"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST

DOCTORAL SCHOOL

MEDICINE

TASTE ACTIVE NATURAL COMPOUNDS AND THEIR BIOLOGICAL POTENTIAL

PHD THESIS SUMMARY

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List with scientific papers

Studies published in Journals

1. **Gradinaru TC** [#], Petran M [#], Dragos D [#], Gilca M. PlantMolecularTasteDB: A Database of Taste Active Phytochemicals. Front Pharmacol. 2022 Jan 12;12:751712. https://doi.org/10.3389/fphar.2021.751712.([#] Equal contribution)
   Factor de Impact 2022=5.6 *(Chapter 6)*

2. Dragoș D [#], Petran M [#], **Gradinaru TC** [#], Gilca M. Phytochemicals and Inflammation: Is Bitter Better? Plants (Basel, Switzerland). 2022 Nov;11(21). https://doi.org/10.3390/plants11212991.([#] Equal contribution)
   Factor de Impact 2022=4.5; *The article was awarded by UEFISCDI*; *(Chapter 7)*

3. **Grădinaru TC**, Gilca M, Vlad A, Dragoș D. Relevance of Phytochemical Taste for Anti-Cancer Activity: A Statistical Inquiry. Int J Mol Sci. 2023 Nov 12;24(22):16227 https://doi.org/10.3390/ijms242216227.
   Factor de Impact 2022=5.6 *(Chapter 8)*

4. **Grădinaru TC,** Vlad A, Gilca M. Bitter Phytochemicals as Novel Candidates for Skin Disease Treatment. Curr Issues Mol Biol. 2023 Dec 30;46(1):299–326. https://doi.org/10.3390/cimb46010020.
   Factor de Impact 2022=3.1 *(Chapter 3)*

Participation in Conferences, Congresses

1. **Grădinaru TC**, Petran M, Dragoș D, Gîlcă M. Anticancer mechanisms of natural compounds. In: International Summer School FOOD SAFETY AND HEALTHY LIVING. Bucharest, 3-6 September 2023 – oral presentation
2. **Gradinaru TC**, Petran M, Dragos D, Gilca M. TOP 20 OF INFLAMMATION-RELATED MOLECULAR TARGETS OF BITTER TASTANTS. In: 22nd International Congress of International Society for Ethnopharmacology & 10th International Congress of Society for Ethnopharmacology. Imphal, Manipur, India, 24-26 February 2023; - poster and poster presentation
3. Dragos D, **Gradinaru TC**, Petran M, Gilca M. PLANTMOLECULARTASTEDB: A NEW TOOL IN CHEMOSENSORIAL APPROACH. In: 21stInternational Congress of the International Society for Ethnopharmacology, 28-31 May 2022, Taichung, Taiwan,. – awarded poster
4. **Gradinaru TC**, Gilca M. CODE OF TASTE IN TRADITIONAL CHINESE MEDICINE- A GENETIC KEY? In: 20th International Congress of the International Society for Ethnopharmacology, 18- 20 April 2021, Thessaloniki, Greece. – awarded poster
5. Dragos D, Petran M, **Gradinaru TC**, Gilca M. DO BITTER PHYTOCOMPOUNDS TARGET INFLAMMATION-RELATED MOLECULES? In: 20th International Congress of the International Society for Ethnopharmacology, 18-20 April 2021, Thessaloniki, Greece. – poster

Particularities

1. Obtaining the Benelux Office for Intellectual Property (BOIP) certificate (i-DEPOT number 122867) for the PMTDB (**PlantMolecularTasteDB**) concept (Marilena-Gîlcă Vasile, Dorin Dragoș, **Teodora-Cristiana Grădinaru**, Mădălina Petran)

2. Obtaining an award from the 6 offered by Giract's European FlavorPhD Research Awards 2019–2020 for the best research projects of the first year of the PhD, award obtained on 20 December 2019

Introduction

Presentation of the field of the doctoral thesis

This doctoral thesis has a multidisciplinary character, being at the confluence of several fields: biochemistry, pharmacognosy, ethnopharmacology, biology.

Although used for millennia in the treatment of various ailments, medicinal plants still represent an area of interest for researchers, being a huge reservoir of compounds with great structural diversity endowed with biological potential [1,2].

The vast majority of phytocompounds are characterized by a taste profile [3]. Given the fact that taste receptors have also been identified extraorally, it is hypothesized that taste may have other physiological roles than the sensory one [4,5].

The purpose of the doctoral thesis

The aim of this PhD thesis is to create a freely accessible online database that includes taste/orosensorially active phytocompounds and to investigate the impact of taste/orosensation of these phytocompounds, as well as the impact of chemical classes regarding the presence of anti-inflammatory and anti-cancer activity.

Content of the doctoral thesis

This PhD thesis is organized into two sections, namely: a general part comprising *Chapters 1, 2 and 3* and a special part comprising *Chapters 4,5,6,7,8 and 9.*

Chapter 1 describes general information about ion channels and taste receptors involved in the perception of taste or taste sensations. Taste active compounds are also given as example.

Chapter 2 includes the detailed description of bitter taste receptors (TAS2R), including the following aspects: TAS2R structure, receptor types, their associated cascade signaling, their expression and localization, their potential roles, followed by the description of TAS2R agonists. *Chapter 2* also focuses on the involvement of bitter natural compounds in inflammation and cancer, as well as the involvement of TAS2R in the same processes.

Chapter 3 includes data obtained in a literature review study on the existence of bitter taste receptors in skin structures. This chapter describes the potential roles of bitter receptors in the skin or skin cells, as well as changes in the expression of these receptors in the skin or skin cells under various conditions. Also detailed here are some examples of phytocompounds, agonists of TAS2R, with beneficial roles in experimental models of dermatological diseases.

Chapter 4 illustrates the working hypothesis and general objectives that have been pursued.

Chapter 5 contains general research methodology (research steps to achieve the objectives, data mining concept, statistical analysis).

Chapter 6 contains the presentation of the online accessible database, called PlantMolecularTasteDB (https://plantmoleculartastedb.org/index.php), a national and international novelty, as it comprises a large number of taste active phytocompounds (over 1500 phytocompounds) that are described in detail regarding the corresponding identifiers from international databases, molecular formulas, chemical structures, SMILES, organoleptic classification, taste receptors on which they act and other sensory data, chemical classes and subclasses to which they belong, as well as anti-inflammatory activity of these compounds that it is documented from three internationally known databases, all

this information being supported by bibliographic references. Furthermore, studies regarding the anti-inflammatory activity of phytocompounds are accompanied by the mention of the experimental model on which the research was carried out (*in vitro*, animal studies or clinical studies). The result of this project - PlantMolecularTasteDB, the realization of which involved a long team effort (that lasted several years), represents the foundation of the doctoral thesis because the following two studies had this database as their starting point.

