Formation, characterization and lab-grade cost analysis of stevia-mushroom hybrid tablets using *Stevia rebaudiana bertuni* and *Agaricus bisporus* to mask bitter aftertaste of stevia

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Biotechnology

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2023

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I certify that this research work titled "***Formation, characterization and lab-grade cost analysis of stevia-mushroom hybrid tablets using Stevia rebaudiana bertuni and Agaricus bisporus to mask bitter aftertaste of stevia***" is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources has been properly acknowledged/referred.

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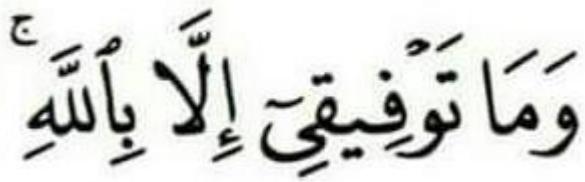
Dedication

To my brother and I, for the unyielding support and shared journey throughout this thesis

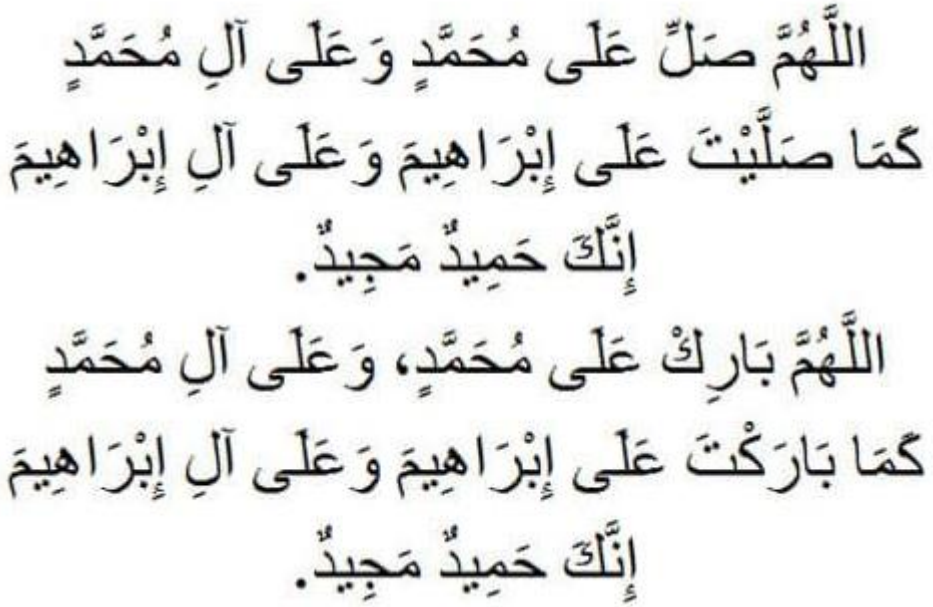
Acknowledgement

All praises to **Almighty Allah**, the most beneficent, the most merciful. It is only because of His help and blessings that I was able to complete my research work. He has undoubtedly granted me much more than I deserve.



"My Success is Only by Allah"

Durood-o-Salaam to our beloved Prophet **MUHAMMAD** ﷺ (PBUH), the last prophet of Allah and the prophet who had brought us from the darkness to the lightness.



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Ayesha Aslam

Abstract

The demand for natural sweeteners has been on the rise due to the growing awareness of the adverse effects of artificial sweeteners. *Stevia rebaudiana bertuni*, a plant species known for its sweetening properties, has gained significant popularity as a natural alternative to traditional sweeteners. In this study, we explore a novel approach to enhance the sweetness and nutritional value of stevia through myceliation using *Agaricus bisporus*, commonly known as the white button mushroom. The aim of this research is to formulate Stevia-Mushroom tablets and evaluate their cost-effectiveness compared to conventional stevia tablets while focusing on the process involving the inoculation of *Stevia rebaudiana bertuni* leaves extract with *Agaricus bisporus* extracts to stimulate the presence of fungal enzymes and enhance the steviol glycoside content while minimizing the bitter aftertaste of stevia, resulting in a more potent and nutritionally enriched sweetener that is more desirable. The study encompasses several stages, including the extraction of steviol glycosides from stevia and proteins from *Agaricus bisporus*. Once both extracts are mixed, it is then processed into tablet form, incorporating appropriate binder and optimizing the tablet recipe and ratio to ensure a stable tablet with intact integrity. The formulated tablets are then subjected to physicochemical characterization, including measurements of hardness, friability, disintegration time, and dissolution rate, GCMS and FTIR to assess their suitability for commercial production and consumption. The cost analysis is conducted to compare the production expenses of Stevia-Mushroom tablets with conventional stevia sweeteners. This evaluation includes factors such as raw material costs, labor, energy consumption, equipment, and packaging. The objective is to determine the economic viability and competitiveness of Stevia-Mushroom tablets in the market.

The results of this study provide insights into the feasibility of utilizing fungal proteins as a promising technique for enhancing the quality and value of stevia sweeteners. Additionally, the cost analysis provides valuable information for decision-making regarding large-scale production and market adoption of Stevia-Mushroom tablets as a natural and sustainable alternative to conventional sweeteners.

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1.1 Tabletop sugars as everyday sweeteners

Tabletop sugars are the most commonly used sweeteners around the globe. These artificial crystalline sugars are a part of daily diet across the globe whether in food, beverages and even some pharmaceutical products. According to statista.com, during just 2022 and 2023, a total of 176 million metric tons were consumed globally. Sugar market already is blooming and daily sugar intake across the globe is one the rise. Daily sugar intake mounts up to around 10% of our daily calorie intake that leads a number of chronic and Non-Communicable Disease such as cardiovascular problems along with obesity and diabetes. (Avena et al., 2015)

According to the global stats issued by the World Health Organization (WHO), a total of around 40 to 41 million people die each year due to Non-Communicable Diseases (NCDs). Not only that, another WHO stats have shown that the number of diagnosed cases of diabetes and prediabetes has been on the rise since 1995. By 1995, a total of 180 million adults were affected by diabetes but by 2014, this number has gone up to 422 million. The ratio has been increased to 8.5% from 4.7% over the span of only 4-5 decades. (WHO, 2021) As for Pakistan, diabetes has become a huge growing burden. The total number of diagnosed diabetes and prediabetes cases in Pakistan has went from between 40-60 million in 1995, all the way to 12-14 million individuals in 2011. (Bahadar et al., 2014)

Among all the regions in Pakistan, Sindh and Khyber Pakhtunkhwa were the least affected, having the highest number of diagnosed cases of diabetes. The leading factor of this outcome could be genetics, age, obesity and other underlying conditions. (Akhtar et al., 2019)

With such an alarming rise, sugar intake should be monitored on an individual level. One of the most effective measure is to avoid these conditions is lowering the daily intake of these sugars. The best way to introduce a sugarless diet is to replace tabletop sugar with a non-caloric sweetener. A natural, a-caloric and vegan alternative to sucrose based sweeteners. (Swiader et al., 2019)

1.2 Natural alternative - *Stevia rebaudiana bertuni*

Stevia rebaudiana bertuni is a naturally occurring plant, native to Brazil, Paraguay, Eurasia, China, Korea and Japan. This plant has a natural sweet taste, which is the reason its leaves or leaf power can be used in place of sweetener. *Stevia rebaudiana bertuni* belongs the Aesteraceae family and has a natural profile of glycosides, conveniently named as steviol glycoside. These glycosides are the reason for sweet natural taste. These glycosides include Steviosde, Steviobioside, Rebaudioside B, C, D, E, F, Dulcoside A, Rubusoside, and Rebaudioside. Glycemic index of stevia plant is unique aspect as well as it a-caloric in nature even in its natural state. In other words, stevia as a sweetener doesn't add any extra calories to you daily intake. (Panpatil et al., 2008)

Figure 1 *Stevia rebaudiana bertuni* plant and leaf extract powder

According to The International Market Analysis Research and Consulting group (IMARC) the stevial global market is estimated to reach approximately 818 million US

dollars by the year 2024. This indicates growing market for stevia as well as a market evolution and public perception towards a more sustainable, vegan and organic sweetener. Along with the distinct taste, steviol glycosides imparts a number of pharmaceutical benefits such as anti-hyperglycemic effects, antihypertensive effects, anticancer effects, antioxidant and antimicrobial activity, anti-tumor effect, effect on glucose absorption and on glucose synthesis, stimulates insulin secretion and effect on glucagon secretion. (Singh et al., 2019)

However, these glycosides are multifunctional as not only do they impart sweet taste; they also impart bitter aftertaste. Stevioside is the one glycoside that mounts up to 60 to 70% of the total glycosidic content of the stevia plant and alone is around 110-270 times sweeter than an average tabletop sugar such as sucrose. But along with the sweetness, stevioside brings about a bitter or licorice-like aftertaste. Rebaudioside A is the other glycoside that takes about 30-40% of the total glycoside content of stevia. Rebaudioside A is also relatively 240-400 times more sweet than artificial tabletop sugar. Rebaudioside A, however, doesn't have as much of bitter aftertaste. (Yadav et al., 2011)

1.3 Constraints in using steviol glycosides

Despite the pharmaceutical advantages along with the commercial and industrial appeal, stevia still isn't being readily accepted as an ideal sweetener. That is because it imparts a certain bitter or licorice-like aftertaste. This off putting after taste is what is offering the reluctance in choosing stevia over other artificial tabletop sugar options out in the market.

This bitter aftertaste is due to the same steviol glycosides that are giving sweet taste. These exact glycosides end up binding to the bitter taste receptor on human tongue and a distasteful flavor on the tongue. ((Hellfritsch et al., 2012) There has been minimal

work done on masking the bitter aftertaste of stevia, even though stevia holds so much potential and global market value.

Most commercial brands who have introduced stevia mix or stevia blend sweeteners rely on other sweeteners that are added to the stevia mix to lessen its bitter aftertaste. Acesulfame K, Sorbitol and lactose are some of the common examples. Adding these artificial sweeteners defeats the overall purpose of switching to stevia or any other natural sweetener. Other than that, a commonly known and used sweetener, Aspartame is under scrutiny as both previous and recent research arises suggesting Aspartame as a potential carcinogen. (Czarnecka et. al., 1957; Landrigan et. al., 2021)

1.4 The masking addition – *Agaricus bisporus*

Agaricus bisporus, commonly known as the White button mushrooms are edible basidiomycetes mushrooms that are commonly used in foods across the globe, dubbed as the most commercially important cultivated edible mushroom. White button mushrooms are known for their umami taste and palatable texture and are usually added to bland or cheesy dishes that relies on texture more than flavor. Other than the taste and texture, *Agaricus bisporus* is rich in carbohydrates, proteins, fibers, minerals, vitamins and phenolic compounds. (Ramos et. al., 2019)

Figure 2 *Agaricus bisporus*

A long range of active ingredients in *Agaricus bisporus* including glycoproteins, peptides, polysaccharides, essential amino acids, nucleosides, triterpenoids, fatty acids and lectins etc. Due to these ingredients, white button mushrooms elucidate a lot of health beneficial properties such as antimicrobial, anticancer, antidiabetics, antihypertensive, antihyperglycemic, antihypercholesterolemic, antioxidant and antihypercholesterolemic properties. (Atila et. al., 2017)

Kingdom	Fungi
Division	Basidiomycota
Class	Agaricomycetes
Order	Agaricales
Family	Agaricaceae
Genus	Agaricus
Species	*A. bisporus*
Binomial name	*Agaricus bisporus*

Figure 3 *A. bisporus* scientific classification

1.5 Potential masking agent - Glutamic acid

Agaricus bisporus has an interesting profile but our compound of interest here the taste masking mastermind. *Agaricus bisporus* has been found to have 32% glutamic acid in its dried form. This makes glutamic acid the most dominant non-essential amino acid found in White button mushrooms. (Jaworska et. al., 2013)

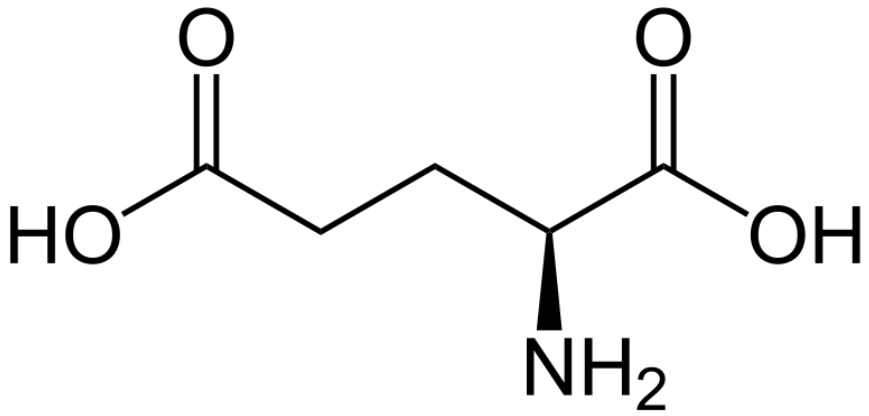

Figure 4 Glutamic acid structure

White button mushroom being rich in glutamic acid makes it a perfect candidate for bitter taste masking for our stevia-based sweeteners. A previous study using electronic tongue showed that potential of a poluglutamic acid complex in masking the bitter taste of diphenhydramine DPH, a very bitter tasking medicine used to cure common cold and fever. Addition of polyglutamic acid or PGA lead to the formation of DPH and PGA complex that alters the molecular size of DPH. This change in the size of the molecule prevents the molecule from binding to the bitter taste receptor, hence, reducing the bitter taste of the drug. Though both compounds bonded amorphously, the complex structures were stale but not in neutral or weak acidic environment. (Agresti et. al., 2008)

Since then, numerous studies have been conducted that depict the potential of glutamic acid in taste masking. A study in 2014 listed multiple amino acid complexes and their

relative binding affinities with hTas2R4 class of bitter taste receptors. Poly-glutamic acid, l-Glutamic acid γ-ethyl ester, had the one of the highest binding affinity ranging to -6.46. (Pydi et. al., 2014)

Need of the study

Both *Stevia rebaudiana bertuni* and *Agaricus bsiporus* have a wide list of research done on them. But bitter taste masking of stevia using a complete organic and vegan and beneficial source is something that hasn't been done yet. Both stevia and white button mushrooms individually have a list of beneficial compounds that exude multiple health benefits but most importantly, both have minimum caloric index. Coming together, stevia and white button mushroom have the potential to birth a product that will have all the benefits of stevia sweetener without any bitter aftertaste.

