, H., et al. "Apigenin, a Component OfMatricaria RecutitaFlowers, Is a Central Benzodiazepine Receptors-Ligand with Anxiolytic Effects." *Planta Medica*, vol. 61, no. 03, June 1995, pp. 213–216, https://doi.org/10.1055/s-2006-958058.

Accordingly, the synthetic production of other flavones from it might be profitable, if one flavone can be extracted from cultivated products in high yields. These flavanones include naringin, which is present in citrus fruits to up to 3 400 µg per gram. [20] [21]

Figure 6. general structure of flavones with numbered carbons[22]

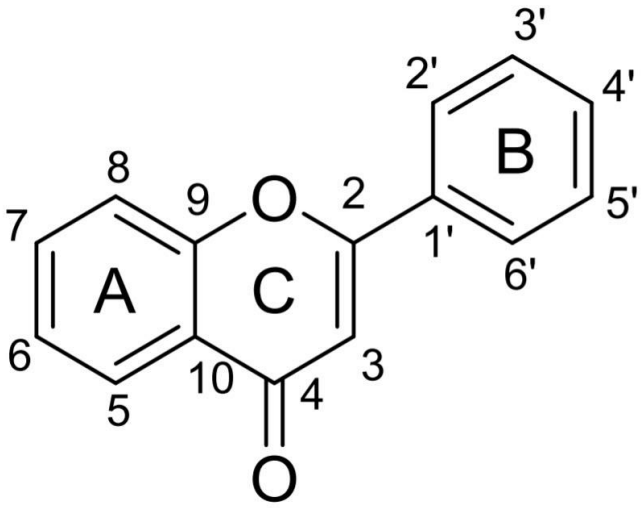


Figure 7. Conversion of naringin to apigenin through rhoifolin

[20] Yusof, Salmah, et al. "Naringin Content in Local Citrus Fruits." *Food Chemistry*, vol. 37, no. 2, Jan. 1990, pp. 113–121, https://doi.org/10.1016/0308-8146(90)90085-i. Accessed 21 May Amin, Insha, et al. "Naringenin (4,5,7-Trihydroxyflavanone) as a Potent Neuroprotective Agent: From Chemistry to Medicine." *Studies in Natural Products Chemistry*, vol. 65, 1 Jan. 2020, pp. 271–300, https://doi.org/10.1016/b978-0-12-817905-5.00008-1

[20]Alkhalidy, Hana, et al. "Dietary Flavonoids in the Prevention of T2D: An Overview." *Nutrients*, vol. 10, no. 4, 31 Mar. 2018, p. 438, https://doi.org/10.3390/nu10040438.

2020.

[21] Amin, Insha, et al. "Naringenin (4,5,7-Trihydroxyflavanone) as a Potent Neuroprotective Agent: From Chemistry to Medicine." *Studies in Natural Products Chemistry*, vol. 65, 1 Jan. 2020, pp. 271–300, https://doi.org/10.1016/b978-0-12-817905-5.00008-1.

[22]Una, Dominica, et al. "Structure-Property Relationship of Flavonoids as Potential Green Inhibitors for Oilfield Scales: A Mini-Review." *Journal of Engineering Research and Reports*, 1 Dec. 2021, pp. 41–51, https://doi.org/10.9734/jerr/2021/v21i617472.