Chapter 7 assesses the impact of taste/taste sensations of phytocompounds on their anti-inflammatory activity through data mining PlantMolecularTasteDB. This chapter also evaluates the existence of associations between the chemical class to which the phytocompounds belong and the anti-inflammatory activity.

Chapter 8 evaluates the impact of phytocompound tastes or orosensations on anti-cancer activity through data mining PlantMolecularTasteDB. Associations between chemical classes of phytocompounds and anti-cancer activity are also illustrated. In this chapter it is also showed that there is a strong association between the anti-inflammatory effect and the anti-cancer effect of phytocompounds.

Chapter 9 presents the conclusions resulting from this doctoral study, as well as the personal contributions.

1. Taste receptors, ion channels involved in the detection of taste or orosensasions and taste active natural compounds

By integrating the data from modern taste science and the information from Traditional Chinese Medicine and Ayurveda results in the following types of tastes - sour, sweet, salty, umami and bitter - and trigeminal orosensory sensations - astringent, pungent [6–8].

The term "taste active compound" was used in the present paper to denote a compound that produces an orosensation (including the sensation of spicy or astringent; spicy or astringent being actually considered trigeminal sensations, in contrast to classical tastes which are orosensations mediated by cranial nerves VII, IX, X)[9].

Both receptors and ion channels are involved in the detection of taste/orosensation.

Phytocompounds have representatives in many chemical classes, such as: flavonoids [10], alkaloids [11], terpenoids [12],coumarins [13], etc

2. Bitter taste receptors (TAS2R), natural bitter compounds, bitter taste receptors agonists, roles in inflammation and cancer

Bitter taste receptors (TAS2R) are present both in the oral cavity and in extraoral locations [4].

Some receptors can bind an impressive diversity of compounds while others can have some selectivity [14].

TAS2Rs are expressed in various systems (for example, in the skin[15,16], in the muscular system [17], in the lung [18,19]).

Natural bitter compounds, bitter taste receptor agonists

Although only 26 types of bitter taste receptors have been described to date, there is an impressive variety of bitter compounds that can bind to these receptors (there are about 270 ligands and about 800 ligand-receptor interactions) [20].

Some compounds are able to bind to multiple TAS2Rs. An example of this is represented by amarogentin which is able to activate seven bitter taste receptors [14].

Bitter natural compounds and bitter taste receptors in inflammation

The potential involvement of TAS2R in the inflammatory process was also issued by Maria Ekoff and colleagues who showed that specific agonists of TAS2R inhibited the release of pro-inflammatory mediators (histamine, PGD2) from preactivated mast cells (umbilical cord blood-derived mast cells)[21].

In a study of nasal mucosa samples taken from patients with chronic rhinosinusitis, it was revealed that in these samples there is a higher expression of TAS2R38 receptors compared to the nasal mucosa of subjects without chronic rhinosinusitis [22]

Bitter phytocompounds belonging to certain chemical classes have been cited more frequently as having anti-inflammatory activity (eg, phytocompounds belonging to the flavonoid class [23].

Bitter natural compounds and bitter taste receptors in cancer

In a study published in 2019, noscapine, via TAS2R, can affect cell survival in ovarian tumor cells by inducing apoptosis [24].

According to the conclusions of several researchers who have comprehensively reviewed the available information related to the expression of bitter taste receptors in cancer, there is in most cases, with few exceptions, a trend of decreased TAS2R mRNA and protein expression in cancer cells compared to normal [25,26].

There are numerous studies in the literature regarding the benefits of agents that are bitter in cancer, both in vitro and in vivo experimental models[27–30].

Bitter phytocompounds belonging to certain chemical classes have been cited more frequently as having anticancer activity (e.g. from the flavonoid class [31]

To what extent anti-cancer effects of bitter agonists are mediated by the direct involvement of TAS2R and their associated signaling pathways remains therefore a direction of future research.

3. Bitter natural compounds, bitter taste receptors (TAS2R) and skin

The bitter taste receptors expression in the skin shows both intra-individual and inter-individual variation. This difference in the bitter taste receptors expression in the skin is due to factors such as age, sex, sun exposure, as well as the location of the areas on the body map [15,32].

It can be stated that bitter taste receptors are expressed in all 3 layers of the skin, as their expression was detected at the level of keratinocytes [33–35], adipocytes [36], fibroblasts [34], lymphocytes [37].

The following roles of TAS2R in skin have been proposed: chemosensory function [35], hair growth modulation [38], modulation of lymphocyte migration in the skin [37], etc.

Numerous bitter phytocompounds, have beneficial roles in inflammation as well as skin carcinogenesis.

An example is represented by apigenin which is a known agonist of TAS2R14, TAS2R39 [39]. In a mouse model of induced psoriasis, apigenin reduced erythema, scaling, reduced protein expression of IL-1β, IL-6, TNF-α in the skin [40].

Given the presence of bitter taste genes and receptors in the skin, the functionality of TAS2R at this level, as well as the involvement of various bitter phytocompounds, agonists of these receptors, in certain experimental models of pathologies (e.g. atopic dermatitis, psoriasis, squamous cell carcinoma), the pharmacological potential of bitter compounds is worth studying in the future, as it may have unsuspected therapeutic implications in dermatological conditions.

4. Working hypothesis and general objectives

The general hypothesis of this doctoral thesis can be succinctly formulated as follows: The taste of (phyto)compounds and/or chemical classes of phytocompounds can play the role of a pharmacological descriptor and can have a predictive potential of biological actions (anti-inflammatory, anti-cancer).

General objectives were represented by:

- developing a database made up of phytocompounds that are taste active and that contains information about their chemical structure, chemical class to which they belong, activated taste receptors, anti-inflammatory activity
- investigation of the database developed in the previous stage regarding potential associations: taste of phytocompounds and biological activity (anti-inflammatory, anti-cancer) as well as chemical class of phytocompounds and biological activity (anti-inflammatory, anti-cancer),

5. General research methodology

In this chapter I described the development of this doctoral project, as well as the general methods that led to the fulfillment of the objectives of this doctoral thesis.