Climate of Pakistan is suitable to grow, sustain and harvest both of the above mentioned crops. A product like a stevia tablet mixed with white button mushroom extracts can not only be an innovation but a business startup idea that is sure to boost the economy of Pakistan.

CHAPTER 2: REVIEW OF LITERATURE

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2.1 Consumption of Sugar

Sugar has always played a significant role as a basic tabletop sweetener in every household. Sugar has also played a major role as a sweetener in the preparation of many commercial food products since the beginning of times. Pakistan's estimated monthly consumption of sugar stands at 0.45 million tons. Consequently, a total of 5.4 million tons of sugar demand needs to be met to meet the local yearly demand. (Sugar Sector Study, 2021)

2.2 Sugar Production in Pakistan

Sugarcane propagation plays an important role in sugar production in Pakistan. Though not the sole parameter, sugarcane production still plays an important role in meeting the sugar demands annually. Data summarized by Punjab Food Department, sugar recovery rate has been around 10% and 11% in central and southern Punjab respectively in 2022 alone. (Pakistan, 2022)

2.3 Rise in the sugar market and intake in Pakistan

The daily sugar consumption in Pakistan is around 20kg per capita. With the increase in the population of Pakistan, it is obvious to conclude that the sugar intake rate has risen as well. During year 2022 and 2023, the sugar market in Pakistan was expected to rise at about 3.3% rate. A lot of the sugar efflux goes to the large corporations and business such as bakeries, beverages, ice-creams, candies etc. Surprisingly, a huge chunk of sugar is taken on by the beverage industry mounting up to a 1.2 million metric tons per annum. (Finance Division, GoP Islamabad, n.d.)

2.4 Cause of Non-Communicable Diseases (NCDs)

Non-communicable diseases or NCDs are the leading cause of death across the globe mounting up to around 41 million deaths a year, as stated by WHO. A number of reports have shown that the NCDs can usually be traced all the way back to the sugar consumption. As for global stats, around 71% if all deaths that occur globally are due to NCDs. Obesity, cardiovascular diseases, respiratory disorders and diabetes all are far behind NCDs in terms of death tolls. All 4 of the above mentioned diseases mount up to a total of 80% of the overall premature NCD deaths. Other than that, other chronic habits such as drinking and smoking increase the risk of demise via a NCD. According to WHO reports, 4 metabolic changes that increase the risk of NCD include Hypertension, Hyperlipidemia, Hyperglycemia and Obesity. (WHO, 2021)

2.5 Added or artificial Sugar- A major reason behind NCDs

Even though sugar is an essential part of everyday diet of a lot of people, the overall reputation of sugar remains controversial. Despite being an essential, sugar can be traced as the leading cause behind cardiovascular diseases. Sugar discussed here is actually artificial, crystalline sugar, sugar naturally occurring in sweet fruits or vegetables or dairy is fine, healthy even. Plant based foods naturally contain essential amino acids, mineral and antioxidants. Dairy on the other hand is loaded with healthy proteins and rich in calcium. These natural produces take longer in the digestive system, which reports have shown actually reduces the risk of onset of cancer, diabetes and cardiovascular disorders. Excessive added sugar, as shelf-life extenders or flavor-boosters, will bring about diabetes. (Swiader et al., 2019)

2.6 Sugar consumption and long-term effects

Added or artificial sugar is not to be associated only with sweetened food such as sweets, soft drinks etc. But excessive sugar is added to some of the less obvious foods as well such as bread, ketchup etc. This makes it difficult to monitor average calorie or sugar intake on a personal level.

Excessive amounts of artificial sugars lead to hypertension as well as severe inflammation and eventually a chronic cardiovascular conditions. Unchecked and monitored sugar intake leads to unhealthy weight gain. Human body is designed to have an appetite control system. But sugar intake, essentially in liquid or beverage form leads to shutting the appetite control system off, leading to gradual rise in average calorie intake and of course, average weight gains too. (McKimm et al., 2020)

2.7 Native stats of Diabetic cases in Pakistan

The total number of diabetic and prediabetic diagnosed cases is on the rise since the last century. This rise can be observed across all regions of Pakistan. Sindh has seen the biggest rise among other regions and provinces whilst KPK has seen the lowest recorded cases among others. Though other than sugar intake, a number of other factors can be seen taken in to account with the higher risk of diabetes such as age, environmental factors as well hereditary factors, BMI, prior medical conditions. Out of the above mentioned factors, added sugar still remains the leading factor contributing to diabetes. (Akhtar et al., 2019)

2.8 Space for natural sweeteners

With the increase in public understanding and trends to a healthier and vegan alternative to diet, companies are either changing or introducing a new line of sugar-

free foods via replacing aspartame based sugars with more concentrated and vegan based sweeteners. (Świąder et al., 2011).

As of now, some of the most commonly used artificial sweeteners vigorously in food industries include cyclamate, aspartame, saccharin and acesulfame K. All of these sweeteners are high intensity, artificial and raise overall health concerns over regular intake of these sweeteners. (Tandel, 2011; Sun et al., 2006; Beltrami et al., 2018; Kant, 2005).

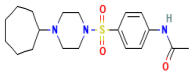
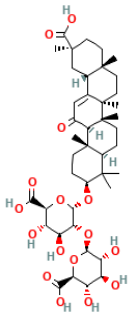
Other than the obvious health concerns, these sweeteners also bitter, metallic or aromatic off-flavors as the quantity of these sweeteners is increased. This off-putting taste is what makes these sweeteners limited. (Świader et al., 2011). Above stated reasons are the main fuel in the ongoing search for a natural and a-caloric sweetener with minimum to none health concerns and no compromise on the taste. (Yadav et al., 20)

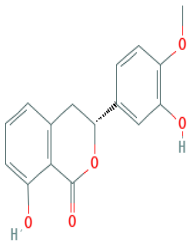
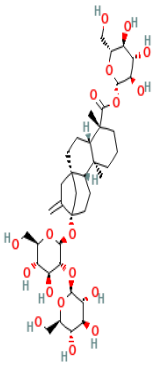
2.9 Plant based sweeteners

With the increase in public awareness and consciousness, what type of product is being consumed is under more scrutiny than ever. The general trend shift towards a healthier and organic diet is pushing the search for organic and vegan sweeteners. Regardless of many options available in the market, the demand for non-caloric or low caloric sweeteners still remains. The conscious decision making regarding sweetener has increased the demand of natural sweeteners. There are multiple compounds that are extracted from plants sources that have the potential to be used to as commercials sweeteners. Such as brazzein (extracted from Pentadiplandra brazzeana, an evergreen shrub), curculin (extracted from Curculigo latifolia), Erythritol (found in many sweet

fruits and fermented food), steviol compounds (extracted from Srevia rebaudiana) etc. (Kumar et. al., 2021)

Table 1 Some examples of natural sweetening compounds

Sr. no.	Class of natural sweeteners	Examples	Plant source	Sweet principle	Structure	References
1.	Sweet proteins	Thaumatin	*Thaumatococcus daniellii*	Thaumatin I and II		Inglet (1976)
2.	Sweet terpenoids	Triterpenoids	*Periandra dulcis mart.*	Glycyrrhizin, oleanane-type triterpenoid glycosides		Negri (2013)

3.	Sweet Polyketide	Dihydroisocoumarins	*Hydrangea macrophylla*	Phyllodulcin		Kinghorn et. al., 1986
4.	Sweet terpenes	Steviol glycosides	*Stevia rebaudiana bertuni*	Steviosside, rebaudioside A		Puri et. al., 2012

2.10 Stevia and its constituents

Stevia rebaudiana bertuni or more commonly known as just stevia is a perfect sugar alternative as it has all the aspects that are needed in terms of both public appeal and health-benefiting properties. Stevia is an edible, safe to consume, FDA approved, vegan and acaloric sweetener. The sweetening compounds, known as steviol glycosides, are non-interactive in nature, suggesting that they do not interact with other constituents in the same medium. This highlights the potential of stevia to be used as a commercial sweetener in beverages, candies, cakes, fondue etc. Unlike stevia, artificial sweeteners currently being used in food industries as of now, raises many health hazards namely obesity, bladder cancer and even brain tumor, all while being non-caloric. (Gupta et. al., 2013)

Regarding the safety of stevia or stevia extracted products, FDA has approved the use of stevia as GRAS (Generally Regarded As Safe). Joint FAO/WHO Expert Committee on Food Additives (JECFA) IN 2008, came together to set a recommended ADI of stevia at 4mg/day. Other than FDA and WHO testimony, stevia has been a common sweetener among Paraguayans for around 1500 years. The very existence and general health profile of the population is proof enough that stevia indeed is safe to consume and doesn't have any serious and long-term health concerns. (Ahmad et. al., 2020)

Another report that supports the claim of safety of stevia is a 2017 study that concluded that steviol glycosides not teratogenic, mutagenic or even carcinogenic. Steviol glycosides cause no toxicity whatsoever. (Abbas et al., 2017).

2.11 Ecology

Stevia classifies as a herbaceous perennial plant, belonging to Asteraceae family, native to west Paraguay and Brazil respectively. It can and currently is being cultivated in Brazil, Thailand, Central America, China, India, and Korea. Ideal temperature to grow stevia falls in the range 40° at night to 48°C during the day. A higher percentage of sweet glycosides were recorded when the plants were grown at higher latitudes. Another research conducted by (Hossain et al., 2017) suggested that vegetative growth of stevia saw a decline when the temperature dropped below 20°. Also when the length of the day receded to less than 12 hours. (Hossain et al., 2017)

2.12 Physical aspects and morphological markers

Stevia thrives in a semi-humid subtropical climate with specific temperature and rainfall conditions. It prefers high light intensity and warm temperatures but is more sensitive to day length. Partial shade is preferred during sunny summers. Most portions

of south Asia that do grow stevia see good yield during January and February session. (Hossain et. al., 2017)

Some of its other morphological properties include,

- Stevia has broad, ovate-shaped leaves.
- The leaves are arranged opposite to each other on the stem.
- The stem of Stevia is typically herbaceous and can be woody at the base in some cases.
- The plant can reach a height of about 30 to 80 centimeters.
- Stevia produces small, white flowers in clusters or spikes.
- The roots of Stevia are fibrous and relatively shallow.

2.12 Compounds of interest in stevia

The compounds of stevia that have captured special attentions of researchers and scientists across the globe are its glycosides. Steviol glycosides are a class of compounds naturally found in Stevia plant and these phytochemicals are the exact compounds that give the stevia its sweet taste. These compounds are stevioside (3-10%), Rebaudioside A (13%), and Rebaudioside B, C, D of more importance. Other related compounds consist of Rebaudioside C (1-2%), Dlucoside A. Other than the previously mentioned major sweet glycosides, some minor glycosides include flavonoid glycosides, cinnamic acids, coumarins, some essential oils, and phenylpropanoids. (Kumari et al., 2017)

Other than the sweetening glycosides, sweetening triterpenes, sterols and esters can also be found in stevia leaves. Dried leaves of stevia can yield these sweetening

compounds, along with our compounds of interest, stevioside and rebaudioside A. Both of these compounds are individually 200-350 times sweeter than artificial sugars commonly used. Extraction from dried leaves led to 5 to 10% yield of stevioside while 2 to 4% yield rebaudioside A. (López-Carbón et al., 2019)

2.13 Medicinal prospects of stevia

One of the leading glycoside compound stevioside, that alone has been known to be around 110 to 270 times sweetener than sucrose based sweeteners that we use in our everyday life, has both commercial and pharmacological potency. Stevioside has been found to induce anti-inflammatory, anti-hypertension, anti-diarrhea, anti-tumor as well as beneficial impact on the glucagon and insulin secretion into the bloodstream and impact overall glucose synthesis. (Chatsudthipong & Muanprasat, 2009)

One the other hand, Rebaudioside A being the second abundant glycoside found in stevia, is found to be around 240 to 400 times sweeter than the average tabletop sugar. Rebaudioside A is known to bring about multiple benefits such as pancreatic stimulation of β-cells to produce insulin. Other pharmacological properties due to synergetic effect of all steviol glycosides include anti-cancer, antioxidant, anti-hyperglycemic and immunomodulatory benefits. (Chatsudthipong & Muanprasat, 2009)

Not only is stevia edible and safe, stevia has many medicinal and pharmacological potential properties. Several reports have included stevia as contributing factors in reducing hypertension, fighting dental cavities and anti-bacterial properties. (Chatsudthipong & Muanprasat, 2009)

2.14 Commercial value and global market

As of 2022, the global market of stevia stands at about 355 Million USD and is only expected to rise. By 2032, this market is to expand to 708.1 Million USD, at a CAGR of 7.2% from just 2022 to 2023, according to Futuremarketinsights.com.

The idea of using stevia as a sweetener is not new at all. As of now, stevia leaves or leaf powder can easily be found in any local market. These powders and leaves are being used as sweeteners not only in households as herbal teas and sweetener. But also on industries as well. As of now, larger corporations such as Pepsi Company and The Coca Cola Company have launched a line of their regular beverage in which stevia is used as a sweetener.