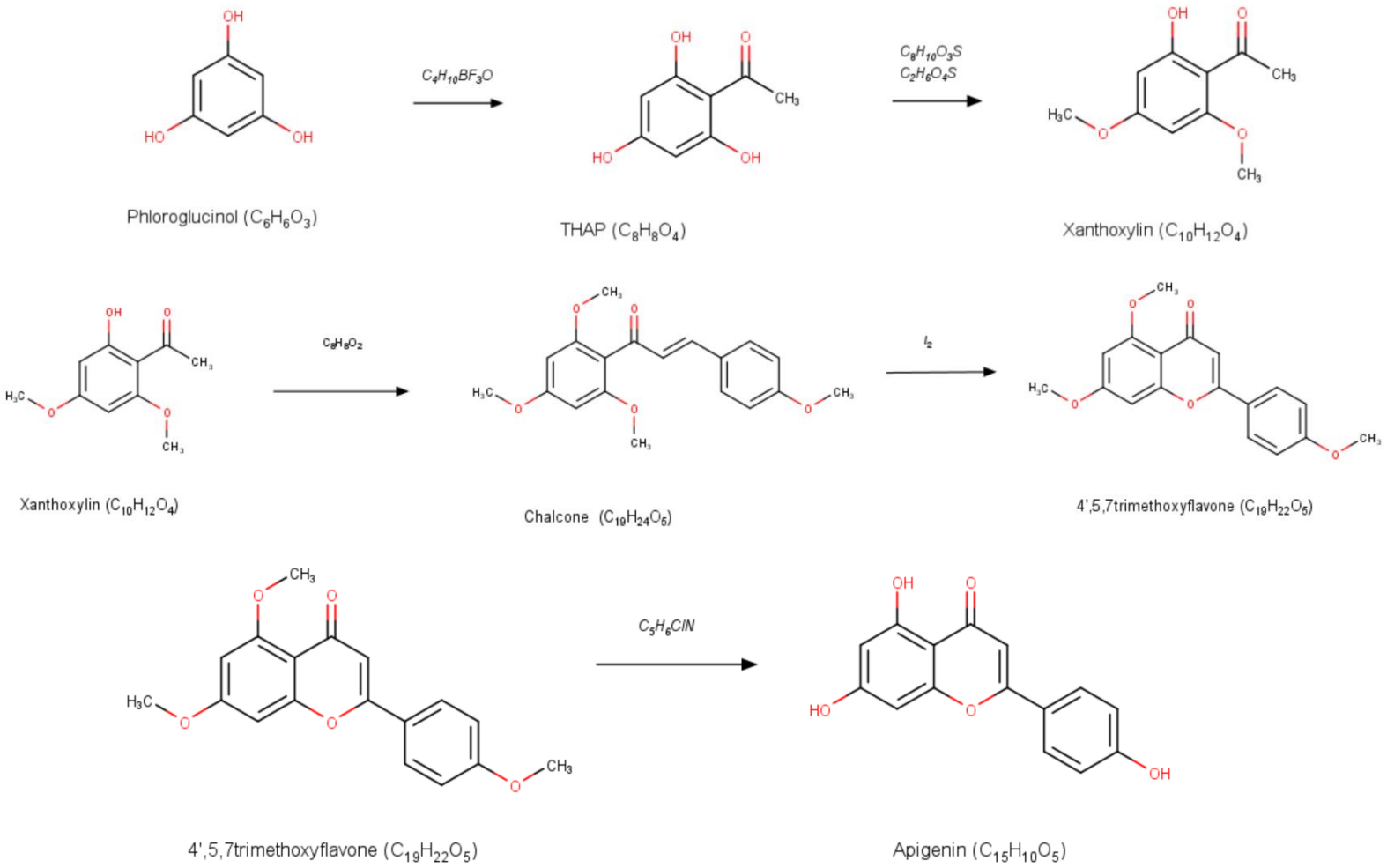
First, naringin is converted to rhoifolin with iodine treatment, which saturates the carbon-carbon double bond between the second and third carbon of the flavone ring C (see figure 6.) After this, the hexagonal functional rings attached to rhoifolin with etheric bonds are attacked by sulphuric acid (H_2SO_4). This reaction is called the acidic cleavage[23] of ethers, where a strong acid attacks the ether complex with a nucleophilic substitution mechanism. This results in the deformation of the etheric bond, which is replaced with hydrogen. Thus, a hydroxyl group is formed, which fulfils the cycle of apigenin creation.

The described method was able to produce 0.41g of apigenin from 3.00g of grapefruit juice, which is a relatively good yield for apigenin synthesis. In addition to sulphuric acid, sodium thiosulphate, and iodine, mainly methanol was used in the extraction of naringin. Additional solvents used included only pyridine, water, and ethanol. Based on the chemicals used the method is overall favourable from an environmental perspective, due to the limited amount of hazardous and unsustainable waste produced. On the contrary, it should be considered the starting ingredient for naringin production is grapefruit, which is a time and water-consuming plant to grow. Although the *Federal University of Bahia* research group used grapefruit peel waste for their research, it is unlikely the method can be rescaled to enable larger-scale production of apigenin. Thus, when evaluating the overall steps of this apigenin production procedure, including the cultivation of citrus fruits, the described method is not likely to be profitable.

