PHYTOCHEMICAL ANALYSIS OF TWO VALUABLE TRADITIONAL UNDERUTILIZED PLANTS OF HIMACHAL PRADESH

Project Report

Submitted to the "**Jaypee University of Information Technology**" in the fulfilment of Major Project for the award of the Degree of

B.Tech

In Discipline **Biotechnology**

Submitted By

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To

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ACKNOWLEDGEMENT

I would like to express my deep and sincere sense of gratitude to our supervisor Dr Hemant Sood (Associate Professor, JUIT) for her unwavering support and guidance throughout the course of the project. Without his expertise in the subject, I would not have been able to complete the project credibly and on time. I would also like to acknowledge with thanks the kind of patronage, motivation and constructive criticism received by me from my supervisor as these contributed immensely to the evolution of our ideas on the project.

Also, I'm extremely grateful to my parents for their constant encouragement and for providing me with all the necessary resources required for the successful completion of the project.

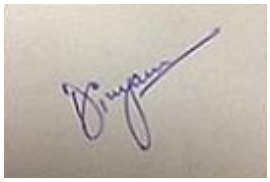
Date: 14th May 2022

DECLARATION

I Divyam hereby declare that the project report titled "Phytochemical analysis of two valuable traditional underutilized plants of Himachal Pradesh" has solely been submitted by me to "Jaypee University of Information Technology" under the guidance and supervision of Dr Hemant Sood and the work for the same has been carried out at Jaypee University of Information Technology, Solan H.P. (173234).

All the information & statement made by me are correct to the best of my knowledge. My candidature is liable to be cancelled and legal action may be initiated against me in case the information provided is found to be false or incorrect in any way.

Place: Shimla
Date: 14th May 2022



Name: Divyam

SUPERVISOR'S CERTIFICATE

This is to certify that the work titled "Phytochemical analysis of two valuable traditional underutilized plants of Himachal Pradesh" by Divyam during their 8th semester (January- May 2022) in fulfilment for the Major Project has been carried out under my supervision.

Signature of Supervisor:

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ABSTRACT

Morchella esculenta (Gucchi) and *Bunium persicum (Kala zeera)* are two exotic wild fungi and plant species found in Himachal Pradesh which are tremendously known in the villages of upper Himachal region particularly by the people living Kinnaur, Rekong Peo, Kullu, Chamba and Shimla. These two species have been extensively used by the people living there as food and food additives. This research has carried out the phytochemical analysis and nutraceutical profiling of these species to identify the compounds present. For this, the methanolic extract of these species were prepared and evaluated for the results that showed the presence of phenolic compounds in *Bunium persicum* and *Morchella esculenta* equal to (**217.368 microgram gallic acid equivalent/ mg of plant extract**) and (**239.747 microgram gallic acid equivalent/ mg of plant extract**) respectively. Flavonoid content for *Bunium persicum* and *Morchella esculenta* as (**36.903microgram quercetin equivalent/mg of plant extract)** and (**202.2667 microgram quercetin equivalent/mg of plant extract)** respectively. In the nutraceutical profiling only carbohydrates and proteins were quantified and the amounts were (**6.89% and 1.234%)** carbohydrates for *Bunium persicum* and *Morchella esculenta* respectively and (**4.09%** and **5.873%)** protein for the same.

INTRODUCTION

In spite of green revolution in India which modernized the agricultural production by latest equipment, modern technologies like genetic engineering to different artificial crossing and hybridisation methods, economic instability in the rural areas of the country is tremendous. In the remote villages of the nation many tribes and people of remote villages are economically weaker to run their livelihood. Himalayas are the regions of a great biodiversity.

Dev bhoomi Himachal Pradesh contributes an important and a major portion to plant diversity in India. Himachal has been reported with over 600 recorded species of plants till now that are edible and are used by the people living in villages and especially by the tribal people like gaddis.

Many more indigenous people living natively in different regions of Himachal from Chamba district to kinnaur and Mandi, Hamirpur to Dharamshala and Shimla.

Native people have traditional knowledge about wild species of different plants and fungi like "*Morchella esculenta* (gucchi), and spices like *Bunium persicum* (Kala zeera, shahi zeera) and many more.

These species and even many more are used as a food by the people in Himachal Pradesh as these carry a wide range of medicinal properties, different herbal compounds, anti-cancer compounds, anti-diabetic compounds and many other enzymes and vitamins out of which few are discovered and a wide range is still to be tested.

Many alkaloids, flavonoids, anti-oxidants, vitamins and phytoremediation components and many more constituents have been reported from these different wild food species.

These species are found in different distribution pattern in Himachal at altitude ranging from (1400-3500) metres above sea level and have a great expected price in the market locally from Rs.150/kg – 20,000/kg).

Thus, Himachal is place full of traditional food that has not been carried out into the rest of the states of the country.

Therefore, the attempt of this research is to focus and carryout these species in front of people and science for the welfare of mankind and the environment as many of these species are critically endangered because of unsuitable methods of harvesting.

[1.1] Morchella esculentum

Figure 1.1.1 Morchella esculentum (Gucchi)

Morchella esculenta commonly known as **true morel, morel mushroom, yellow morel** *and* **sponge morel** belongs to the kingdom fungi, Phylum Ascomycota, Class Pezizomycetes, Order Pezizales, Family Morchellaceae, Genus Morchella and Species M. esculenta.
Gucchi grows in shady areas usually found in forests at an altitude range of 2500-3000 meters above the sea level at places commonly like Shimla, Kinnaur, Kullu, Sirmaur and Chamba.