Other than the sweetening properties of stevia, is also being used as flavor enhancer and colorant in salads and ice-creams etc. (Ur et. al., 2017) Other than that, stevia is also being actively used in mouth wash and drinkable chlorophylls. They are also a rich source of phytosterols that yields multiple benefits in healthcare, skincare and supplements. (Dawid et al., 2015)

Extraction of stevia is a green process. Large scale extraction and production of stevia-based products will leave behind waste just as all factories and industries. But the waste after stevia extracts would be used as an animal feed additive and even fertilizer. This makes the whole stevia approach far less detrimental to the environment than any other industry. (Dawid et al., 2015)

2.15 Stevia in Pakistan

Stevia rebaudiana bertuni, a naturally sweet herb, is grown as an alternative sugar crop and cash crop in various countries worldwide. However, the cultivation of *Stevia*

rebaudiana bertuni on a large scale encounters challenges due to its low seed germination rates. This lack of mechanized production technology poses a significant obstacle to the successful domestication of *Stevia rebaudiana bertuni*. (Savita et al., 2004)

As of now, stevia plant is being used and sold as a household plant or a small farm plant. In other words, stevia as of now has had domesticated use. Till recently, stevia has yet to be reintroduced as a commercial crop. Agricultural institutions such as Pakistan Agricultural Research Council (PARC) and Punjab Agricultural Research Board (PARB) are working together to unravel and circulate more knowledge and troubleshooting methods to ensure safe and high yield production of stevia in Pakistan. (Ijaz et. al., 2015)

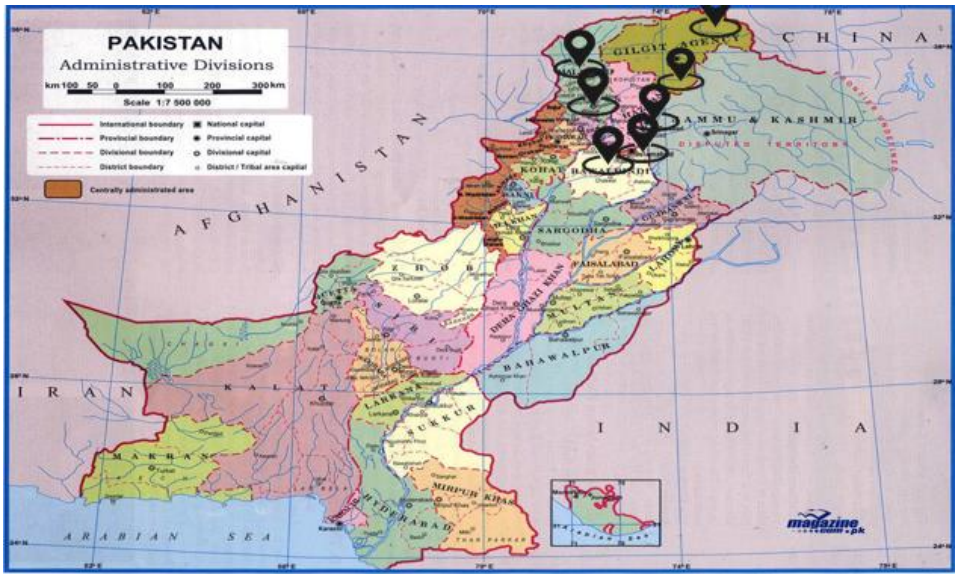


Figure 5 Areas of Pakistan growing stevia

2.16 Stevia tablets

Stevia tablets are meant to be a marketable product and its reliability relies on multiple factors including dissolution and disintegration in different beverages. The powder mix showing the best dissolution and disintegration had stevia extract, crospovidone, lactose, and magnesium stearate in ratio by weight 15/2.5/32/0.5. (Yousaf, A. M. et al., 2019). This research showed the stable and marketable stevia tablets can be developed however, the bitter or licorice aftertaste of stevia wasn't lessened or removed in these tablets.

The mechanical strength of the tablet is what helps to keep the tablet compact and sturdy. Mechanical strength of any tablet depends upon the structural changes of the constituents of the tablets. Long-term structural changes i-e Plastic deformations are what keeps the tablet sturdy. When compared to sweet potato powder, compaction of stevia tablets produced a much more stable and high mechanical strength tablet. (Shamsudin, I. S. et al. 2012)

Stevia leaf powder was also found to be a great artificial sugar substituent in turmeric effervescent tablet to incorporate the sweet and acceptable flavor to the otherwise earthy bitter taste of turmeric alone. Addition of stevia leaf powder was found to increase the overall antioxidant activity and total phenolic content, decrease total microbial number and add to effect on the hygroscopicity of the tablet. (Belgis, et al., 2023) Hence, proving that stevia leaf powder has the potential to be used as a commercial sweetener.

Key sweetening glycoside in Stevia i-e Rebaudioside A has also been seen to have an impact on the flow properties of sorbital, a sugar alchohol. Flow properties of sorbitol, namely compressability index, Hausner ratio and a mass flow rate lessened whilst the

overall cohesion increased due to addition of Rebaudioside A. The overall plasticity and total energy for compression was also found to be increased. Due to that, the overall tensile strength of the tablet increased than a control pure sorbitol tablet. Higher tensile strength allowed a longer disintegration time as well. (Hurychová et al., 2018)

As has been indicated before, stevia has the potential to be a potent commercial or industrial sweetener. Extracts of Curcuma xanthorrhiza Roxb. were made into an effervescent tablets mixed with different ratios of stevia to develop a stable tablet that fulfills the physical tablet test. Physical tablet test included visual tests, uniformity test, hardness test, friability test, pH test and solubility test. 3 and 4% of stevia in of *Curcuma xanthorrhiza Roxb.* effervescent tablet fulfilled all physical tablet test. (Lynatra et al. 2018)

As mentioned above, stevia is acaloric in nature and can serve as a substitute for a great alternative to other low-caloric to acaloric sweeteners for type 2 diabetes such as sucralose. As of now, sucralose is being widely used as a sweetener for diabetic patients. A double blinded experiment, where one group was given stevia extract as a sweetener while the other group was given sucralose. Glycemic response and lipid profile was analyzed after eight weeks. Comparison of blood sugar level, insulin, glycosylated hemoglobin and lipid level profiles of both groups were found to have no noticeable variations. In conclusion stevia has all the equipment to be used a sweetener for diabetic individuals. (Ajami et al., 2020)

2.17 Stevia taste masking

The only thing standing in the way of stevia being marketed as the ultimate and universally used sweetener is the bitter aftertaste of the stevia. This is the only reason, that despite having so many health benefits and being a healthier and vegan alternative,

we still have yet to see stevia in the market as a sugar alternative. Stevia and masking of the aftertaste of stevia are of common interest and many studies are being conducted to remove or lessen the bitter aftertaste to improve the overall public perception and compliance of stevia.

Encapsulation is one practical technique under study to mask the unpleasant aftertaste of stevia. A study conducted used 2 different encapsulation methods in hopes to lessen the bitter aftertaste of stevia, spray drying and electrohydrodynamic method. Both methods utilized maltodextrin-inulin and zein respectively. Neither encapsulating matrices showed any chemical interaction between glycosides of stevia, meaning that both matrices were ideal. Encapsulation efficiency measured via HPLC measured at about

Not only has the work been done to mask the bitter aftertaste of stevia, stevia itself has also been studied to see if it can mask the bitter taste of other pharmaceutical medicines. Studies have already shown that addition of a small amount to a bitter concoction can reduce the bitterness. (Bakke et al., 2018) One such study used stevia as a sweetener to tone down the bitterness of Metoclopramide hydrochloride to improve patient compliance. (Savita et al., 2014).

Objective

Objectives of this study can be broken down into following 3 pointers,

1. To acquire aqueous extracts of both *Stevia rebaudiana bertuni* and *Agaricus bisporus*. The reason behind aqueous extracts is so the tablets are safe to consume. Phytochemical screening of the final product is important as well as it will give a generic perspective regarding phytochemicals in the tablet.

2. Characterization of the final product using GCMS techniques as well as FTIR technique to get a deeper perception of constituents in the tablet.

3. Cost analysis of the final product to evaluate the possible revenue and gross margin of the hypothetical sales of the product.

CHAPTER 3: MATERIALS AND METHODOLOGY

CHAPTER 3: MATERIALS AND METHODOLOGY

Following are the steps followed to formulate the tablet and evaluate the parameters that the tablet is able to sustain. Further characterization and quantification of the tablets was also done in order to see phytochemical constituents and variation among the initial plant materials i-e *Stevia rebaudiana bertuni* and *Agaricus bisporus*.

3.1 Aqueous extraction

Since tablet are meant to be edible, an edible prototype was to be developed. For this purpose, edible solvents were to used. Distilled water as a solvent is considered a green extraction method. Reports have shown that water used as a solvent for the extraction of steviol glycoside has been quite effective, along with other factors such as growing conditions and drying methods. (Stramarkou et. al., 2021)

The method of extraction used here is “Maceration” to get maximum yield while also getting edible extracts. Maceration has been known to yield a good amount of stevioside and rebaudioside A. Maceration extraction is the easiest and greenest extraction procedure that doesn’t require sensitive instruments and chemicals.

Both stevia extracts and mushroom extracts were subjected to a total of 7 minutes together. Then they were transferred into clean petri dishes and put into incubator. Each extract was subjected to dehydrator to remove excess water solvent leaving behind a stevia and white button mushroom mix. This settled stevia and mushroom mix was then scraped off of the petri plates using a new, clean and sterilized blade. The extract mix was coming off as dried powder. This dried powder was then collected and stored separately for the next step.

3.2 Recipe and ratio management of tablets

After the extract mix is obtained, it is essential to look for a binder that not only holds the plant extracts together and non-interactive with other compounds in the extraction mix but is also safe and edible. Binder of choice here is α-cellulose 500g from Sigma Aldrich.

α-cellulose is a white, colorless, tasteless powder that is naturally found in maize and rice straw. α-cellulose is generally used in many foods ranging from shredded cheese to dietary supplements, as a stabilizer, emulsifier, binder, bulking agent adhesive, disintegrant and anti-caking agent. (Shokri et. al., 2013) α-cellulose was also seen to work well with water as a diluent unlike other binders available within the resources. All the above mentioned attributes are what prompted the use of α-cellulose as a binder in the tablets. (Ohwoavworhua et. al., 2007)

The quantity of Stevia and that of white button mushroom was combined based on 09 different ratios. The quantity of stevia and White button mushroom was set as 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6 respectively. Each extract mix was stored differently.

3.3 Direct compression method

3 ingredients i-e extract mix of Stevia and White button mushrooms, binder and diluent were all there is in the tablets. Next step is to find the perfect ratio of all additives to ensure a stable tablet. The prototype batch of tablet was set at a total weight of about 0.25g each. Each 0.25g tablet contained 0.8g of binder i-e α-cellulose, a drop of distilled water and 0.17g of extract mix. 08 tablets, with each tablet having a different ratio of extract mix were created.

Each tablet mix i-e extract mix and binder were then added to the manual pill press machine; size 2 die was used. Lower punch was inserted in the middle die first, the

tablet mix was then added. The upper punch was then gently lowered in the middle die. Each punch was then struck by hammer 8 times. A few tablets of each mix were then formed.

Figure 6. Manual die with upper and lower punch, size 2

3.4 Molecular Docking

One of the main reasons White button mushrooms were used was due to the abundance of glutamic acid content found in them. As mentioned above, numerous research has concluded that glutamic acid containing compounds have good taste-masking potential. For this purpose, glutamic acid binding tendencies with both bitter taste receptors i-e hTAS2R4 and hTAS2R14 that steviol glycosides were reported to activate.

For molecular docking, compound structures were downloaded from pubchem in SDF format. The structures of both human bitter taste receptors were taken from swisssmodel in PDB format. For docking, autodock was used. For visualization of the docked structures, BIOVIA Discovery Studio was used.

3.5 Mechanical testing

After the formation of tablet, the tablet was subjected to mechanical testing to evaluate the sturdiness and durability of the tablet. For this purpose, SHIMADZU Autograph AGX Plus testing machine was used. Stress and strain the tablet was able to withstand was calculated to ensure that these tablets are sturdy enough to be used as commercial and pharmaceutical tablet.

The dimensions of the tablets were also calculated using a Vernier caliper.

Figure 7 Universal Hardness testing machine

3.6 GCMS

GCMS technique is performed to see the phytochemical profile of a sample extract. GCMS allows for an in-depth analysis of the sample, separating compounds based on their compound structures, metabolite category, retention time and signal intensity.

CHAPTER 3: MATERIALS AND METHODOLOGY

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique widely utilized in various scientific disciplines for the identification and quantification of complex mixtures of organic compounds. It combines the separation capabilities of gas chromatography (GC) with the selective and sensitive detection of mass spectrometry (MS), offering researchers an invaluable tool for elucidating the composition of diverse samples with high precision and accuracy. (Bouchonnet,et. al., 2013.)

In recent years, the application of GC-MS has witnessed remarkable growth due to its versatility and broad range of applications, encompassing fields such as environmental analysis, pharmaceuticals, food science, forensic investigations, metabolomics, and natural product research, to name a few. The continuous advancements in instrumentation, software, and method development have further enhanced the capabilities of GC-MS, making it an indispensable technique in modern analytical laboratories.

The fundamental principle of GC-MS lies in the sequential combination of two distinct analytical methods, each contributing to a specific aspect of compound analysis. Gas chromatography serves as the first step in the process, where a sample containing a mixture of volatile and semi-volatile compounds is vaporized and injected into the GC column. Within the column, the compounds undergo separation based on their differing physicochemical properties, such as boiling points and polarity. As they traverse through the column, the individual compounds elute at different times, resulting in a chromatogram that represents the distribution of components in the sample.

Following the separation in the gas chromatograph, the effluent is introduced into the mass spectrometer. The mass spectrometer further scrutinizes the eluted compounds by

ionizing them, fragmenting the ions, and measuring the mass-to-charge ratios (m/z) of the resulting ions. This process generates mass spectra, unique to each compound, which serve as fingerprints for compound identification. The mass spectra are then compared to spectral libraries or databases to identify the compounds present in the sample. (Hübschmann et. al., 2015)

The ability of GC-MS to identify compounds based on their mass spectra, along with the chromatographic separation, offers several distinct advantages over other analytical techniques. It allows for the detection and quantification of minute quantities of compounds within complex matrices, even in the presence of interfering substances. ((Hübschmann et. al., 2015))

The aim of this thesis is to explore the principles, instrumentation, methodologies, and applications of GC-MS. It will delve into the various components of GC-MS systems, such as the injection systems, chromatographic columns, mass analyzers, and detectors, highlighting their contributions to the overall performance of the technique. Additionally, this thesis will present case studies and real-world examples to demonstrate the versatility and utility of GC-MS in different research domains.

GCMS of designed tablet was performed using the SHIMADZU GCMS QP2020 equipment, the column specifications were Shimadzu SH-Rxi-5Sil MS with dimensions of L=30m, ID=0.25, DF=0.25. GCMS technique was applied only one time using n-hexane as a solvent.