[23] Farmer, Steven, and Dietmar Kennepohl. "18.3: Reactions of Ethers- Acidic Cleavage." *Chemistry LibreTexts*, 26 Aug. 2015, chem.libretexts.org/Bookshelves/Organic_Chemistry/Organic_Chemistry_(Morsch_et_al.)/18%3A_Ethers_and_Epoxides_Thiols_and_Sulfides/18.03%3A_Reactions_of_Ethers-_Acidic_Cleavage.

4.2. The synthetic production of apigenin from phloroglucinol[24]

Figure 8. The synthetic pathway from phloroglucinol to apigenin



Faculty of Life Science and Technology of the *Kunming University of Science and Technology* used phloroglucinol as the starting reactant for apigenin production.

Phloroglucinol, or *benzene-1,3,5-triol* by its IUPAC name, *1-(2,4,6-trihydroxyphenyl)ethan-1-one* is a commonly used and relatively inexpensive starting reactant for further synthesis.

Phloroglucinol was acetylated with the Fires rearrangement method by using Boron trifluoride etherate (Figure 9.) as the other reactant. The obtained THAP was then methylated to xanthoxylin *1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone* with the use of Methyl p-toluene sulfonate and dimethyl sulphate (Figure 9.) Xanthoxylin was condensed to chalcone *(2E)-3-(4-methoxyphenyl)-1-(2,4,6-tarimethoxyphenyl)prop-2-en-1-one* with anisaldehyde (Figure 10.) The chalcone was treated with iodine to obtain 4',5,7trimethoxyflavone. With the use of pyridine hydrochloride, the methyl groups of

[24] Wang, Jin, et al. "Total Synthesis of Apigenin." *Journal of Chemical Research*, vol. 36, no. 3, Mar. 2012, pp. 121–122, https://doi.org/10.3184/174751912x13285269293913. Accessed 8 Feb. 2023.

the flavone were replaced by hydrogens forming hydroxyl groups. This demethylation step resulted in the formation of apigenin.

Phloroglucinol is an easily available starting reactant, as the molecule is cheap to synthesise or extract from plants. In addition, the prices of synthetically produced further reactants and solvents used are relatively low. Hence, this method is cost-efficient, and it does not encounter difficulties of agricultural production, unlike the method **4.1.** Thus, this method is feasible and more time-efficient on a large scale than the combination of extraction and synthesis processes, resulting in a more profitable outcome. However, most of the chemicals used are either severely toxic, corrosive, hazardous for the environment, or all the previously mentioned. Therefore, the problems of this method arise from its unsustainability and the great extent of hazardous waste produced.

Figure 9. The structures of boron trifluoride etherate, methyl p-toluene sulfonate and dimethyl sulfate

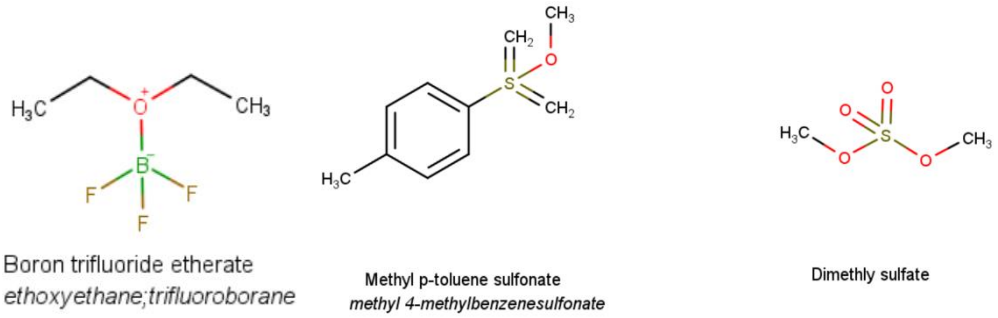
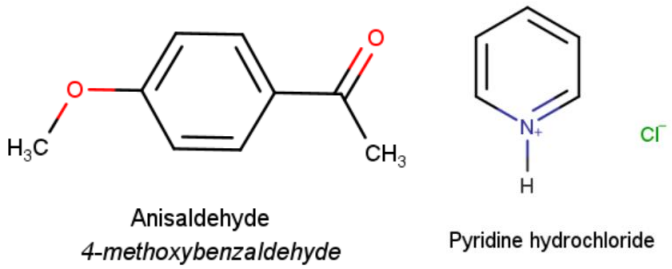


Figure 10. The structures of anisaldehyde and pyridine hydrochloride



4.3. Metabolic engineering of apigenin[25].

As it has been stated previously, only low yields of apigenin can be reached when apigenin, or other flavones that are to be converted to apigenin, are extracted from plants. However, due to the hazardous waste problem of the chemical synthesis of flavones, the cultivation of apigenin products is to be developed to be a more sustainable alternative for apigenin production.