Data mining is the process of searching and analyzing a large group of raw data to identify patterns and extract useful information [41].

Statistical analysis of the data from the studies presented in Chapter 7 and Chapter 8 was performed using the R Foundation for Statistics language and programming environment, version 4.0.3.

The results were considered statistically significant if the p-value was below 0.05 (universally accepted threshold). If multiple comparisons were used, the Bonferroni correction was applied, which determined the occurence of a new level of statistical significance represented by dividing the value of 0.05 by the number of comparisons used [42].

The term association was used to describe the statistical relationship between two variables.

6. PlantMolecularTasteDB – an innovative database

6.1. Introduction

The first objective of the doctoral studies was to create a database containing phytocompounds cited in the literature as taste active, information related to chemical structure, orosensorial properties, associated taste receptors, as well as information about their anti-inflammatory potential.

6.2. Material and method

The data collection regarding phytocompounds was carried out both through the systematic search of electronic resources and through the investigation of documents in physical format (books).

The creation of the list of taste active phytocompounds was achieved in a first step by identifying and adding them from internationally recognized databases: BitterDB [20,43], FoodDB [44], PhytoMolTasteDB [3], PubChem [45], SuperSweetDB [46]. The list previously obtained was later enriched by searching with keywords in other internationally recognized databases:: Google Scholar, PubMed și ScienceDirect.

The search was performed by using the phrases: "(phytochemical) AND (taste)", "(specific phytochemical name) AND (taste)", "(phytochemical) AND (astringent OR bitter OR pungent OR salty OR sour OR sweet OR umami)", "(specific phytochemical name) AND (specific taste)", "(phytochemical) AND (specific taste receptor/chemosensor name)" [47].

After this electronic search, whenever needed, a manual search also took place, mostly for sensorial characteristics or for chemical structures of compounds that were not available in the mentioned databases.

In order to be included in this database, natural compounds had to fulfill two conditions:

- The chemical structures availability in PubChem[45], HMDB[48], FooDB[44], ChEMBL[49] or in studies/articles
- Phytocompounds to be described as taste active (sensorial or experimental – ligand-receptor affinity tests)

The distribution by chemical classes of the phytocompounds was achieved by searching the specific phytocompounds in PubChem[45], FooDB[44], HMDB[48].

For the evaluation of the anti-inflammatory potential, a systematic search was performed in three internationally recognized databases (PubMed, GoogleScholar, ScienceDirect) using the following key phrase: "[specific phytochemical name] AND (antiinflammatory OR anti-inflammatory OR inflammation)" [47], for example: (Apigenin) AND (antiinflammatory OR anti-inflammatory OR inflammation).

The "molecular taste of plants" or "phytomolecular taste" represents the virtual taste profile for a certain plant which is made up of all the tastes/orosensations given by the main gustatory/orosensorially active compounds that enter the composition of the plant of interest [50]. Phytomolecular taste is a newly original described concept [50]. This can be generated in the database in the form of a pie chart for a specific plant of interest. The contribution of each taste of the major constituent phytocompounds of the respective plant is taken into account.

6.3. Results

PlantMolecularTasteDB is a unique database at national and international level, for the uniqueness and originality of this concept, the Benelux Office for Intellectual Property (BOIP) i-DEPOT certificate was obtained with the number 122867. This database is also accessible online for free (https://plantmoleculartastedb.org/index.php, accessed on 01.04.2024).

PlantMolecularTasteDB contains 1527 taste active natural compounds derived from plants. There are compounds that exhibit multiple tastes/taste sensations (recorded with multiple tastes/taste sensations). Regarding the number of records of phytocompounds in each taste/taste sensation category, they are: 1114 records with a bitter taste, 263 - sweet taste, 224 – with pungent , 189 - with astringent, 61 - with a sour taste, 25 - with an umami taste, 7 - salty taste (Figure 6.1).

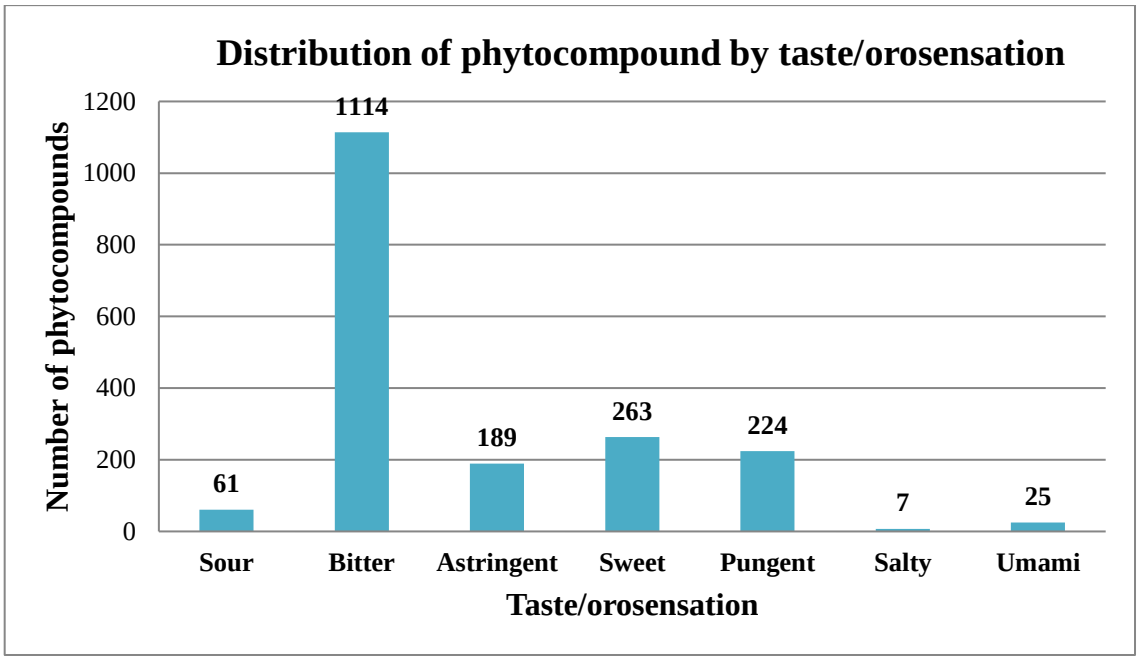

Figure 6.1. Distribution of phytocompounds by taste/orosensation

In terms of chemical classes represented in this database, the first three best represented chemical classes are the alkaloid class, the terpenoid class, and the glycoside class.