Figure 8 SHIMADZU GCMS machine QP2020

3.6 FTIR analysis

Fourier Transform Infrared Spectroscopy (FTIR) is a widely used analytical technique that plays a crucial role in the characterization and identification of a vast range of organic and inorganic compounds. By exploiting the interactions between matter and infrared radiation, FTIR enables researchers to obtain valuable information about molecular structure, functional groups, and chemical bonds within complex samples. Its non-destructive nature, high sensitivity, and ability to analyze a wide variety of sample forms have made it an indispensable tool in multiple scientific disciplines, including chemistry, materials science, pharmaceuticals, environmental analysis, and forensic investigations. (Abidi et. al., 2021)

The principle of FTIR spectroscopy lies in the interaction of molecules with infrared light, which leads to the excitation of molecular vibrations. When a sample is exposed to a broad range of infrared radiation, specific wavelengths of light are absorbed by the sample, corresponding to the characteristic vibrations of different chemical groups. The molecular vibrations result from the oscillation of atoms around their equilibrium

positions, and the energy levels involved in these vibrations are unique to each compound. As a result, the absorption of infrared radiation produces a distinctive pattern known as an infrared spectrum, which provides a molecular fingerprint for the compound under investigation.

The essential components of an FTIR spectrometer include a broadband infrared light source, an interferometer, a sample holder, and a detector. The interferometer, employing a Michelson interferometer or a related design, modulates the intensity of the infrared radiation, allowing for the rapid acquisition of an interferogram. The interferogram is then transformed into a spectrum using a mathematical technique known as the Fourier transform. This transformation generates the final FTIR spectrum, which displays the absorption peaks corresponding to the vibrational modes of the molecules in the sample. (Abidi et. al., 2021)

FTIR spectroscopy offers several distinct advantages over other analytical techniques. It allows for the analysis of solids, liquids, gases, and even thin films without the need for extensive sample preparation. Additionally, FTIR is capable of performing quantitative analysis, enabling the determination of compound concentrations in mixtures. Moreover, FTIR can provide qualitative information about sample purity, the presence of impurities, and the identification of unknown substances, making it a valuable tool in routine quality control and research applications. (Grdadolnik et. al., 2002)

The scope of this thesis is to delve into the principles, instrumentation, and applications of FTIR spectroscopy. It will explore the theory behind molecular vibrations, infrared absorption, and the mathematical foundations of Fourier transformation. Furthermore,

this thesis will examine the different modes of FTIR, such as transmission, reflection, and attenuated total reflection (ATR), and their respective advantages and limitations.

FTIR detection allows us to visualize in the form of a graph the amount and the types of functional groups present in our sample. FTIR detects the presence of functional groups based on the level of absorption on a certain wavelength spectrum.

FTIR was also performed using Carry 630 FTIR Spectrophotometer.

Figure 9 Cary 630 FTIR Spectrometer with ATR sampling module

3.7 Cost analysis

Cost analysis is a crucial aspect of any business strategy, including the marketing of stevia-mushroom tablets. It involves assessing and evaluating all the expenses associated with the production, distribution, and promotion of the product. By examining these costs in detail, businesses can make informed decisions that lead to more efficient operations, improved profitability, and a better understanding of their competitive position in the market. (De Rus, et. al., 2021)

3.7.1 Importance of Cost Analysis

Pricing Strategy: Understanding the cost of manufacturing and marketing stevia-mushroom tablets helps in setting an appropriate pricing strategy. It ensures that the product is competitively priced while still generating enough revenue to cover expenses and achieve desired profit margins.

- Profit Maximization: Cost analysis allows businesses to identify areas where costs can be optimized and reduced. By minimizing production and distribution expenses, the company can increase its profit margin per unit sold.
- Resource Allocation: Knowing the cost breakdown helps in allocating resources effectively. The marketing budget can be allocated more efficiently to the most impactful promotional strategies, ensuring a higher return on investment.
- Competitor Comparison: Cost analysis provides insights into how your production and marketing costs compare to your competitors. This information is valuable when positioning the product in the market and formulating strategies to gain a competitive advantage.
- Decision Making: It helps in making informed decisions about various aspects of the business, such as production volume, product design, marketing channels, and resource allocation. (De Rus, et. al., 2021)

3.7.2 Use of Cost Analysis in helping Stevia-Mushroom Tablet Marketing

- Identifying Cost-Effective Promotions: Cost analysis helps identify which marketing strategies are yielding the best results for the least expense. This allows you to focus on the most cost-effective promotional efforts to maximize impact.

- Competitive Pricing: By analysing the cost of production, you can determine a competitive yet profitable pricing strategy for the stevia-mushroom tablets. This ensures that your product offers value to customers while remaining economically viable for your business.
- Manufacturing Efficiency: Cost analysis can highlight areas of production where efficiencies can be improved. By streamlining the manufacturing process, you can reduce production costs and potentially offer a more competitive price to consumers.
- Profit Margin Optimization: Understanding the overall cost structure of the product enables you to set a profit margin that aligns with market demands and your business objectives.
- Product Development: Cost analysis helps in assessing the feasibility of introducing new variants or formulations of the stevia-mushroom tablets. It can guide decisions about which product features are worth investing in based on their impact on the cost structure.

In summary, cost analysis is a fundamental tool in guiding the marketing strategy of stevia-mushroom tablets. It empowers businesses to make data-driven decisions, optimize their resources, and enhance their competitive advantage in the market.

CHAPTER 4: RESULTS

CHAPTER 4: RESULTS

4.1 Tablet formation

A stable tablet was prepared using the recipe mentioned above. The prototype batch of tablets was set at a total weight of about 0.25g each. Each 0.25g tablet contained 0.8g of binder i-e α-cellulose, a drop of distilled water and 0.17g of extract mix. 08 tablets. Each tablet had Stevia and White Button Mushrooms that created a potent and health-enhancing formula that had a significant change in the bitter profile of the said tablet.

Figure 10 Stevia-mushroom pressed tablet

4.2 Basic phytochemical screening

Phytochemical screening is a fundamental process used in research to identify and analyze the presence of various biologically active compounds in plants. "Phyto" refers to plants, and "chemical" denotes the study of chemical compounds. This screening technique plays a crucial role in scientific investigations because of its significance in understanding the potential health benefits and medicinal properties that plants offer.

During phytochemical screening, extracts are examined for a wide range of secondary metabolites in tablet and stevia and white button mushroom plants, such as glycosides, alkaloids, flavonoids, terpenoids, tannins, phenolic compounds, and many others. These compounds are responsible for the diverse biological activities exhibited by different plant species, including flavor enhancing, antioxidant, anti-inflammatory, anticancer, antimicrobial, and other therapeutic properties.

Table 2 Phytochemical Screening Comparison of *Stevia rebaudiana bertuni* and Agaricus bisporus and tablet

Sr. No.	**Metabolites Tested**	**Results of *Stevia rebaudiana bertuni***	**Results of *Agaricus bisporus***	**Results of tablet**
	Alkaloids	+	+	+
	Flavonoids	+	+	-
	Glycosides	+	-	+
	Phenols	+	+	+
	Reducing sugars	+	-	-
	Leucoanthocyanins	-	+	-
	Triterpenoids	+	+	-
	Phlobatannins	-	+	-

4.3 Molecular docking

Molecular docking is a powerful computational technique used in drug discovery and molecular biology to predict how two molecules, typically a ligand (e.g., a drug candidate) and a receptor (e.g., a protein), interact and bind together at the molecular level. By simulating the docking process, researchers can gain insights into the binding affinity and orientation of the ligand within the receptor's binding site. This enables the identification of potential drug candidates and aids in understanding the molecular mechanisms of biological processes. The principle of molecular docking is based on evaluating the complementary shape, electrostatics, and other intermolecular forces between the ligand and receptor, providing valuable information for rational drug design and the study of biomolecular interactions. Following is the interaction of taste masking glutamic acid with both bitter taste receptors in human triggered by steviol compounds. The binding affinity mounted -5.4 and -5.1 between glutamic acid and hTAS2r4 and glutamic acid and hTAS2R14 respectively.

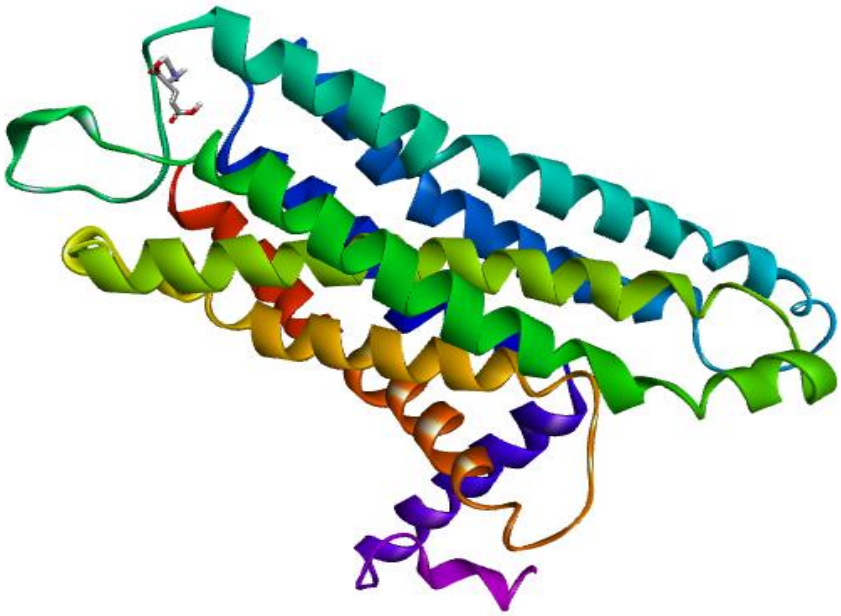


Figure 11 3D hTAS2R4 and Glutamic acid visual

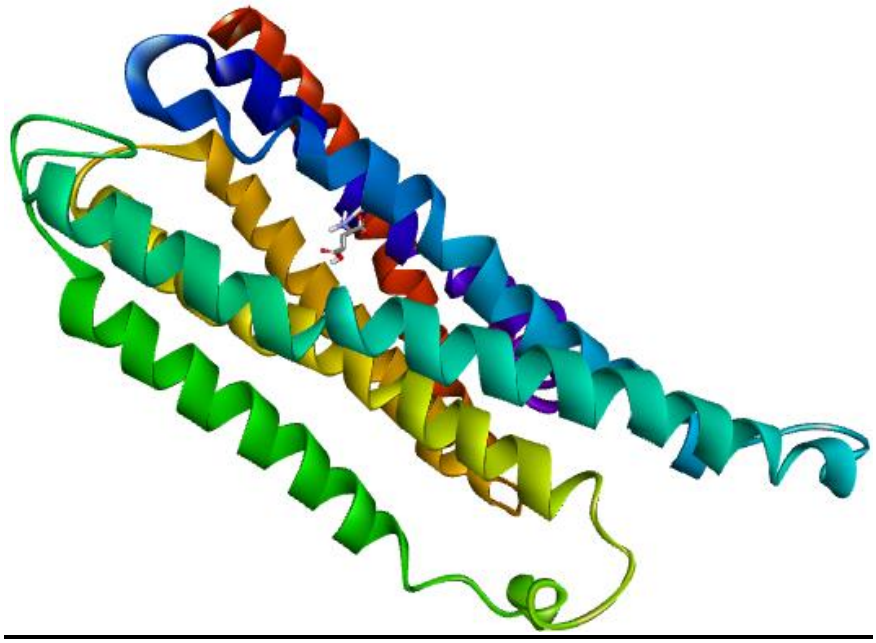


Figure 12 3D hTAS2R14 and Glutamic acid visual

Simultaneously, GCMS report pointed out the presence of certain phytochemical constituents in the tablet. Some phytochemicals were as it is in the tablet as they were in the raw material sample while other were predecessors of phytochemical compounds present in the raw material plants. One such compound of importance is L-Glutamine, N2-[(phenylmethoxy)carbonyl]. Glutamic acid is a precursor of this compound that leads into the investigation if this compound is one of our masking agent. For this purpose, molecular docking using the same software tools were conducted. The binding affinities mounted to be -7.6 and -7.7 between L-Glutamine, N2-[(phenylmethoxy)carbonyl] and hTAS2R4 and L-Glutamine, N2-[(phenylmethoxy)carbonyl] and hTAS2R14 respectively.

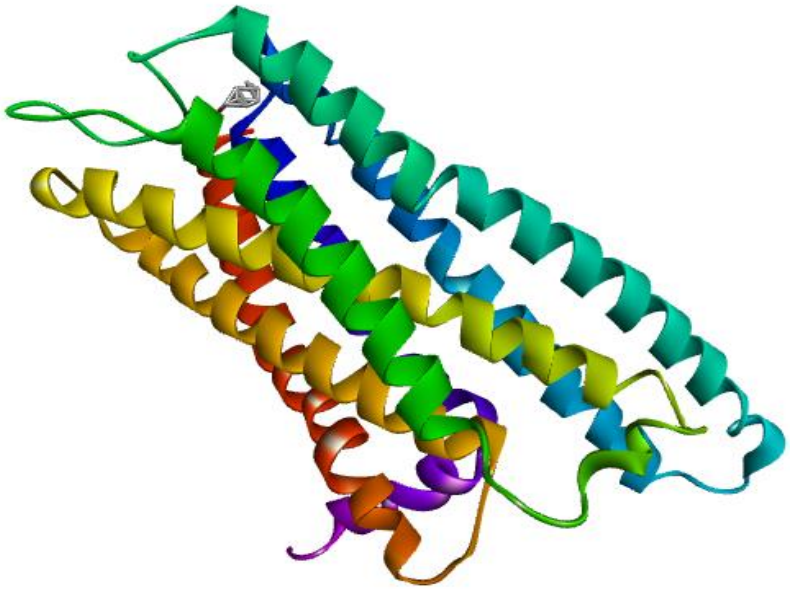


Figure 13 3D hTAS2R4 and L-Glutamine, N2-[(phenylmethoxy)carbonyl]visual

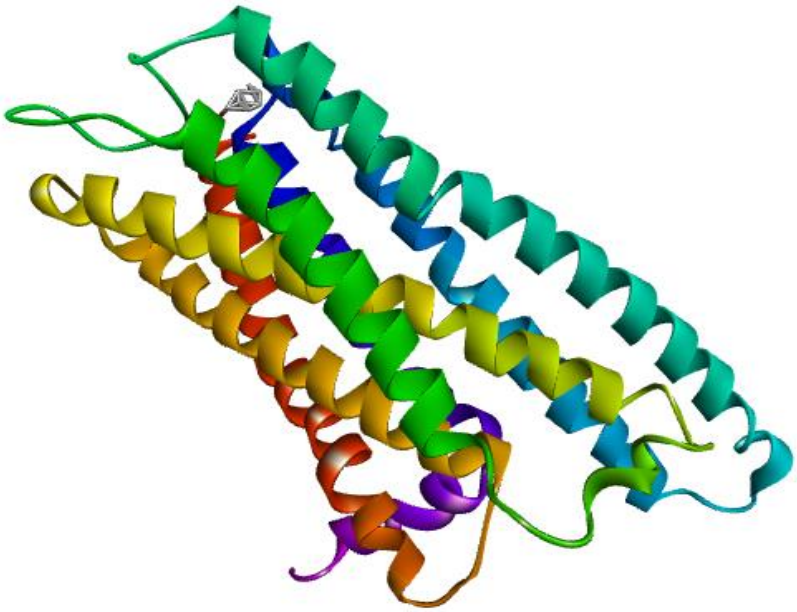


Figure 14 3D hTAS2R14 and L-Glutamine, N2-[(phenylmethoxy)carbonyl]visual

4.4 Interpretation of GCMS analysis

GCMS detailed the presence of phytochemical constituents present in our tablet. Following are the lists of phytochemicals found.