One of the most recent methods includes the biological modification of plants to improve their apigenin content. These solutions include methods like the Flavone Synthase (**FNS**), which is claimed to be more environmentally friendly and cost-efficient than other methods of flavone production. The bioengineering process of apigenin often utilizes plants that are genetically engineered to produce the wanted flavone in large quantities. Apart from chamomile, high contents of apigenin are also found in celery. Because of this celery is often used as a host plant in the biosynthesis of the drug[26]. In detail, the **FNS** method utilizes genetically engineered bacteria. *Escherichia coli (E. Coil)* bacteria cells are engineered to produce the necessary enzymes for flavone synthesis in their metabolic cycle. The reaction pathway substances native to the micro-organism phenylalanine and tyrosine are converted to 4-Coumarate-CoA ligase (**4CL**) via a lengthy pathway. **4CL** product is then converted to naringenin chalcone and naringenin, which are the starting products of **FNS**. The *E. coil* can be modified to produce apigenin because the bacteria has already resources to produce other flavone compounds, which are relatively easy to convert to apigenin. Moreover, genetical engineering allows the improvement of the yields of apigenin reached with this method.

It is assumed that genetically engineered bacteria could be the future key to cost-efficient and quick flavone synthase. However, from now on, the technology is still under development, due to the hindrances of rescaling constantly observed laboratory experiments to standardized large-scale production plans of genetically modified substances.

5. Extraction and detection of apigenin from German chamomile

For my own experiment, I used the method from the *South China University of Technology* to extract apigenin from *Adinandra nitida*. *Adinandra nitida* is a plant native to China, that is also claimed to contain high contents of apigenin. I chose this method since it was feasible in the school laboratory. Additionally, the research group used fresh plant leaves for apigenin extraction, so I supposed, that the method would also work for fresh German chamomile plants. Both the flowers and the stems of German chamomile were used. Furthermore, the method used water as a solvent. Thus, the results

[25] Sheng, Huakang, et al. "Metabolic Engineering of Microorganisms for the Production of Flavonoids." *Frontiers in Bioengineering and Biotechnology*, vol. 8, 2020, p. 589069, pubmed.ncbi.nlm.nih.gov/33117787/, https://doi.org/10.3389/fbioe.2020.589069.

[26] Yan, Jun, et al. "Apigenin Accumulation and Expression Analysis of Apigenin Biosynthesis Relative Genes in Celery." *Scientia Horticulturae*, vol. 165, Jan. 2014, pp. 218–224, https://doi.org/10.1016/j.scienta.2013.11.018.

can be compared to the apigenin content of chamomile infusions. Hence, the method provides a good estimate of the extent of medical benefits of products like chamomile tea.

The chamomile plants were collected in mid-August from a local field with coordinates (60.23; 24.86). The flowers were then stored in a dry, dark environment before the experiment.

5.1. The separation of apigenin from 150g of *Matricaria Chamomille*

Table 1. Concise information about the extraction steps and conditions

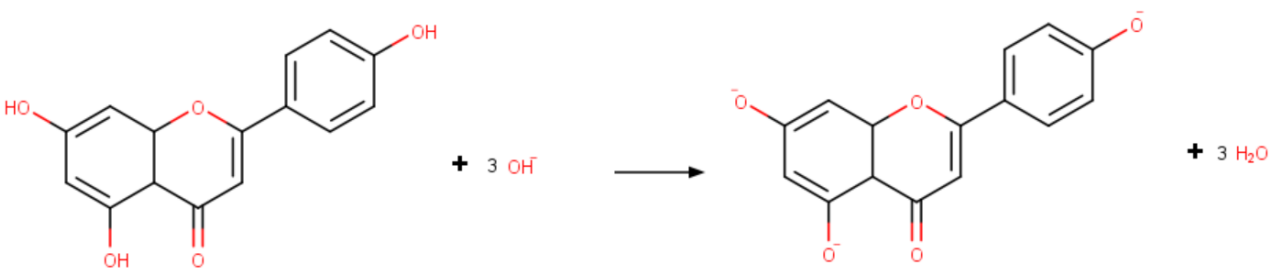
1.	Dissolution with distilled water	Distilled water	100 °C
2.	Precipitation with acid	Concentrated sulphuric acid	90 °C
3.	Crystallization with ethanol	80% Ethanol	Dissolution at 70 °C transferred to 6 °C