6.4. Discussions

Although at the time of PlantMolecularTasteDB's launch there were other online available databases dedicated to taste active compounds, PlantMolecularTasteDB presents several items that represented an international novelty.

PlantMolecularTasteDB is innovative through the information it contains (includes the complete taste profile of each phytocompound, pharmacological activity – anti-inflammatory activity); more than that it only includes natural plant compounds [47]. Another novelty element is the generation of phytomolecular taste for a plant of interest.

SuperSweetDB is made up of only sweet compounds (both synthetic and natural) [46].

BitterDB contains only bitter compounds, both natural and synthetic [20,43].

FlavorDB includes compounds that are sensorial characterized without a distinction between taste and odor, and data on physicochemical properties, data on functional groups are also available [51].

6.5. Conclusions

PlantMolecularTasteDB is a unique database that includes taste active phytocompounds from all categories of tastes/orosensations.

Currently, it is the database that contains the largest number of taste active natural compounds (derived from plants), which describes the complete taste profile of phytocompounds and brings together physicochemical information, qualitative and/or quantitative data, biological activity (anti-inflammatory potential).

PlantMolecularTasteDB represents the fundamental element of the doctoral thesis as the following studies were carried out using as a selection site the set of phytocompounds included in this database.

7. Assessing the impact of taste and the impact of chemical class on the anti-inflammatory activity of phytocompounds by miming data in PlantMolecularTasteDB

7.1. Introduction

Objectives

The main objective of this research was to verify whether or not phytocompound taste/orosensation or chemical class could exhibit predictive power of anti-inflammatory activity.

Other objectives related to the present research are represented by: the evaluation of a potential association between phytocompounds belonging to a certain chemical class and their anti-inflammatory activity, the evaluation of a potential association between the tastes/orosensations generated by the phytocompounds and their anti-inflammatory activity, establishing superiority in terms of the ability to predict anti-inflammatory activity taking into account the two properties of phytocompounds (taste/orosensation, respectively the chemical class), evaluating the involvement of phytocompound-induced tastes/orosensations in the relationship between the supposed association of the class chemical of the phytocompounds and of their anti-inflammatory activity, evaluating a potential association between the tastes/orosensations determined by the phytocompounds and their interaction with the molecular targets involved in inflammation.

7.2. Material and Method

The previously developed database (PlantMolecularTasteDB) was the source from which the phytocompounds investigated in this study were selected [47].

To ensure that the phytocompounds taken into account in the present research were previously sufficiently studied, including from the point of view of the role in inflammation, a threshold of at least 40 studies when searching the name of the phytocompound in PubMed was applied in the process of selection.

Thus, the inclusion criteria for the considered phytocompounds were represented by: their existence in PlantMolecularTasteDB and the existence of a minimum number of 40 articles in PubMed using the name of the natural compound as a search phrase.

In order for a compound to be considered as having anti-inflammatory activity in the scientific literature, we considered the existence of at least one study (*in vitro*, on animal model or clinical study) as positive evidence.

The second essential part of the present research was represented by the validation of the data obtained from the first search. The SwissTargetPrediction was used for this purpose [52]. This bioinformatic tool makes a prediction of the molecular targets of the investigated bioactive molecules. The prediction is based on adding to this program SMILES type characters for the item to be analyzed. Since there were phytocompounds that were not compatible with this tool. Four international databases served as search support for the molecular targets indicated by the previous programme. These are: National Library of Medicine/Protein [53], PubChem [45], The Human Protein Atlas [54], UniProt [55]. This was necessary to validate the involvement of those molecular targets in the inflammatory process. The search at this stage involved the use of the name of the molecular target and keywords that have in their structure ”inflammat” (for example, „inflammation”, „inflammatory”, „antiinflammatory/anti-inflammatory”, „proinflammatory/pro-inflammatory”).

The chemical classes taken in the statistical calculation were: short-chain aliphatic acids, phenolic acids, alkaloids, amino acids, sulfur compounds, coumarins, diterpenoids, other phenylpropanoids*(* - others than the main groups of hydroxycinnamic acids, coumarins, (iso)flavonoids, lignans, stilbenes), flavonoids, glycosides of flavonoids, glycosides of monoterpenoids, monoterpenoids, monoterpenoids, sesquiterpenoid monoterpenoids, tannins, triterpenoids, saccharides.

Regarding the statistical means used in generating the results, they were Chi-square test, Fisher exact test, Mann-Whitney test and Bonferroni correction.

7.3. Results

Of the 1527 compounds contained in the PlantMolecularTasteDB database, 592 met the criterion of having more than 40 studies when each of them was searched in PubMed.

Of these 592 phytocompounds, the majority (452, representing 76.35%) met the criterion of having at least one study related to the anti-inflammatory effect. 140 phytocompounds, representing 23.64%, had no studies related to the anti-inflammatory effect according to the search process.

According to the Bonferroni correction, the statistically significant results were for the associations bitter taste and anti-inflammatory activity, saccharides and lack of anti-inflammatory activity.