4.4.1 Alkaloids

Sr. No.	Compounds
1	2,6-Lutidine 3,5-dichloro-4-dodecylthio
2	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]
3	4-(2-Methoxyphenyl)piperidine
4	2-(2-Hydroxy-cyclohexylamino)-pyridine, N-oxide
5	(5S,6aR,10aS)-5-Propyldecahydrodipyrrolo[1,2-a:1',2'-c]pyrimidine
6	Uridine, 5-heptafluoropropyl
7	1,6-Dihydropyridine-3-carboxylic acid, 5-cyano-2-ethyl-6-oxo-4-phenyl-, methyl ester
8	2-Ethylacridine
9	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro-7-imino-, ethyl ester
10	4-Amino-7-[2,3-dihydroxypropyl]pyrrolo[2,3-d]pyrimidine
11	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]
12	6-Methyl-5-[1-piperidinyl]-2,4-pyrimidinediamine
13	2-Pyridinemethanol 3,5-dichloro-4-hydroxy-6-methyl
14	N-Nitroso-2,4,4-trimethyloxazolidine
15	2-Carbamoylaziridine-1-carboxylic acid, t-butyl ester
16	Piperidine, 4-(2-aminoethyl)-4-benzyl-1,2,5-trimethyl
17	p-N-[3-[N-Aziridyl]propyl]anisidine
18	2-Methylsulfanyl-6-oxo-4-(3-phenoxyphenyl)1,4,5,6-tetrahydropyridine-3-carbonitrile

19	3-Butyl-6-methyl-4-prop-2-en-1-yl-2,6-dioxo-4,5,6,7-tetrahydro-1,2,3-triazolo[4,5-d]pyrimidine
20	1,1,3,3-Tetra-tert-butyl-2-phenylsulfonylthiaguanidine
21	Cycloheptano[d]imidazolidine, 1,3-dihydroxy-2-methyl
22	4H-1,3-Dioxino[4,5-c]pyridine-5-methanol, 2,2,8-trimethyl
23	Nickel, [(1,2,6,7-.eta.)-1,6-heptadiene](pyridine)
24	Nickel, (.eta.-2-2-diallyl ether)(pyridine)
25	Pyridine, 3-ethoxy-2-nitro
26	4-Methyl-2-mercaptopyridine-1-oxide
27	Guanidine, 1-[4-(2-pyridyl)thiazol-2-yl]
28	3,5-Dimethylpiperidine-1-thiocarboxylic acid, 2-[1-[2-pyridyl]ethylidene]hydrazide
29	Cycloheptano[d]imidazolidine, 1,3-dihydroxy-2-methyl
30	6-[N-p-Chlorophenylthiocarbamoyl]-2,4-diamino-6,7-dihydro-5H-pyrrolo[3,4-d]pyrimidine
31	4-Furfurylidene-5-oxo-2-thiooxoimidazolidine
32	3-Hydroxy-6-methyl-2-nitropyridine
33	Pyridine, 2-carbothioamide
34	Pyridine, 2-(methylthio)-3-nitro
35	1-t-Butyl-2-(hydroxyphenylmethyl)aziridine-2-carbothioic acid, S-phenyl ester
36	Pyrrolidine-2,5-dione, 3-(4-formylpiperazin-1-yl)-1-(4-methoxyphenyl)
37	2,4-Dimethoxy-5-methyl pyrimidine
38	2-Brom-uridine

39	2,4(1H,3H)-Pyrimidinedione, 6-amino-1,3-di-2-propenyl
40	5-Bromo-2-mercaptopyridine-1-oxide
41	(5S,6aR,10aS)-5-Propyldecahydrodipyrrolo[1,2-a:1',2'-c]pyrimidine
42	2,6-Lutidine 3,5-dichloro-4-dodecylthio

4.4.2 Glycosides

Sr. no.	**Compounds**
1	Galactopyranoside, 1-octylthio-1-deoxy
2	Sarreroside
3	Ethyl 1-thio-.alpha.-d-arabinofuranoside
4	beta.-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate
5	Methyl 2-O-mesyl-3,4-isopropylidene-.beta.-d-arabinoside
6	alpha.-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate
7	beta.-D-Galactopyranoside, methyl 2,6-bis-O-(trimethylsilyl)-, cyclic butylboronate
8	1,2-O-Isopropylidene-3-O-methanesulfonyl-5-O-methoxycarbonyl-d-xylofuranose
9	Digitoxin
10	Desulphosinigrin

4.4.5 Terpenoids

Sr. no.	Compounds
1	Thiophene-2-carboxaldehyde, phenylsulfonylhydrazone
2	Benzenepropanoic acid, TBDMS derivative
3	1,2-Bis(trimethylsilyl)benzene
4	2,6-Nonadienoic acid, 7-ethyl-9-(3-ethyl-3-methyloxiranyl
5	1-Heptene, 2,6,6-trimethyl
6	Benzene, (3,3-dimethylbutyl)
7	p-Xylene
8	Benzene, (2,3-dimethyldecyl)
9	1,2-dihydro-8-hydroxylinalool
10	Benzeneethanol, .alpha.,.beta.-dimethyl
11	1-Cyclohexene, 1-ethynyl
12	Cyclopentene, 1-ethenyl-3-methylene
13	D-Mannotridec-6-ene-1,2,3,4,5-pentaol
14	1-Hexene, 4-methyl
15	1,4-Bis(trimethylsilyl)benzene
16	1H-4-Azacycloprop[cd]indene, octahydro-4-methyl
17	1-Cyclohexene-1-methanol
18	Propanoic acid, 2-[[(1-methylethylidene)amino]oxy]-, ethyl ester

19	N-Benzylidene-2-[N-aziridyl]ethylamine
20	Cyclopropane, 1-methylene-2-(4,4-diethoxybutyl)
21	2-Heptenoic acid, 7-(methylenecyclopropyl)-, methyl ester
22	1-Pentanol, 5-(methylenecyclopropyl)
23	3-Methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene 1,1-dioxide
24	alpha.-d-Glucofuranosyl benzenesulfonate
25	4-Penten-1-ol, 2-methylene
26	Cyclohexene, 1-nitro
27	1,2,4-Benzenetricarboxylic acid, cyclic 1,2-anhydride, nonyl ester
28	4-Cyclopentene-1,3-diol, trans
29	:Hydrazinecarboxamide, 2-(2,6-cyclooctadien-1-ylidene)
30	2,5-Cyclohexadiene-1,4-dione, 3-hydroxy-5-methyl-2-(1-methylethyl)
31	1H-4-Azacycloprop[cd]indene, octahydro-4-methyl
32	Bicyclo[3.3.0]octan-2-ol, 7-methylene
33	(7R,8RS)-Ethyl 8-hydroxy-cis-bicyclo[4.3.0]-3-nonene-7-carboxylate
34	Thiophene-2-carboxylic acid, 5-methylsulfonyl-4-nitro
35	1,2-trans-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-hydroxy-1-isopropyl)cyclohex-3-ene
36	Naphthalene, decahydro-2,3-dimethoxy-, (2.alpha.,3.beta.,4a.alpha.,8a.alpha.)
37	Lup-20(29)-ene-3,21,28-triol, 28-acetate, (3.beta.,21.beta.)

38	9-Methyl-10-methylenetricyclo[4.2.1.1(2,5)]decan-9-ol
39	Bicyclo[10.1.0]trideca-4,8-diene-13-carboxamide, N-(4-fluorophenyl)
40	6-Heptene-1-nitrile
41	Thiophene, 2-(1,1-dimethylethoxy)-5-methyl
42	4-Cyclopentene-1,3-diol, cis
43	p-Octyloxynitrobenzene
44	3-Thiophenecarboxylic acid, 3-aminotetrahydro-2,2-dimethyl-, (R)
45	Caryophyllene oxid
46	1,5-Methano-1H-indene-1,4-diol, octahydro-, (1.alpha.,3a.beta.,4.beta.,5.beta.,7a.beta.)
47	Cyclopentanone, 2-cyclopentylidene
48	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydro-2H-picene-4a-carboxylic ac
49	Cyclohexane,ethenylidene
50	Piperazine-1,4-diamine, N,N'-bis(pyridin-2-ylmethylene)
51	(2-Phenethylcarbamoyl-ethyl)-carbamic acid benzyl ester
52	3-Methylenecyclopropane-trans-1,2-dicarboxylic acid
53	Cyclohept-4-enecarboxylic acid
54	Benzaldehyde, 3,4-methylenedioxy-2-nitro-, thiosemicarbazone
55	Azabicyclo[3.2.2]noname-3-thiocarboxylic acid 2-[2-pyridyl-methylene]hydrazide

56	7-epi-trans-sesquisabinene hydrate
57	3-Benzoyl-5-(2,4-dimethoxybenzylidene)rhodanine
58	Urea, N,N"-(2-methylpropylidene)bis
59	Benzene, (1,2,2-trimethylpropyl)
60	2,3-Bis(p-tolylsulfinyl)-1,3-butadiene
61	3-Dodecene, (Z)-
62	7-Tetradecene
63	4,5-Trimethylene-2H-1,3-oxazine-3H-2,6-dione
64	2,5-Cyclohexadiene-1,4-dione, 2-ethyl
65	1,5:2,4-Dimethanopentalene-3,6-diol, octahydro
66	N-[2-(2-Hydroxy-1-naphthylmethyleneamino)-4-methoxyphenyl]acetamide
67	Cyclohexanol, 2-methylene-3-(1-methylethyl)-, cis-
68	Naphthalene, decahydro-1,4-dimethoxy-, (1.alpha.,4.beta.,4a.alpha.,8a.beta.)
69	Cyclohexene, 6-butyl-1-nitro
70	trans-Z-.alpha.-Bisabolene epoxide
71	Z,Z,Z-4,6,9-Nonadecatriene
72	2-Cyclopentene-1-thioacetic acid, 1-hydroxy-, S-t-butyl ester
73	Benzene, p-di-tert-butoxy
74	2-Pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (E)
75	2,4-Heptadiene, 5-diethylboryl-2-methyl-4-trimethylsilyl

76	Silane, [[(3.alpha.,5.beta.,20S)-pregn-11-ene-3,11,17,20-tetrayl]tetrakis(oxy)]tetrakis[trimethyl
77	7-Heptadecene, 7-methyl-, (E)
78	4-Undecene, 7-methyl
79	2-((2R,4aR,8aS)-4a-Methyl-8-methylenedecahydronaphthalen-2-yl)acrylaldehyde
80	2-Amino-5-iodotoluene
81	4.alpha.,5.beta.-Epoxy-9.alpha.-hydroxygermacra-1(10),11(13)-dien-6,12-olide 8.alpha.-(4-hydroxysenecioate)
82	7-Acetyl-9-(2-chloroethyl)-7,8-diazatetracyclo[4.3.0.0(2,4).0(3,5)]non-8-ene
83	Benzenepropionic acid, 4-tridecyl ester
84	Benzenepropionic acid, 4-tetradecyl ester
85	6-Methyl-2-phenethyl[1,3]dioxan-4-one
86	Z,Z,Z-1,4,6,9-Nonadecatetraene
87	4-Methyl-E-9-octadecene
88	1-Cyclohexene-1-methanol
89	1-Eicosene
90	3-Amino-2,3-dihydrobenzoic acid, N-dimethylaminomethylene-, methyl ester
91	Pregn-5-ene-3,8,11,12,14,20-hexol, (3.beta.,11.alpha.,12.beta.,14.beta.,20R)
92	1,5-Cyclooctadiene, 1-t-butyl
93	Cyclohexane-1,3-dione, 2-allylaminomethylene-5,5-dimethyl
94	Pentaleno[1,2-b]oxirene, octahydro-, (1a.alpha.,1b.beta.,4a.alpha.,5a.alpha.)