I aimed for a 450 to 750-milligram yield of apigenin. For this reason, I used 150 grams of German chamomile. First, the plants were cut into small pieces and 150 grams of the plant was measured. Due to the limited capacity of the school apparatus, I separated my sample into three Erlenmeyer flasks. The samples were boiled in distilled water at 100 °C for one hour to dissolve apigenin from the flowers. The solution was then cooled down. After this, the extract was separated from the mixture by vacuum filtration. The extract was saved, and the flowers were boiled again, to ensure that most of the wanted solutes had been extracted successfully, after which the same vacuum filtration process took place. Next, the volume of the extract was halved to 760ml, by evaporating the rest of the solvent out.

After this step, 15 ml of 95% sulphuric acid (H_2SO_4) was added to the solution. The solution was then heated for 20 minutes at a temperature of 90 °C. This was done carefully to avoid the acidic mixture boiling and thus overflowing. During the acid addition and heating step, a dark precipitate was observed to form on the bottom of the flask.

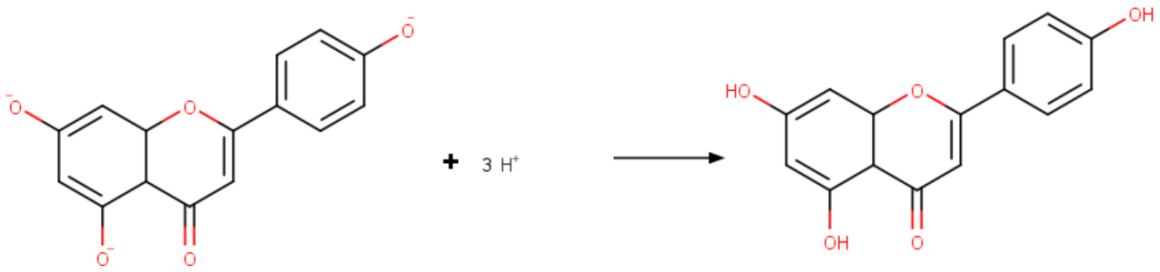
The strong acid H_2SO_4 was used to decrease the solubility of apigenin. Apigenin is soluble in basic solutions, due to the deprotonation of hydroxyl groups. Within basic and relatively neutral environments the highly polar bond between the oxygen and the hydrogen of the hydroxyl group may break, forming H^+ ions and apigenin with anionic properties (Figure 11.). The anionic properties explain why apigenin is greatly soluble in basic solvents due to the strong ion-dipole interactions between the solute and the polar solvent.

Figure 11. the deprotonation of apigenin in basic solution.



However, in strongly acidic environments, the anions are protonated back to apigenin due to interactions with H^+ ions that force the O^- -groups to react with the H^+ ions (Figure 12.). This reduces the solubility and apigenin precipitate is formed.

Figure 12. the protonation of apigenin in acidic solution



According to the instructions I followed, the next step would have been to filter the precipitate out of the solution. However, it turned out that none of our school filters had the capacity to separate the fine precipitate from the solution. Instead, the precipitate got stuck in the filters. After this unsuccessful attempt, the filters were washed with a concentrated sodium hydroxide solution, which dissolved the particles in question. The following step was to turn the solution back to highly acidic with sulphuric acid which caused the particles to precipitate out again.

Thus, I had to separate the precipitate from the acidic solution in another way. I ended up centrifuging the samples and decanting the solvent out. However, this approach was unideal, since all of the solvent could not be decanted out without sample loss.

After this, the precipitate was washed by centrifuging until a neutral pH was obtained. According to the article I followed, the sample should be quite pure after being washed. However, the powder I obtained was dark brown, which was alarming, as pure apigenin is light yellow in colour. This suggested that my sample was highly impure.

Figure 13. washed sample

Figure 14. Recrystallized sample

The final step was to re-crystallize the precipitate with ethanol. Approximately 70-degree ethanol was added to the samples that were kept in a warm water bath. The precipitate was dissolved into the ethanol after which the solution was taken to a refrigerator. The sample was kept in ethanol in the refrigerator for 28 hours. The cool environment causes a decrease in the solubility in ethanol, forcing

the sample to be recrystallized from the solution. The solid was then extracted from the solution by decanting most of the ethanol out, after which the remnants of the solvent were let to evaporate.