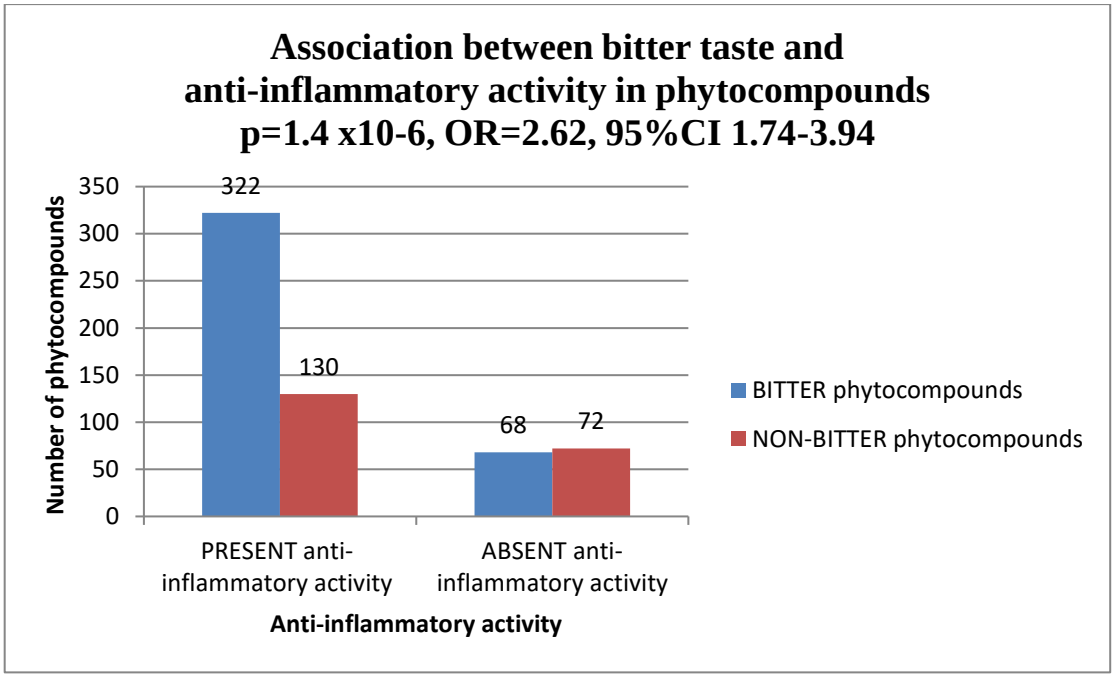

Figure 7.1. Association between bitter taste and anti-inflammatory activity in phytocompounds

Saccharides have a negative association with anti-inflammatory actions, a statement supported by a p-value of 0.0003, an OR (Odds Ratio) of 0.25 and a 95% CI (Confidence Interval) of 0.11- 0.56 (does not include value 1).

Flavonoids had a p-value (Fisher's exact test) of 0.003, OR of 3.35, 95%CI 1.41-9.7, so a positive association with anti-inflammatory activity, but applying the Bonferroni correction the level of statistical significance dropped to 0.002.

For the 548 compounds that could be searched for their molecular targets, 1836 possible targets resulted. Of these, 363 are related to the inflammatory process, 1245 are not related to the inflammatory process, and for the remaining 228 targets, the link to the inflammatory process cannot be accurately stated.

Bitter phytocompounds have a greater number of molecular targets related to inflammation compared to non-bitter phytocompounds.

7.4. Discussions

Taste is a better predictor of the anti-inflammatory activity of phytocompounds than chemical class of phytocompounds. The most relevant example from the present research is related to the bitter taste of phytocompounds which is a more important predictor than any chemical class considered, including the flavonoid class.

There are *in vitro, in vivo* studies in the literature that show that bitter phytocompounds from various chemical classes exhibit anti-inflammatory effects (for example, modulating the secretion of some cytokines involved in inflammation) [56–59].

A possible explanation for the association of anti-inflammatory activity with a certain class could also be due to the activation of the corresponding taste receptors. There is a study suggesting that polyphenols (the most representative class are flavonoids) bind to oral and extraoral TAS2Rs that modulate signaling pathways that are involved in anti-inflammatory processes, thus demonstrating beneficial effects [60].

Another study shows that although certain bitter-tasting compounds (eg, caffeine, theobromine) share structural similarities, they may have different promiscuity profiles [61]. Thus, for the complete characterization of the biological activities of some natural plant compounds, their chemical structure seems not to be sufficient.

The negative association between anti-inflammatory activity and sweet taste is supported by other researches that have shown that a diet high in sugars (fructose, sucrose) is associated with chronic inflammation [62–65]. There are sweet-tasting phytocompounds that exhibit anti-inflammatory properties [66,67].

7.5. **Conclusions**

The taste of phytocompounds is a variable more strongly associated with the anti-inflammatory activity than the chemical class of phytocompounds.

Bitter taste has a statistically significant positive association with anti-inflammatory activity.

Sweet-tasting phytocompounds and sour-tasting phytocompounds have a statistically significant negative association with anti-inflammatory activity.

Saccharides show a statistically significant negative association with anti-inflammatory activity.

8. Assessing the impact of taste and the impact of chemical class on the anti-cancer activity of phytocompounds and the possible relationship between anti-cancer activity and anti-inflammatory activity of phytocompounds by data mining in PlantMolecularTasteDB

8.1. Introduction

Objectives

The general objective of the present study was to identify the existence of an association between the tastes/orosensations of phytocompounds and their anti-cancer activity, as well as between the chemical class of phytocompounds and anti-cancer activity.

The identification of a potential association between the anti-inflammatory action of the taste active phytocompounds and their anti-cancer action, the finding of a potential involvement of the different tastes/orosensations generated by the phytocompounds regarding the association of the anti-inflammatory activity - anti-cancer activity, the identification of a potential differential involvement of the chemical classes of phytocompounds regarding the association anti-inflammatory action - anti-cancer action, identifying a superiority regarding the impact of taste or chemical class in anti-cancer activity and finding the hierarchical relationship between the variable taste/orosensation and the chemical class of phytocompounds regarding the association anti-inflammatory action - anti-cancer action are other objectives that we proposed in the present study.

8.2. Material and method

PlantMolecularTasteDB (https://plantmoleculartastedb.org/index.php, accessed on 01.02.2023) served as a selection site for the compounds included in the statistical analysis [47].

Applying the same phytocompound selection methodology as in the previous study, a minimum number of 40 articles found by compound name search in PubMed was required for a given phytocompound to be included in the study [68].

Thus, the inclusion criteria in this study were: the presence of the phytocompound in PlantMolecularTasteDB and the criterion of the existence of at least 40 studies when specifically searching the name of each phytocompound in this database.

In order to investigate the presence of studies in the scientific literature related to anti-cancer effects, a search was made for each phytocompound that met the inclusion criteria in three internationally recognized databases, namely PubMed, Science Direct, Google Scholar.

In order for the time variable to not influence the statistical results, a new search was performed regarding the anti-inflammatory activity of phytocompounds. The criteria stated in the previous study were used [68].

For anti-cancer activity, search phrases for each phytocompound that met the considered inclusion criteria included the name of the phytocompound and keywords such as, "antiproliferative," "cancer," "chemopreventive," "chemotherapy," "cytotoxic," "neoplastic," "tumor".

The existence of at least one study (*in vitro*, on animal model or clinical study) for the respective phytocompound was considered as ”positive evidence” for the investigated pharmacological effect.