95	Cyclopentanecarboxylic acid, 3-methylene-2,2-dimethyl-5-[(E)-1-propenyl]-, methyl ester
96	Naphthalene, decahydro-1,4-dimethoxy-, (1.alpha.,4.beta.,4a.alpha.,8a.alpha.)
97	Bicyclo[10.1.0]trideca-4,8-diene-13-carboxamide, N-(3-chlorophenyl)
98	1-Methyl-7-azabicyclo[4.1.0]hepta-2,4-diene-7-carboxylic acid, 3,17-diacetoxy-4,4,10,13-tetramethylhexadecahydrocyclopenta[a]phenanthren
99	Naphthalene, decahydro-1,4a-dimethyl-7-(1-methylethyl)-, [1S-(1.alpha.,4a.alpha.,7.alpha.,8a.beta.)]
100	9,10-Diazatetracyclo[6.2.0(2,7).0(3,6).0(5,8)]dec-9-ene, N1-oxide
101	2-Octene, 1-bromo-1,1,2-trifluoro
102	2,6-naphthalenedicarboxylic acid, decahydro
103	N-[4-Sulfophenyl]-2-[1-[2-pyridyl]ethylidene]hydrazinecarbothioamide
104	10-Methylene-tricyclo[4.3.1.1(2,5)]undecane
105	2,3-Diazabicyclo[3.2.0]hept-2-ene, 1,6,6,-trifluoro-4-spirocyclopropane
106	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine
107	5-Bromo-8-(5-nitrosalicylideneamino)quinoline
108	1-Azacyclotridecane-1-thiocarboxylic acid, 2-[1-[2-pyridyl]ethylidene]hydrazide
109	Methyl 3-(1-pyrrolo)thiophene-2-carboxylate
110	Naphthalene, 1,4,5,8-tetrahydro
111	3a,10a-dihydroxy-5-(hydroxymethyl)-7-(2-hydroxypropan-2-yl)-2,10-dimethyl-4,6a,7,9,10,10b-hexahydrobenzo[e]azulene-3,8-dione

112	3-Hydroxy-2-methyl-6-phthalimidohexane p-toluenesulfonate
113	2-(n-Pentyl)oxybenzylidene acetophenone
114	2-(2-Methylpropyl)oxybenzylidene acetophenone
115	1.alpha.-(Hydroxymethyl)-7.alpha.,8.alpha.-dimethyl-7-(2-(3-furyl)ethyl)bicyclo[4.4.0]dec-2-ene 2-carboxylic acid, methyl ester
116	2-Amino-5-phenyl-1-[((E)-phenylmethylindene)amino]-1H-pyrrole-3,4-dicarbonitrile
117	2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,8a-hexahydro-gamma-chromene
118	Naphth[1,2-b]oxirene, decahydro-1a,7-dimethyl

4.4.4 Amino acids

Sr. No.	**Compounds**
1	L-Glutamine, N2-[(phenylmethoxy)carbonyl]
2	2-Acetylamino-3-cyano-propionic acid
3	l-Methionine, N-methoxycarbonyl-, isohexyl ester
4	4-Furfurylidene-5-oxo-2-thiooxoimidazolidine
5	Acetic acid, 2-(1-piperidyl)-, [4-aminofurazanyl-3-(amino)methylidenamino

4.4.5 Others (Unspecified)

Sr. No.	Compounds
1	6,8-Dimethyl-2-[4-(thiophene-2-carbonyl)piperazin-1-yl]quinoline-3-carbonitrile
2	7-Aza-1-oxaspiro[4.5]decane-2,8-dione, 10-t-butoxycarbonylaminoacetyl
3	l-Methionine, N-(2-chloroethoxycarbonyl)-, hexyl ester
4	benzeneacetic acid, 4-(methoxycarbonyl)-.alpha.-[4-(methoxycarbonyl)phenyl]-, ethyl ester
5	4-(4-Ethoxycarbonylbuta-1,3-dienyl)-1-methyl-2,5-diphenyl-1H-pyrrole-3-carboxylic acid, ethyl ester
6	Carda-4,20(22)-dienolide, 3-[(6-deoxy-3-O-methyl-.alpha.-D-allopyranosyl)oxy]-1,14-dihydroxy-, (1.beta.,3.beta.)
7	Benzenesulfonamide, N-[[5-(aminomethyl)-2-furanyl]methyl]
8	Benzenepropanoic acid, TBDMS derivative
9	3-[(2-Fluoroanilino)methyl]-5-(2-methoxyphenyl
10	Silane, bromomethyltripropyl-
11	2-(2-Methylpropyl)oxybenzylidene acetophenone
12	Cyclotetrasiloxane, octamethyl-
13	Cyclotrisiloxane, hexamethyl-
14	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
15	Phenylboronic acid, 2TMS derivative

16	1,3,5-Benzetriol, 3TMS derivative
17	2-Methylthiomethyl-4-(1-methylthioethyl)thietane
18	1,3,5-Benzetriol, 3TMS derivative
19	2-Methylthiomethyl-4-(1-methylthioethyl)thietane
20	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
21	3-Nitrophthalhydrazide
22	3,3,5,5,7,7,9,9,11,11,13,13-Dodecamethyl-1,15-bis(1,3,3,5
23	Cyclopentene-1-carboxylic acid, 4-[2-(diphenylmethyl)-2-propen
24	5-Bromo-4-nitroimidazole-2-[2-thioacetic acid]
25	Cyclotrisiloxane, hexamethyl-
26	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-
27	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
28	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-
29	Anthracene, 9,10-dihydro-9,9,10-trimethyl-
30	Cyclobarbital
31	1,4,12-Trioxa-7,9,15,17-tetraazacyclononadecane-8,16-dithione
32	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
33	3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide
34	4-tert-Butylphenol, TMS derivative
35	6-Dimethyl(chloromethyl)silyloxypentadecane
36	tert-Butyldimethylsilyl 2,3-dimethylbenzoate

37	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl
38	p-Menth-1-en-3-one, semicarbazone
39	Pentasiloxane, dodecamethyl-
40	Ethyl tris(trimethylsilyl) orthosilicate
41	Cyclobarbital
42	2-Myristynoyl-glycinamide
43	Methyl 3-cis,9-cis,12-cis-octadecatrienoate
44	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy
45	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl
46	1,2-Bis(trimethylsilyl)benzene
47	1-Propoxynonane
48	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl
49	Cyclotrisiloxane, hexamethyl-
50	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-
51	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane
52	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate
53	4,6-di-tert-Butylresorcinol
54	1,2-Bis(trimethylsilyl)benzene
55	Bendazol
56	5H-Cyclohepta-1,4-dioxin, 2,3,4a,6,7,9a-hexahydro-, cis-
57	Silanamine, N-[2,6-dimethyl-4-[(trimethylsilyl

58	Benzestrol, 2TMS derivative
59	Thymol, TMS derivative
60	(E)-1-(2-Hydroxy-4,6-dimethoxyphenyl)-3-phenylprop
61	5-Ethyl-5-methylnonadecane
62	13-Methyl-Z-14-nonacosene
63	2,3-Dihydroxy-6-nitroquinoxaline
64	Cystathionine, 2TMS derivative
65	2,4,6-Trichlorobenzonitrile
66	Trisiloxane, 1,1,1,5,5,5-hexamethyl-3,3-bis[(trimethylsilyl
67	Silicic acid, diethyl bis(trimethylsilyl) ester
68	1,2,4-Benzenetricarboxylic acid, cyclic 1,2-anhydride, nonyl
69	Benzothiazole-2,3-dicarboxylic acid, 2,3-dihydro-6-nitro-,
70	Benzenepropanoic acid, 4-benzoyl-, methyl ester
71	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-
72	Cyclodecasiloxane, eicosamethyl-
73	2-pentene, 3-(chloroethylboryl)-2-(chlorodimethylsilyl)-, (e)-
74	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
75	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
76	Benzene, 1,4-dichloro-2-nitro-
77	3,4-Dimethylbenzoic acid, TBDMS derivative
78	Ethyl homovanillate, TMS derivative

79	2-Oleoylglycerol, 2TMS derivative
80	Silane, dimethyldimethyl((dodec-9-ynyloxy)silyloxy)propoxy
81	4,7-Dimethoxy-2-methylindan-1-one
82	1H-Pyrazole, 1-(3-methylbutyl)-4-(4,4,5,5-tetramethyl-1,3,2
83	Octadecane, 3-ethyl-5-(2-ethylbutyl)-
84	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
85	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
86	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)
87	1-(.beta.-d-Ribofuranosyl)-4-difluormethoxy-uracil
88	Trinexapac-ethyl, TMS derivative
89	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
90	Tricyclo[4.2.1.0(2,5)]non-7-ene, 3,4-di(tris(trimethylsilyloxy
91	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid, 4,7-dihydro
92	5-Methylsalicylic acid, 2TMS derivative
93	2,6-Lutidine 3,5-dichloro-4-dodecylthio-
94	3-Butoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
95	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl
96	Decanenitrile
97	2-Ethylacridine
98	3-Carbazolyl methyl ketone thiosemicarbazone
99	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane

100	Uridine, 5-heptafluoropropyl-
101	Trimethylsilyl 3-methyl-4-[(trimethylsilyl)oxy]benzoate
102	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-
103	Terephthalic acid, 2-adamantyl propyl ester
104	Tris(tert-butyldimethylsilyloxy)arsane
105	Silanol, trimethyl-, phosphite (3:1)
106	Docosane, 1,22-dibromo-
107	(3-Benzenesulfonyl-4-isobutyrylcyclopent-2-enyl)acetic acid
108	5-Methoxy-3,3-dimethyl-pyrrolidin-2-one
109	Benzoic acid, 4-[[(trimethylsilyl)oxy]methyl]-, trimethylsily
110	Carbonic acid, decyl pentadecyl ester
111	2-Octene, (Z)-
112	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl]-
113	Pentanal, 5-hydroxy-, (2,4-dinitrophenyl)hydrazone
114	3,5-Dichloro-4-hydroxybenzoic acid
115	Di-n-decylsulfone
116	d-Glucitol, 1-S-heptyl-1-thio-
117	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
118	2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,8a-hexahydro-gamma
119	1-n-Octadecyloxy-1-methyl-1-silacyclobutane
120	Urea, 1-(4-fluoro-3-nitrophenyl)-3-(1H-indazol-5-yl)-

121	Dimethylmalonic acid, di(2-ethylhexyl) ester
122	2,2,4,4-Tetramethyl-6-(1-oxo-3-phenylprop-2-enyl)-cyclohexane
123	4-Chloro-bicyclo[2.2.1]heptan-2-ol
124	1,2-Propanediol, 3-(octadecyloxy)-, diacetate
125	1,1,3,6-tetramethyl-2-(3,6,10,13,14-pentamethyl-3-ethyl-pentadecyl
126	1.alpha.-(Hydroxymethyl)-7.alpha.,8.alpha.-dimethyl-7-(2-(
127	6-Dimethyl(chloromethyl)silyloxytridecane
128	3-Hydroxy-2-methyl-6-phthalimidohexane p-toluenesulfonate
129	1-Ethyl-trans-2-butenyl 2,4,6-trimethylbenzoate, (.+/-.)-
130	2,3-Diazabicyclo[3.2.0]hept-2-ene, 1,6,6,-trifluoro-4-spirocyclopropane
131	Pyridine, 1,2,3,6-tetrahydro-1-methyl-4-[4-chlorophenyl
132	Nona-3,5-dien-2-ol
133	4-[5-(3-Methoxyphenyl)-1H-1,2,4-triazol-3-yl]aniline
134	2-Nitrobenzyl alcohol, trifluoroacetate
135	1-Azacyclotridecane-1-thiocarboxylic acid, 2-[1-[2-pyridyl
136	Cyclobarbital
137	2-Heptenal, 2-propyl-
138	2-Octadecyl-propane-1,3-diol
139	2,3-Diazabicyclo[3.2.0]hept-2-ene, 1,6,6,-trifluoro-4-spiro
140	Butanal, 3-methyl-, (2,4-dinitrophenyl)hydrazine
141	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-

142	.beta.-d-Glucofuranosiduronic acid, methyl, .gamma.-lactone
143	3-Nonynoic acid
144	3(2H)-Thiophenone, dihydro-, oxime, 1,1-dioxide
145	Succinic acid, heptadecyl 2-methylallyl ester
146	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4
147	1,5,9-Cyclododecanetriol
148	2(1H)-pyridinone, 1-octyl-
149	Cyclopentanecarboxylic acid, 3-methylene-2,2-dimethyl-5-[(
150	Cyclopentanecarboxylic acid, 3-methylene-2,2-dimethyl-5-[(E)-1-propenyl]-, methyl ester
151	Bicyclo[3.3.1]nonane-2,9-diol, exo-anti-
152	.alpha.-Linolenic acid, TMS derivative
153	1-(3,3-Dimethyl-but-1-ynyl)-2,2,3,3-tetramethylcyclopropanecarboxylic acid
154	1-(4-Amino-furazan-3-yl)-5-methyl-1H-[1,2,3]triazole-4-carboxylic acid tert butyl ester
155	Valeric acid, 2,7-dimethyloct-7-en-5-yn-4-yl ester
156	Benzenepropionic acid, 4-tridecyl ester
157	1-Cyclopropyl-3,4-epoxyhex-5-en-1-yne
158	1-Cyclohexene-1-methanol
159	6-(1,4-Benzodioxan-2-ylcarbonylhydrazonomethyl)uracil
160	2-Butanone, (2,4-dinitrophenyl)hydrazone

161	2-Pentyl-cyclohexane-1,4-diol
162	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl
163	Benzene, p-di-tert-butoxy-
164	4,4-Dimethyl-cyclohex-2-en-1-ol
165	Pyrrole, 2-(1-naphthyliminomethyl)-
166	Cyclopentanol, 1-(methylenecyclopropyl)-
167	Methyl tetrahydroionol
168	N-[2-(2-Hydroxy-1-naphthylmethyleneamino)-4-methoxyphenyl
169	Dimethylmalonic acid, monochloride, 2-octyl ester
170	Propanoic acid, 2-chloro-, 2-propenyl ester
171	Cyclooctasiloxane, hexadecamethyl-
172	2-(2-Butoxyethyl)cyclopentan-1-one 2,4-dinitrophenylhydrazone
173	3-Pentanone, 1-hydroxy-2-methyl-1-phenyl-
174	1-Cyclopropyl-3,4-epoxyhex-5-en-1-yne
175	Z-2-Octadecen-1-ol acetate
176	1-Fluorononane
177	Cyclooctane-1,4-diol, cis
178	Decane, 1-chloro-
179	2-Butyndiol dimethyl ether
180	dl-Citrulline
181	2-Adamantanone oxime

182	E-8-Methyl-9-tetradecen-1-ol acetate
183	Z-26-Pentatriaconten-2-one
184	1,1,3,3-Tetra-tert-butyl-2-phenylsulfonylthiaguanidine
185	Tricyclo[3.3.1.1(3,7)]decanone, 4-hydroxy-, (1.alpha.,3.beta
186	3-Nonanol, 1,2:6,7-diepoxy-3,7-dimethyl-, acetate
187	Cycloheptasiloxane, tetradecamethyl-
188	8,11-Octadecadiynoic acid, methyl ester
189	7,8-Dibromo-4,4,7-trimethyl-hexahydro-benzo[1,3]dioxin-2
190	Phenol, 4-amino-2-nitro-
191	6-(3,5-Dimethyl-1H-pyrazol-1-yl)-N-(2-hydroxyethyl)-1,2,
192	2,2,2-Trichloro-1-(2-nitrophenylthioamino)ethanol
193	10-Acetoxy-2-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4
194	Cyclopentanone, 2-cyclopentylidene-
195	Arachidonic amide, N-[5-hydroxy-n-pentyl]-
196	Propanoic acid, 5-hexen-1-yl ester
197	p-Octyloxynitrobenzene
198	4-Methylfurazan-3-carboxylic acid
199	1,8-Nonadien-3-ol
200	Cyclohexasiloxane, dodecamethyl-
201	Octadecane, 5-methyl-
202	Heptadecanoic acid, heptadecyl ester