5.2. Identification of apigenin with thin-layer chromatography (TLC)

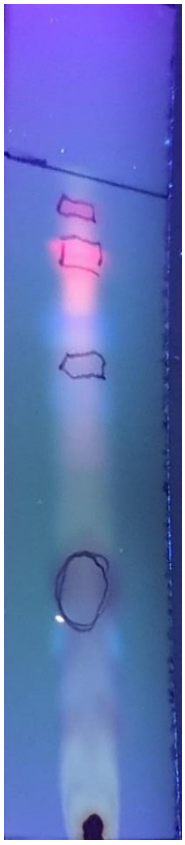
I followed the method established by the Faculty of Medicine of *Siriraj Hospital, Mahidol University*[27] for apigenin identification.

The recrystallized particles were analysed with thin-layer chromatography (TLC). Hexane, ethyl acetate, and acetic acid were used with the 31:14:5 ratio as a mobile phase. This was done in the ventilated hood for safety reasons.

Five thin-layer chromatography trials were done. Table 1. presents the data obtained from each trial.

A trend of two blue dots and two red dots was observed (Figure 16.).

Figure 15. (left) plate obtained by research of the *Mahidol University* **Figure 16.** (right) my TLC results



The red and blue colour pattern obtained with TLC was observed to be almost identical between the results of *Mahidol University* (Figure 15.) and my results (figure 16.). This suggests that apigenin was

[27] Phadungrakwittaya, Rattana. "Identification of Apigenin and Luteolin in Artemisia Annua L. For the Quality Control." *Siriraj Medical Journal*, vol. 71, no. 3, 1 May 2019, pp. 240–245, https://doi.org/10.33192/smj.2019.37.

present in both samples. However, many of the samples had a dark spot, which could be a result of impurities present in the sample. Additionally, the dots obtained in my experiment were a lot higher than in the research compared to. In addition, the pattern is not exactly similar in every sample as the distances between red and blue dots were not constant in comparison to the other samples (Table 2.). For this reason, they were interpreted as a singular dot by the University research, but they appeared as two separate dots on my TLC analysis.

5.3. calculating the retention factor (RF) value.

The distances between the dots were collected by using a ruler (uncertainty $\pm\ 0.10\ cm$).

Table 2. presents the distances travelled by different point patterns on the TLC plate in centimetres. Yellow row refers to the impurities, blue rows to the blue lines and red rows to the red line patterns.

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.
Height travelled by solvent	*4.5*	*4.8*	*5.2*	*5.1*	*5.1*
Dot 1.	1.7	1.6	1.8	-	-
Dot 2.	3.1	-	-	-	2.9
Dot 3.	3.3	3.8	-	3.2	3.1
Dot 4.	4.0	4.2	4.1	4.0	4.1
Dot 5.	4.3	4.4	4.8	4.7	4.7

From these distances, the RF values were calculated with the following formula.

$$RF = \frac{distance\ travelle\ by\ solute}{distance\ travelled\ by\ solvent}$$

$$RF_{sample1.height2.} = \frac{3.10cm\ \pm 0.10cm}{4.50cm\ \pm 0.10cm} = 0.69cm\ \pm 0.20cm$$

Table 3. presents the RF values of different points on the TLC plate in centimetres.

	Sample 1.	Sample 2.	Sample 3.	Sample 4.	Sample 5.	Average
Dot 1.	0.37	0.33	0.35	-	-	0.35
Dot 2.	0.69	-	-	-	0.57	0.63
Dot 3.	0.73	0.78	-	0.63	0.61	0.69
Dot 4.	0.89	0.86	0.82	0.77	0.80	0.83
Dot 5.	0.95	0.92	0.92	0.92	0.92	0.93

Moreover, the obtained RF values do not match the ones provided by the research. Both experiments used the same solvent combination. However, the original report used Silica gel 60 RP-18 F_{254s} but the plates accessible in our school were Silica gel 60 F_{254s}. Even though the obtained colour pattern was similar the RF values I obtained differed greatly from what was found in the research, considering that

both the TLC plate used, and solvent combinations were replicated according to the reference article. The RF values presented by the article were 0.24, 0.21 and 0.09, whereas the values we got for our patterns were on average 0.63, 0.69, 0,83 and 0.93.