The chemical classes taken in the statistical analysis were: short-chain aliphatic acids, phenolic acids, fatty acids, alkaloids, phenolic others (other than phenolic acids, esters of phenolic acids, amides of phenolic acids), amino acids, sulfur compounds, coumarins, diterpenoids, esters of phenolic acids, phenylpropanoids (others) (other than the classes of (iso)flavonoids, hydroxycinnamic acid derivatives - amides, esters -, cinnamic acid esters, coumarins, lignans, methoxyphenols, stilbenes), flavonoids, flavonoid glycosides, steroid glycosides, triterpenoid glycosides, monoterpenoid glycosides, monoterpenoids, sesquiterpenoids, tannins, triterpenoids, saccharides.

8.3. Results

Only 624 phytocompounds out of 1527 were included in statistical analysis. It should be noted that a phytocompound can have several tastes/orosensations (Figure 8.1).

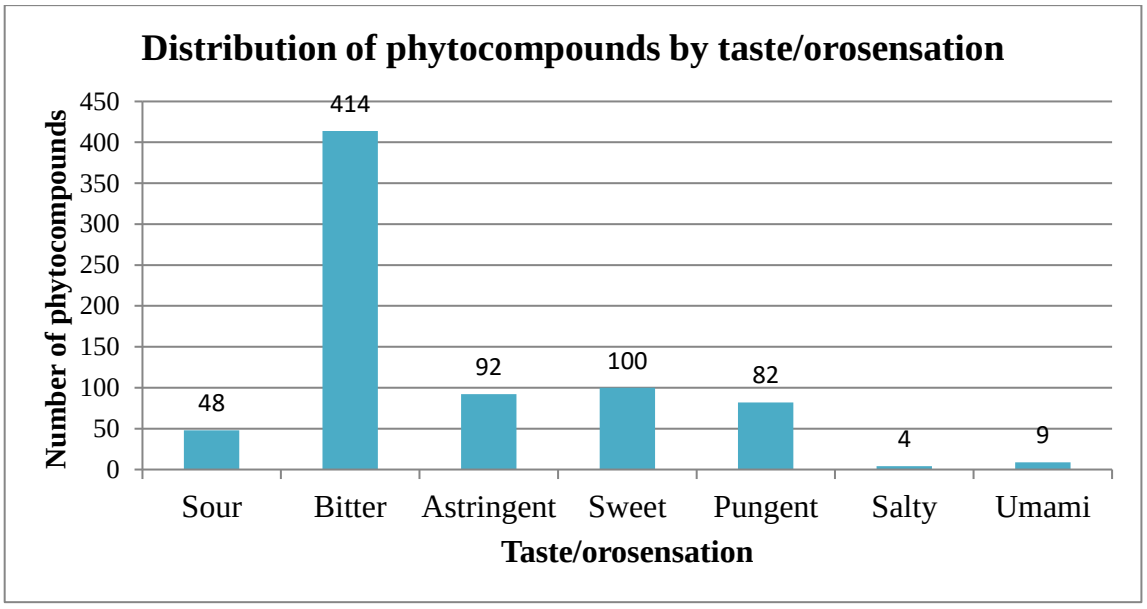

Figure 8.1 Distribution of phytocompounds by taste/orosensation

A statistically significant positive association was found for bitter taste and anti-cancer activity, a claim supported by a p-value of 7 x 10^{-4}, OR 1.92 and 95% CI 1.32-2.77(Figure 8.2). A statistically significant positive association was found for bitter taste and anti-inflammatory effect, supported by a p value of 0.003, OR 1.9 and 95% CI 1.32–2.77.

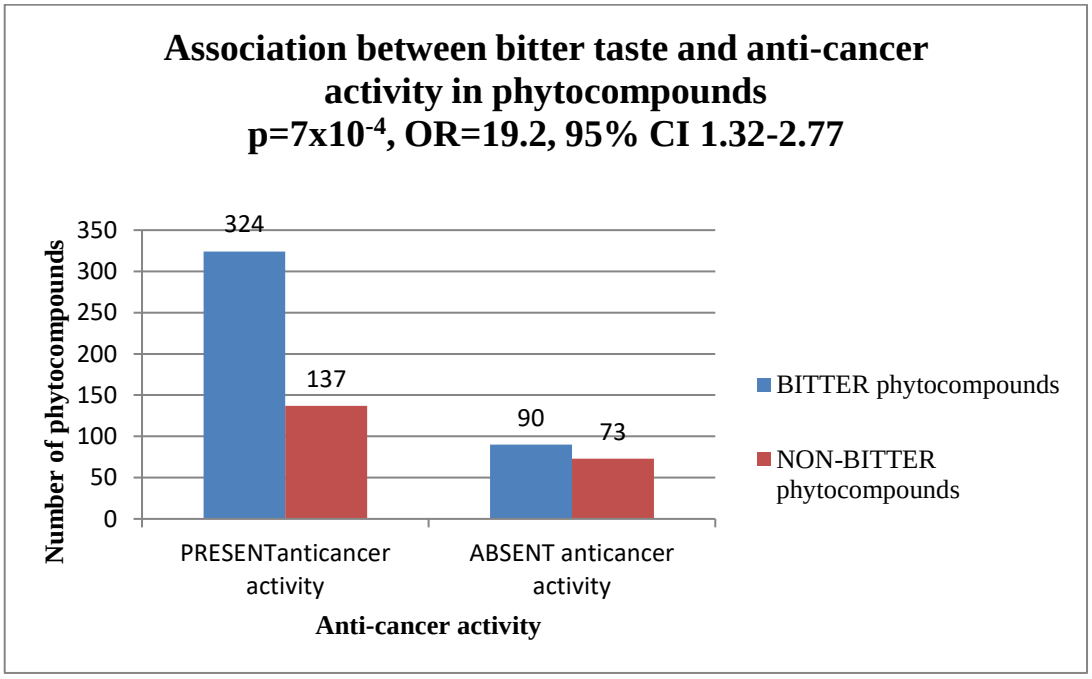

Figure 8.2. Association between bitter taste and anti-cancer activity in phytocompounds

A negative association was found between sweet taste and anti-cancer activity, a statement supported by a p-value of 10^{-5}, OR 0.36 and 95% CI 0.23-0.56 (Figure 8.3). There is a negative trend of association between sweet taste and the anti-inflammatory effect, as

the p-value in this case (0.016) did not reach the new threshold of statistical significance taking into account the Bonferroni correction (threshold value of 0.0035).