203	2-Heptyn-1-ol
204	cis-Decalin, syn-2-methoxy-
205	1-(2,3-Dihydro-benzo[1,4]dioxin-2-ylmethyl)-3-ethyl-thiourea
206	Thiophene-2-carboxylic acid, 5-methylsulfonyl-4-nitro-
207	Butyric acid, 4-phenyl-, isobutyl ester
208	2-Methylsulfanyl-6-oxo-4-(3-phenoxyphenyl)1,4,5,6-tetrahydropyridine
209	cis-2-Ethyl-2-hexen-1-ol
210	trans-3,4-Epoxynonane
211	Hydrazinecarboxamide, 2-(2,6-cyclooctadien-1-ylidene)-
212	Ethanone, 2-azido-1-(4-methyl-3-furazanyl)-, oxime
213	Cyclohexyl propionate
214	d-Mannitol, 1-decylsulfonyl-
215	Cyclohexene, 1-nitro-
216	Cyclopentasiloxane, decamethyl-
217	Cyclopropane, 1-methylene-2-(4,4-diethoxybutyl)-
218	Undecane, 4,7-dimethyl-
219	1-Propanone, 1-(3-cyclohexen-1-yl)-2,2-dimethyl-
220	Benzene, (2,3-dimethyldecyl)-
221	cisZ-11,12-Epoxytetradecan-1-ol
222	Nonane, 4,5-dimethyl-
223	Paromomycin

224	3-Nonanol, 1,2:6,7-diepoxy-3,7-dimethyl-, acetate
225	1-Hexanol, 2-ethyl-
226	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl
227	Bicyclo[2.2.0]hex-1-yl-methanol
228	Ethenamine, N-methyl-N-nitroso-
229	Tris(tert-butyldimethylsilyloxy)arsane
230	1-Butanol, 2-methyl-
231	3-Pentanol, 2-chloro-4-methyl-, (R*,S*)-(.+/-.)-
232	Heptane, 2,2,3,5-tetramethyl-
233	1,3-Dioxane, 4,4-dimethyl-
234	Bicyclo[2.1.1]hexan-2-ol, 2-ethenyl-
235	Tert-butyl N-benzylcarbamate
236	(2,2-Dimethylcyclobutyl)methylamine
237	2-Buten-1-ol, propanoate
238	Morpholine
239	Hexane, 2,4-dimethyl-
240	2-Hexanol, 3,4-dimethyl-

4.4.6 Others (Specified)

Sr. no.	Classification	Compounds
1	Carboxylic acid	Formic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo[1,5-a]pyrimidin-6-yl)ethyl ester
		Benzoic acid, 4-(1,3-dioxolan-2-yl)-, methyl ester
		1-Hexanol, 5-methyl-2-(1-methylethyl)-, acetate
		Stearic acid, 3-(octadecyloxy)propyl ester
		4-Acetyloxyimino-6,6-dimethyl-3-
2	Phenol	Hexahydropyridine, 1-methyl-4-[4,5-dihydroxyphenyl]-
3	Phenylureas	2-Oxazolamine,4,5-dihydro-5-(phenoxymethyl)-N-[(phenylamino
4	Coumarins	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11(1H
		7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin
5	Non-reducing sugars	l-Gala-l-ido-octose
6	Quinuclidones	1-Azabicyclo[2.2.2]octan-3-one
7	Styrenes	Butanoic acid, 3-methyl-, 3-phenyl-2-propenyl ester
8	Phenol ethers	3-Butyl-5-(phenoxymethyl)-2-oxazolidinone
9		3-Isopropyl-5-(phenoxymethyl)-2-oxazolidinone
10	Anisoles	Ethyl homovanillate, TMS derivative

11	Ketals	1,3-Dioxolane, 4-ethyl-5-octyl-2,2-bis(trifluoromethyl)-, trans
12	Oxazolidinones	Carbamic acid, N-(4-pyridyl)-, 2-(2-oxazolidinone-3-yl)ethyl
13	Xanthines	Purine-2,6-dione, 8-(3-ethoxypropylamino)-1,3-dimethyl-3
14	Alkylarylamines	(3,5-Dimethylpyrazol-1-ylmethyl)(4-methylfurazan-3-yl)amine
15	Napthoquinone	2-[4-Cyclohexylbutanoylamino]-3-chloro-1,4-naphthoquinone
16	Amines	n-Dodecylpyridinium chloride
17	Anilides	Butanedioic acid, monoamide, N-(3-fluorophenyl)-, allyl ester
18	Indanes	1H-inden-1-amine, 2,3-dihydro-3-phenyl-
19	Thiazoles	Acetic acid, 2-[4-(4-chlorophenyl)-2-thiazolyl]-, ethyl ester
20	Triazoles	4-Allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione
21	Alkyl aryl ethers	Disulfide, bis(5-methoxy-2-pyrimidinyl)
		Thiophene, 2-(1,1-dimethylethoxy)-5-methyl-
22	Oxime ethers	3-Hepten-2-one, O-methyloxime
23	Trialkylheterosilanes	(4-Octylcyclohexyl)methyl trimethylsilyl ether

24	Dibenzazepines	Carbamic acid, N-[10,11-dihydro-5-(2-methylamino-1-oxoethyl
25	Benzothiazoles	Ethanone, 2-(2-benzothiazolylthio)-1-(3,5-dimethylpyrazolyl
26	Iridoids	3-(1,5-Dimethyl-hexa-1,4-dienyl)-2,2-dimethyl-4-trimethylsilylcyclopentanol
27	Imidolactams	1H-1,2,3-Triazole-4-carboxylic acid, 1-(4-amino-1,2,5-oxa
		Furazan-3-carbohydrazide, 4-amino-
28	Imidazoles	1H-imidazole-2-methanol, 1-decyl-
		Imidazole, 2-bromo-4-methyl-5-nitro-

4.5 Intepretation of FTIR analysis

FTIR graph thus obtained is indicating the presence of certain functional groups in the tablet. Interpretation of this graph is as follow,

4.5.1 OH (Hydroxyl group):

Absorption peak around 3500-3200 cm^-1: This indicates the presence of the hydroxyl group (OH) in the sample. The peak is broad and usually strong.

4.5.2 CO (Carbonyl group):

Absorption peak around 1750-1650 cm^-1: This indicates the presence of a carbonyl group (C=O) in the sample. The peak is typically sharp and strong.

4.5.3 C=C (Double bond):

Absorption peak around 1680-1620 cm^-1: This indicates the presence of a carbon-carbon double bond (C=C) in the sample. The peak is usually sharp and strong.

4.5.4 CH (Aliphatic groups):

Absorption peaks around 3000-2800 cm^-1: These indicate the presence of aliphatic (saturated) carbon-hydrogen bonds (CH) in the sample. The peaks are typically broad and strong.

It's important to note that the exact positions and intensities of the absorption peaks may vary depending on the specific molecule or compound present in the sample, as well as the resolution and conditions of the FTIR analysis.

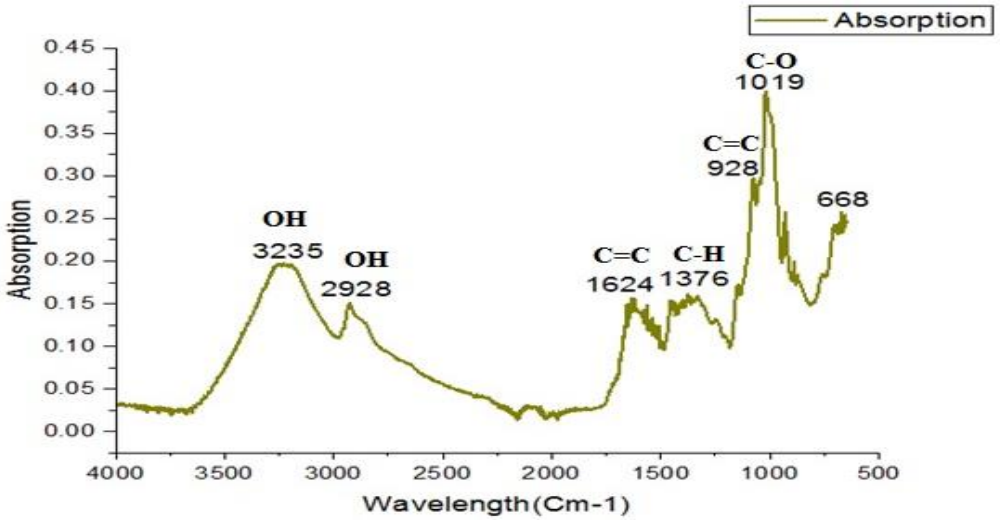


Figure 15 FTIR graph plot of stevia-mushroom tablet

4.6 Mechanical strength

As consumer demand for innovative and functional food products increases, the formulation of Stevia-Mushroom tablets presents a novel approach to provide both sweetness and potential health benefits in a convenient and portable form. However,

before these tablets can be considered for commercial use, it is essential to assess their mechanical properties, particularly their hardness and mechanical strength.

The hardness of a tablet determines its ability to withstand external forces during handling and transportation without crumbling or breaking. It is an essential parameter that influences the tablet's disintegration and dissolution rates, impacting its overall efficacy and consumer experience. Mechanical strength, on the other hand, measures the tablet's ability to resist deformation or breakage under pressure, ensuring its stability and shelf-life.

In this study, we aim to investigate the hardness and mechanical strength of Stevia-Mushroom tablets using standardized testing methods. By understanding the mechanical properties of these tablets, we can assess their suitability for various applications and provide valuable insights for optimization and developments of commercial tablets.

As can be seen in the graph, Strain/Stress Max, could indicate the tablet's ability against external force to maintain integrity. Stress, Strain percentage, Tensile strength of said tablet was found to be ≈1Kg, 58% and0.8 MPa. Diameter of the tablet was found to be

9.8 x 3.47 mm using the Vernier caliper.

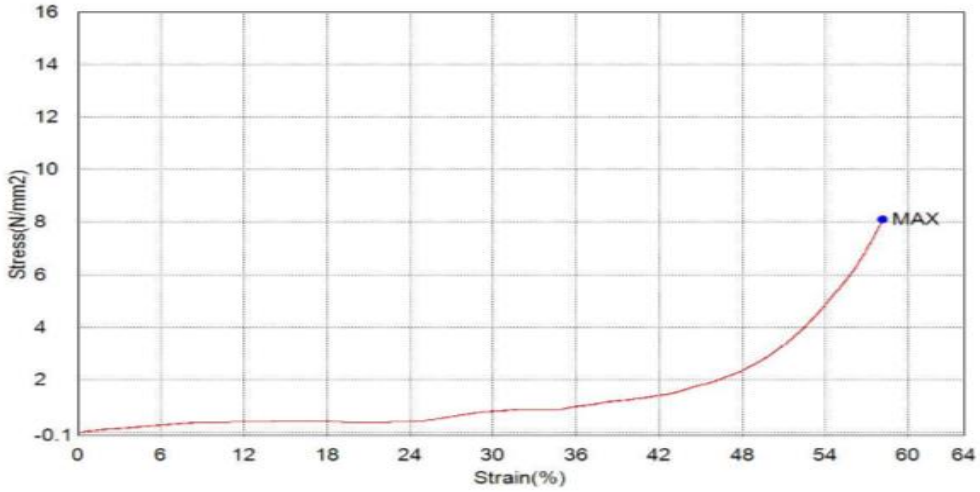


Figure 16 Mechanical stress/strain graph plotted of Stevi-O tablet

4.7 Cost analysis

To make sure that the evaluation of stevia-mushroom tablet as per market competitors, a basic lab-grade analysis was conducted as per reported by (Kunta, 2018). A parallel was drawn by accessing the market price of all such popular competitors of stevia blend sweeteners. For this purpose, TROPICANA Slim Stevia mix, CANDEREL Stevia and STEVIANA Stevia sweetener were chosen.

Figure 17 Price analysis of available stevia products available in local market

From the lab-grade production cost and calculation an estimation of bulk prices was also evaluated to mimic the value at a more industrial scale. The weight estimation of stevia-mushroom tablet was calculated to be 60mg using water as solvent was estimated to be at PKR 34,365. This is the production cost of production of 60mg stevia-mushroom extract mix.

Evaluation of cost of labor, revenue, gross margin and mark-up were also calculated all of which pointed that a reduced dependence on international market and companies is possible, given that all raw materials are grown in Pakistan. This cost analysis can serve as a means to further evaluate and analyze the potential of stevia grown in Pakistan to be a lead provider to not only local industry but international as well.

Here is a summary of all cost utilized in this research project. All prices are taken from daraz.pk, all equipment were taken at face value.

Table 3 Breakdown of the variable and fixed cost of the entire extraction and pressing process

Unit Op.	Material/equipment	Amount	Variable cost	Fixed cost
Loading	Stevia leaf powder	200g	PKR 4,000	--
	WB Mushroom	200g	PKR 4,000	--
Grinding	Kenwood Kitchen Machine Blender			PKR 38,500
Extraction	Water	25L	PKR 150	--
Drying	Dehydrator	--	--	PKR 75,000
Binding	Alpha Cellulose	500g	PKR 24,728	

Pill pressing	Manual pill press machine			PKR 1500
Labor	17500 x 5/20		PKR 1487	--
Product per cycle	40 tablets	60g	PKR 38,365	PKR 115,000
Revenue	Price * quantity	--	PKR 70,784	
Gross margin	Revenue – total variable	--	PKR 33,238.05	

A pie chart of all the equipment used in the extraction and tablet making summarizing the whole ordeal is given below. Pie chart simplifies the usage contribution of each factor in each step of tablet making, strictly depending on the credit value.