6. Evaluation

Almost no product was obtained using the method. The mass of the impure washed sample remained to be around 0.002g before recrystallization. For this reason, only TLC analysis was conducted. Considering that the theoretical yield of my experiment was between 450mg and 750mg of apigenin, the mass of the final product was alarmingly low. The yield of the sample obtained was too small for any further analysis to be conducted.

Nevertheless, the results of my research show, that *Matricaria Chamomille* does not have enough apigenin to provide any significant tranquillizing effects, as only a small amount of possible apigenin product was found from chamomile. Based on this data, the health benefits of the flower in question are rather common misconceptions than scientifically proven. Thus, it is evident based on the outcome of this research, that enjoying a cup of chamomile tea, or even a Liter, does not contain enough apigenin to cause an individual to fall asleep.

However, the method I used included several problematic assumptions, that might have resulted in the low yield of the final product. The issues included the assumptions of solubility properties of apigenin. Apigenin is only a little water-soluble[28]. This might have caused issues when apigenin was first dissolved in water. Moreover, even the presence of strong acid does not necessarily result in all the apigenin precipitating out. This is because an acidic environment only reduces the solubility of the substance to a certain extent, which does not make apigenin completely insoluble in water. Moreover, none of the steps prescribed allowed the separation of apigenin from other flavones that are present in the plant. Most green plants have numerous different flavones, which have chemically similar properties. Thus, eliminating and separating the unwanted products would have been crucial in obtaining pure apigenin as the final product. This could have been done with for example solid-phase extraction, enzyme-assisted extraction, or pressurized liquid extraction[29].

In addition, I could have chosen the used plants better to maximise the content of the wanted chemicals. The flowers used were fresh, and a large proportion of their weight came from water. To maximise the proportion of apigenin, I could have dried the flowers before my experiment. Additionally, I differed from the original method a couple of times, either due to practicality or available apparatus reasons. This included for example decreasing the volume of water before acid was added to the solution, to obtain the lowest possible solubility. However, to maximise the amount of precipitate forming, the water content could have been reduced even further.

[28] PubChem. “Apigenin.” *Pubchem.ncbi.nlm.nih.gov*, 4 Sept. 2004, pubchem.ncbi.nlm.nih.gov/compound/apigenin#section=Structures.

[29] Chaves, Jaísa Oliveira, et al. “Extraction of Flavonoids from Natural Sources Using Modern Techniques.” *Frontiers in Chemistry*, vol. 8, 25 Sept. 2020, https://doi.org/10.3389/fchem.2020.507887.

Moreover, the TLC results might have suffered, due to the samples absorbing moisture as they were not kept in a desiccator. This could result in the solutes travelling as narrow vertical lines, instead of clear horizontal patterns, which makes the data interpretation difficult. Additionally, I had to construct the mobile phases from chemicals available in the school. However, the purity of these chemicals is likely to be a lot lower than the chemicals for mobile phases used in actual research. These could explain the large differences between my RF values and the RF values of *Mahidol University.*

Even though the issues regarding the quality of the school apparatus complicated my ability to follow the method of apigenin extraction by *The South China University of Technology*, the overall low yield of the product, and its impurity cannot be explained by the modifications I made to the method. Instead, I would argue that this method is not suitable for apigenin extraction from *Chamomile Matricaria* due to the misleading assumptions of chemical properties and biological complexity of the flower made.

7. Extension

The final conclusion of my research is that the apigenin extraction method Preparing *Apigenin from Leaves of Adinandra nitida* by the *South China University of Technology* is not sufficient for the determination of the apigenin content of *Chamomile Matricaria*. Although unequivocal product identification results were not obtained with TLC, other methods that require also small amounts of product could have been tried. These include infrared spectroscopy (IR), proton nuclear magnetic resonance (H-NMR), carbon nuclear magnetic resonance (C-NMR) and mass spectroscopy (MS) analysis.

However, obtaining unsuccessful results based on another scientific article taught me that open communication and honest peer reviews are extremely significant regarding progress in the scientific world. I understood that improvements in developing areas of inquiry can be only made when other scientists communicate their misfortunes in research honestly so that others can adopt the methods in a better direction. Unfortunately, all scientific schools do not follow the stated ideology due to hopes of a better reputation and success for publications.

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