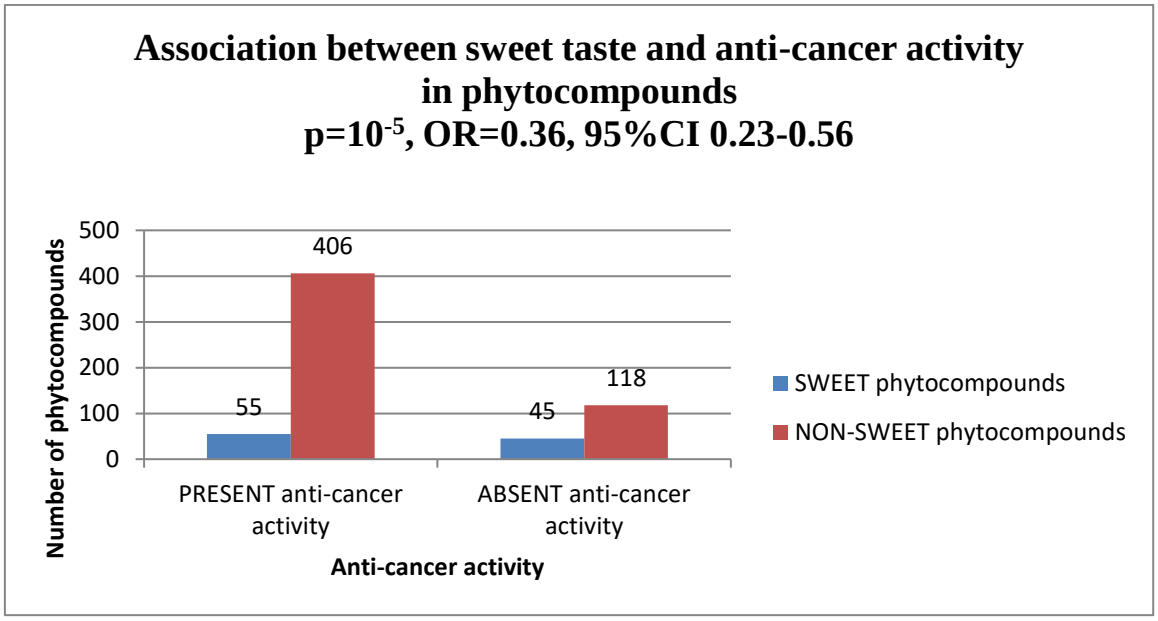

Figure 8.3. Association between sweet taste and anti-cancer activity in phytocompounds

Flavonoids had a statistically significant positive association with the anti-cancer activity, a statement supported by a p-value of 7 x 10^{-7}, OR 12.48 and 95% CI 3.58-76.76 (Figure 8.4). Regarding the same chemical class of phytocompounds and the anti-inflammatory activity, there is a statistically significant positive association, a statement supported by a p-value of 5 x 10^{-5}, OR 15.72 and 95% CI 3.03-324.34.

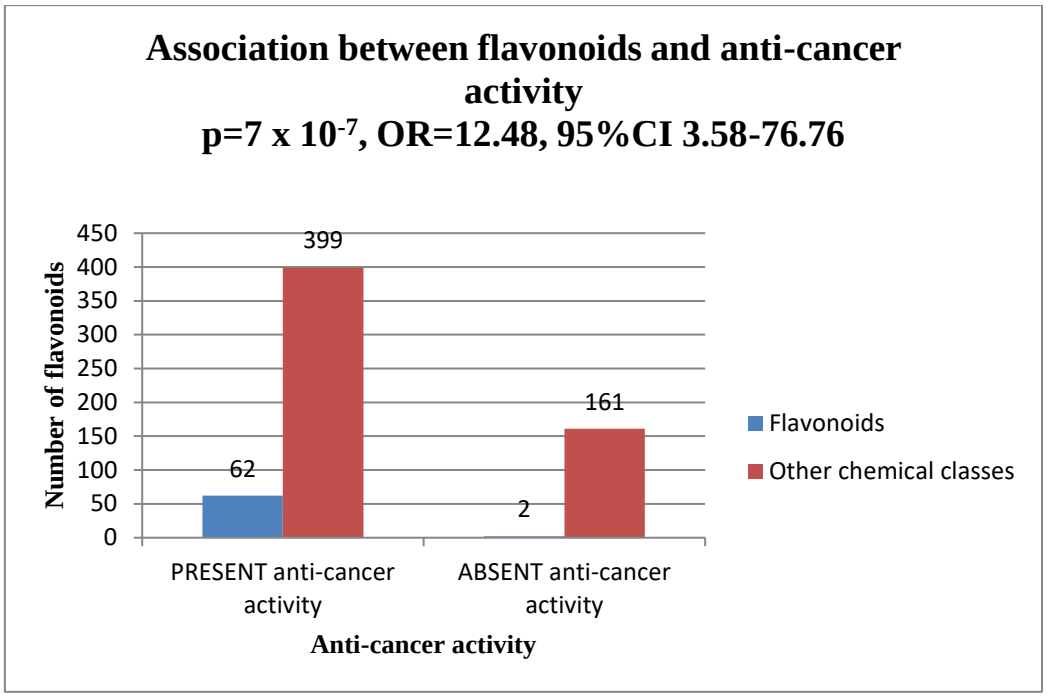

Figure 8.4. Association between flavonoids and anti-cancer activity

A statistically significant negative association was found for saccharides and the anti-inflammatory effect, supported by a p-value of 0.0002, OR 0.25 and 95% CI 0.12–0.52. A statistically significant negative association was also found for the saccharide group, but this time with the anti-inflammatory effect, supported by a p-value of 2 x 10^{-5}, an OR of 0.2 and a 95% CI of 0.09-0 ,41.

A statistically significant negative association was found between alkaloids and anti-inflammatory effect (p-value 0.0003, OR 0.41 and 95% CI 0.25-0.66).

A statistically significant negative association was found between aminoacids and anti-cancer activity (p-value 3 x 10^{-7}, OR 0.14 and 95% CI 0.06-0.3).

These statistically significant associations were also preserved when chemical classes were regrouped.

There is a strong association between the anti-inflammatory effect and the anti-cancer effect of the phytocompounds for all tastes and all chemical classes (with the exception of aminoacids).