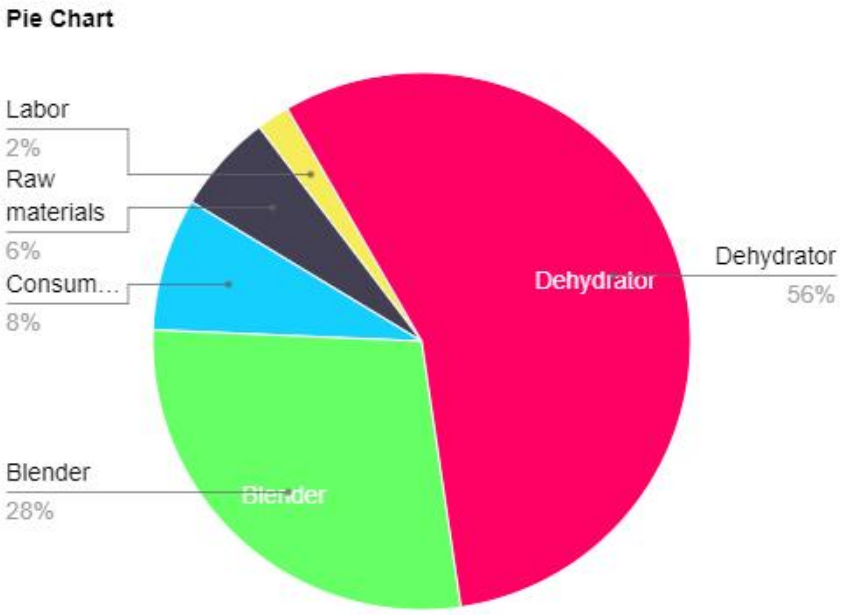


Figure 18 Share of each equipment/material in total cost of single Tablet batch

Table 4 Total depreciation and the salvage value of each piece of machinery to be used in tablet making process

Equipment	Original cost	Salvage value	Useful lifespan	Depreciation/ year	Total Dep.
Kitchen Blender Machine	38,500	0	10	3,850	38,500
Year 1	38,500	0	9	-3,850	34,650
Year 2	38,500	0	8	-3,850	30,800
Year 3	38,500	0	7	-3,850	26,950
Year 4	38,500	0	6	-3,850	23,100
Year 5	38,500	0	5	-3,850	19,250
Year 6	38,500	0	4	-3,850	15,400
Year 7	38,500	0	3	-3,850	11,550
Year 8	38,500	0	2	-3,850	7,700
Year 9	38,500	0	1	-3,850	3,850
Year 10	38,500	0	0	-3,850	0
Manual pill press machine	1,500	1,050	3	150	450
Year 1	194,460	1,050	2	-150	1,350
Year 2	194,460	1,050	1	-150	1,200
Year 3	194,460	1,050	0	-150	1,050
Dehydrator	75,000	45000	4	7,500	30,000
Year 1	75,000	45000	3	-7,500	67,500

Year 2	75,000	45000	2	-7,500	60,000
Year 3	75,000	45000	1	-7,500	52,500
Year 4	75,000	45000	0	-7,500	45,000

4.7.1 Effect of price on Revenue

As per any techno-economic analysis, after setting a benchmark or baseline price for a product, its direct effect on revenue generation potential and gross margin potential is evaluated. This part is to highlight the profitability of the product. Baseline value set for stevia-mushroom tablet is PKR 1769.6.

Table 5 Variation in price affecting Revenue

Price	Variation	Revenue
PKR1504.16	-15%	PKR 601664
PKR 1592.64	-10%	PKR 637056
PKR 1681.12	-5%	PKR 672448
PKR 1769.6	0	PKR 707840
PKR 1858.08	+5%	PKR 743232
PKR 1946.56	+10%	PKR 778624
PKR 2035.04	+15%	PKR 814016

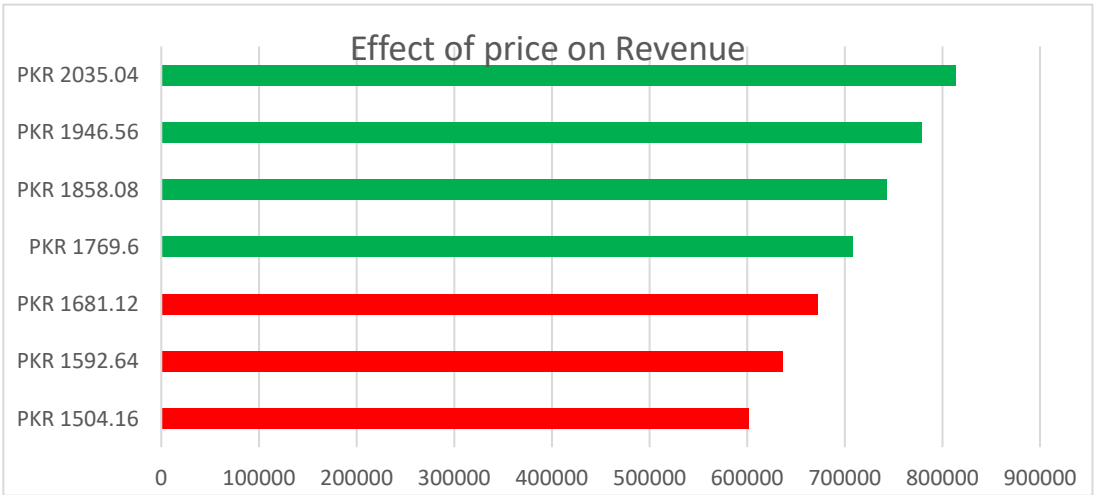


Figure 19 Graphical representation of variation effect on revenue

4.7.2 Effect of price on Gross Margin

Similarly, effect if variation in price is seen on gross margin.

Table 6 Variation in price affecting gross margin

Price	Variation	Gross Margin
PKR1504.16	-15%	PKR 567298.05
PKR 1592.64	-10%	PKR 602690.05
PKR 1681.12	-5%	PKR 638082.05
PKR 1769.6	0	PKR 673474.05
PKR 1858.08	+5%	PKR 708866.05
PKR 1946.56	+10%	PKR 744258.05
PKR 2035.04	+15%	PKR 779650.06

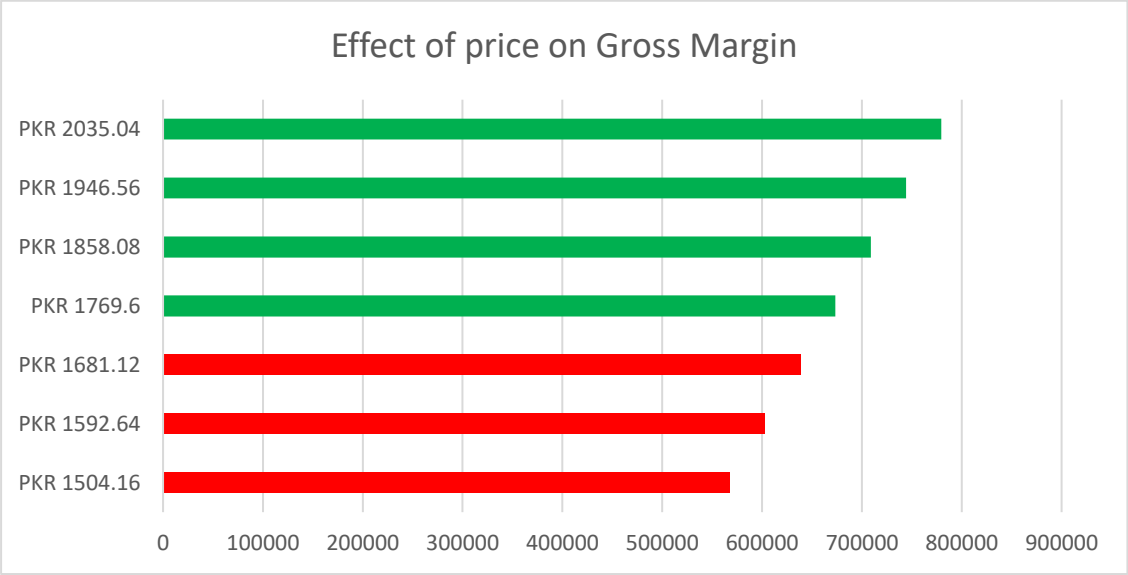


Figure 20 Graphical representation of variation effect on gross margin

CHAPTER 5: DISCUSSION

CHAPTER 5: DISCUSSION

The global demand for healthier sweeteners has spurred research on natural alternatives, leading to the popularity of stevia. While stevia is a zero-calorie sweetener, its bitter aftertaste has hindered its widespread acceptance. To address this issue, researchers have successfully developed a stevia-white button mushroom tablet, incorporating l-glutamine derivatives to mask the bitter taste. The formulation of the tablet involves carefully selected ingredients. White button mushrooms, known as Agaricus bisporus, are used due to their rich umami compounds, which complement the sweetness of stevia. These mushrooms are processed into a fine powder to ensure even distribution within the tablet.

The critical challenge faced in this development was the bitter taste of stevia. To overcome this, l-glutamine derivatives were incorporated into the formulation. L-glutamine is an amino acid naturally found in the human body, and its derivatives are known for their ability to reduce bitterness perception. By using these derivatives, the researchers successfully masked the bitter taste of stevia, making the tablet more palatable. One of the significant benefits of this tablet is its stability. Through meticulous research and testing, the researchers achieved a stable formulation that ensures the tablet's integrity throughout its shelf life during shipping and packaging. This stability is crucial for consumer satisfaction and product effectiveness.

Apart from taste masking, the inclusion of white button mushrooms offers additional nutritional benefits to the tablet. These mushrooms are a rich source of vitamins, minerals, and antioxidants, contributing to the overall nutritional profile of the product. This blend of sweetness from stevia and nutrition from white button mushrooms makes the tablet an attractive option for health-conscious consumers.

As for the cost analysis, the researchers evaluated various factors to ensure the tablet's economic viability. The cost of raw materials, manufacturing processes, and packaging were carefully considered. The abundance of white button mushrooms in the market helped in keeping the material cost within a reasonable range. Moreover, the use of l-glutamine derivatives was found to be cost-effective, given their potency in small quantities. In the long run, the cost analysis indicated that the stevia-white button mushroom tablet could be competitively priced compared to other natural sweeteners in the market. Additionally, the tablet's stable formulation would reduce potential losses due to product degradation, further enhancing its cost-effectiveness.

The successful development of this tablet has opened new possibilities for the food and beverage industry. The use of natural sweeteners, like stevia, is becoming increasingly important as consumers seek healthier alternatives to refined sugars. By combining the sweetness of stevia with the nutritional benefits of white button mushrooms, the tablet offers a compelling solution that caters to both taste and health preferences.

The formation and cost analysis of the stevia-white button mushroom tablet have proven to be a significant breakthrough in the realm of natural sweeteners. The successful masking of the bitter taste of stevia through l-glutamine derivatives and the incorporation of nutritious white button mushrooms have resulted in a stable and cost-effective product. As consumer demand for healthier options continues to rise, this innovative tablet holds great promise in meeting those expectations and revolutionizing the sweetener market

CHAPTER 6: CONCLUSION

CHAPTER 6: CONCLUSION

The production of stevia-mushroom tablets has emerged as a remarkable solution to address the inherent bitter taste of stevia while offering a healthier and more cost-effective alternative to traditional table sugar. Through an optimized lab protocol and a comprehensive techno-economic analysis, the viability and advantages of this production process have been substantiated.

One of the primary challenges with using stevia as a sweetener has been its characteristic bitter aftertaste, which can be off-putting to some consumers. However, by incorporating mushroom extracts known for their natural umami flavors, the stevia-mushroom tablets can effectively mask the bitterness, resulting in a more palatable and enjoyable taste profile. This breakthrough not only enhances the overall consumer experience but also opens doors to a wide array of potential applications in various food and beverage products without compromising on taste.

Beyond taste improvement, stevia-mushroom tablets offer significant health benefits compared to conventional table sugar. Stevia, derived from the leaves of the *Stevia rebaudiana bertuni* plant, is a natural, non-caloric sweetener that does not raise blood sugar levels, making it an ideal option for individuals with diabetes or those looking to reduce their sugar intake. Additionally, stevia has been associated with potential health advantages, including aiding in weight management, promoting oral health, and acting as an antioxidant, contributing to overall well-being. The incorporation of mushroom extracts further enhances the nutritional value of the tablets, as mushrooms are rich in vitamins, minerals, and bioactive compounds that complement the health benefits of stevia.

From an economic standpoint, the production of stevia-mushroom tablets proves to be a highly cost-effective alternative to using traditional table sugar. With the increasing

global demand for healthier sugar substitutes, the market potential for these tablets is vast. The techno-economic analysis conducted on the production process reveals that the cost of manufacturing stevia-mushroom tablets in bulk is competitive, indicating a potential reduction in reliance on expensive imported sweeteners.

In conclusion, the development of stevia-mushroom tablets represents a significant advancement in the field of natural sweeteners. By effectively masking the inherent bitterness of stevia and synergistically combining it with the nutritional benefits of mushroom extracts, these tablets offer a healthier, more sustainable, and cost-effective alternative to table sugar. As consumers increasingly prioritize health-conscious and environmentally sustainable choices, stevia-mushroom tablets have the potential to revolutionize the sweetener industry and cater to the needs of a broad spectrum of health-conscious individuals seeking a natural, low-calorie sugar substitute that enhances overall well-being

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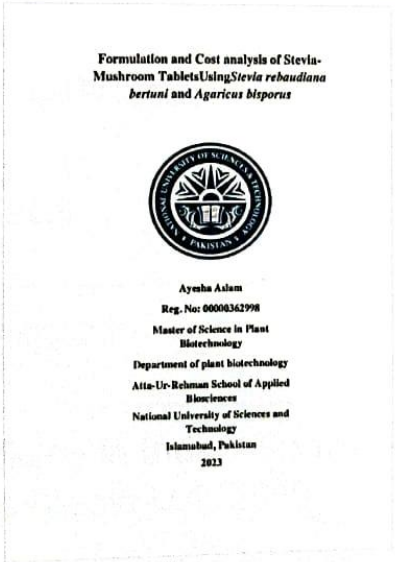

Formulation and Cost analysis of Stevia-Mushroom TabletsUsing*Stevia rebaudiana bertuni* and *Agaricus bisporus*

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