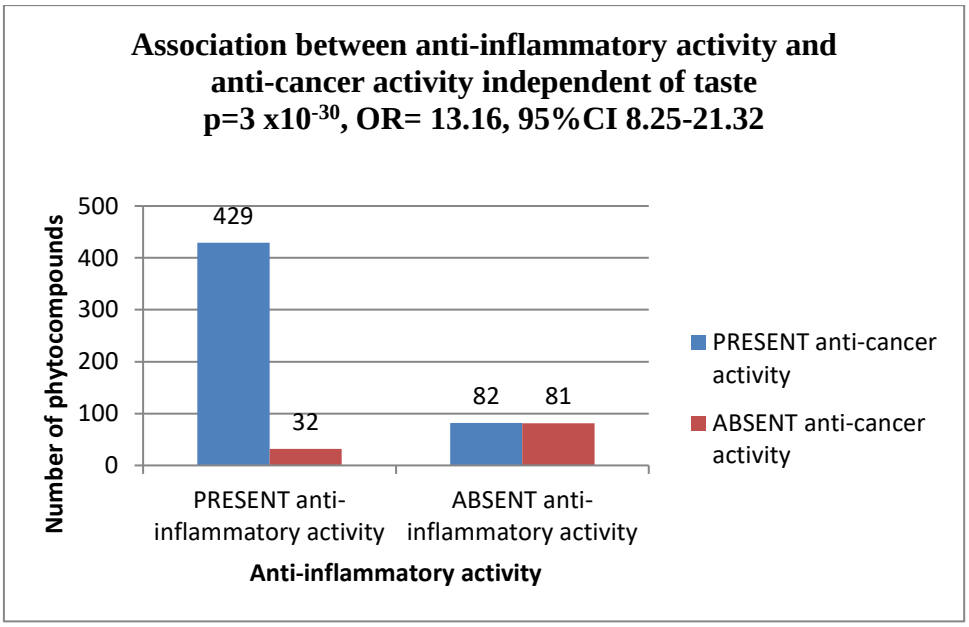

Figure 8.5. Association between anti-inflammatory activity and anti-cancer activity independent of taste

8.4. Discussions

In the previous study, bitter phytocompounds were more likely to exert anti-inflammatory effects compared to non-bitter phytocompounds [68]. Also, in the previous study, sweet-tasting phytocompounds were less likely to have anti-inflammatory activity compared to non-sweet phytocompounds [68]. These trends are also preserved in the current study.

Anti-inflammatory agents are known to have beneficial effects related to carcinogenesis as well. An example in this regard is the non-steroidal anti-inflammatory drugs, which in experimental studies, as well as in epidemiological studies, but also in randomized clinical trials, had a chemopreventive effect [69–78].

The positive association of bitter taste - anticancer activity is not unexpected, since bitter phytocompounds show numerous anticancer effects [27–30].

Another finding is that sweet taste has a negative association with anti-inflammatory activity and a significant negative association with anticancer activity. According to a meta-analysis, there is a significant positive association between higher consumption of fruit juice and the risk of prostate cancer [79]. The same study shows that there is a statistically significant positive association between higher consumption of sugar-sweetened beverages and the incidence of prostate cancer, as well as a statistically significant positive association

between the higher consumption of sugar-sweetened beverages and the incidence of breast cancer [79].

Another major observation from the present study is that the taste/orosensation of a phytocompound is a more important predictor of its biological effects than its chemical class. This result is in line with previous research, in which the phytocompound taste/orosensation as a variable was more strongly associated with the anti-inflammatory effect of the phytocompound than belonging to a certain chemical class[68].

8.5. Conclusions

Bitter phytocompounds are more likely to have anti-cancer and anti-inflammatory activity compared to non-bitter phytocompounds.

Sweet phytocompounds show a higher probability of negative association with anticancer activity, but also with anti-inflammatory activity compared to non-sweet compounds.

There are strongly statistically significant associations between the anti-inflammatory activity and the anti-cancer activity of a taste active natural compound, in other words a phytocompound with anti-inflammatory properties is very likely to have anti-cancer actions as well and conversely, a phytocompound with anti-cancer activity is very likely to show anti-inflammatory activity as well. This finding supports the concept that targeting inflammatory processes represents a chemopreventive and even chemotherapeutic strategy.

The only chemical class for which there was a statistically significant positive association with each of the two pharmacological actions studied is the flavonoid class. Even more interesting is the fact that most of the compounds from this chemical class have a bitter taste, in other words, the taste has a supremacy over the chemical class in terms of predictive power of biological action.

However, experimental studies are needed to confirm the conclusions mentioned above.

General conclusions

The conclusions that emerge from this doctoral study and that reflect the novelty of the research are the following:

- The taste of phytocompounds is a variable with a higher predictive power of anti-inflammatory activity compared to the chemical class
- The taste of phytocompounds is a variable with a higher predictive power of anti-cancer activity compared to the chemical class
- Bitter phytocompounds are more likely to have anti-inflammatory effects compared to non-bitter phytocompounds.
- Bitter phytocompounds are more likely to have anti-cancer actions compared to non-bitter phytocompounds.
- Sweet phytocompounds are more likely to lack anti-inflammatory activities compared to non-sweet phytocompounds
- Sweet phytocompounds are more likely to be devoid of anti-cancer activities compared to non-sweet phytocompounds
- If a phytocompound has anti-inflammatory activity, it is very likely that this phytocompound to have an anti-cancer effect, and, conversely, if a phytocompound has anti-cancer activity it is very likely that it also has anti-inflammatory activity, regardless of the taste variable; it is true that taste as a variable could have an impact on the strength of the association.
- Among all the chemical classes, the flavonoid class has a greater probability to have anti-inflammatory actions as well as anti-cancer activity

Future perspectives

Some of the possible future research directions are mentioned below:

- Using PlantMolecularTasteDB to identify new ligands for TAS2R or to identify ligands for orphan TAS2R (through molecular docking studies)
- Selecting some phytocompounds from the database and investigating them *in vitro* (cell/cell line studies) or on an animal model regarding the stages of carcinogenesis, respectively the intervention of these compounds in the chronic inflammation – cancer axis
- Investigation of a potential correlation between the degree of activation of bitter taste receptors for different phytocompounds from the database (EC50) and their ability to inhibit the development of a tumor clone (IC50).
- Bioinformatic tool creation for estimating the taste profile of a (phyto)compound or estimating the anti-inflammatory activity, or the anti-cancer activity

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