It is usually seen in the months from March to mid-August every year and indigenously known to grow in these months when lightening occurs and the humidity level in the environment is high. People use gucchi in the villages as food and report that it is a high source of protein and has very useful effects on health.

Autochthonous people cultivate gucchi from the forests while taking their cattle for grazing and cook gucchi as normal mushroom for their meal. Some villagers also make pickle of gucchi with added spices and a wide band of flavours in it making it more delicious.

Some studies have stated the use of gucchi as a purgative, laxative, body tonic and reported to be very helpful in stomach related problems and wound healing (Vivek Kumar Raman., Manish Saini et al., 2018) while (Syed Lal Badshah et al.,2021) has reported the presence of Polysaccharopeptides and polysaccharides which are used as anti-cancer drugs.

It is also reported to have anti-inflammatory and anti-tumour properties found in a research by (B.Nitha et al., 2006) seen in the ethanolic extract. Moreover gucchi is also reported to have anti-microbial and anti-oxidant properties (Sandrine A.Heleno et al., 2013).

[1.2] Bunium persicum

Figure 1.2.1 Bunium persicum (Kala zeera/ Shahi zeera)

https://www.researchgate.net/publication/352789825/figure/fig1/AS:1048242049523712@1626931788252/Black-cumin-Bunium-persicum-seeds.png

Bunium persicum commonly known as **Kala zeera, Shahi zeera** belongs to Kingdom Plantae, Phylum Tracheophytes, Class Angiosperms, Order Apiales, Family Apiaceae, Genus Bunium, Species B. persicum is found in forests, grassy slopes and low alpine pastoral lands in the state

of Himachal Pradesh particularly in high mountain regions like Kinnaur, Kullu, Chamba, Shimla, Sirmaur, Lahaul Spiti, Pangi and Bharmaur at an altitude range of (1800-3500) metres.

It is indigenously used in curing urinary tract disorders, in treatment of diarrhoea, piles, insomnia, hiccups, high cholesterol, fever and even gynecological problems. Also it is used for culinary purposes and has hypoglycemic activity. It is known anciently to support and give a good heart health.

According to the ayurvedic usage of Kala zeera, it is actively used for the stomach related specifically digestion related problems, used as analgesic, anti- spasmodic, lactation stimulant and having anti- inflammatory activity.

The seeds of *Bunium persicum* are reported to have 40-50 percent of volatile oil, principle constituents of which are Cuminaldehyde (45.4%), p-cymene (35%), limonene, carvone especially γ-Terpinene as mentioned by (Hassan Hassanzadazar et al., 2018), (https://www.bimbima.com/herbs/black-caraway-kala-jeera-information-benefits-and-uses/44/).

These seeds are also reported to have anti-fungal properties (Takayuki Sekine et al., 2007), anti- oxidant activity reported by (Neda Shahsavari et al., 2008) and hyperglycemic activity reported by (Statti Giancarlo et al., 2006).

LITERATURE REVIEW

Morchella esculenta is an edible mushroom known for its distinct flavor and other health promoting abilities. Polysaccharides, polyphenols, tocopherols and protein hydrolysates have been reported to be the bioactive compounds responsible for the mushroom's strong bioactivities. Additionally, the mushroom is rich source of essential amino acids and contains glutamic acid in the highest concentration.

Commercial artificial planting has not been successful in the case of *Morchella esculenta* so far and submerged fermentation is being explored as alternative for the large scale production of mycelium.

Antioxidant activity of *Morchella esculenta* can be traced back to primary and secondary metabolites. The mushroom has also been reported to have hypolipidemic, hypoglycemic, and anti-tumor activities (Wu et al., 2021).

According to a study and research it is claimed out to be that *Morchella esculenta* will grow on agar media or in liquid culture or on a synthetic media supposed to be composed of suitable carbon and nitrogen sources and desired mineral salts (Thomas D Brock et al., 2018). They stated that in their research a survey pf many nitrogen and carbon sources was made and the fungi grew very well on starch, maltose, fructose, turanose, glucose and sucrose (Thomas D Brock et al., 2018).

(B. Nitha., C. R. Meera., K.K. Janardhanan., 2006) have carried out a research in which anti-tumor and anti- inflammatory activities of *Morchella esculentum* was investigated by making an ethanolic extract of the cultured mycelium of the sample which resulted in a considerable and significant dose-dependent inhibition of both acute and chronic inflammation.

The activity was compared against the standard drug Diclofenac. On the other hand the anti-tumor activity of the sample was determined using DLA cell-lined induced solid tumor and EAC cell line- induced ascites tumor model in mice which showed significant anti-tumor activity against both the solid and ascites tumor (B. Nitha., C. R. Meera., K.K. Janardhanan., 2006).

(Hua-Li Cui et al., 2011) have investigated for the presence of immunomodulatory activity in the polysaccharides isolated from *Morchella esculenta* in which they a water-soluble polysaccharide, MEP was obtained from fermentation broth of *M. esculenta.*

(Ch Ramesh et al., 2010) in a study talked about the medicinal properties of mushrooms which include several bioactive compounds like alkaloids, flavonoids, phenolic compounds and vitamins. Because to the increasing resistance of artificially synthesized drugs in physical structures of humans, the main focus of several researchers and molecular biologists is to identify the unique bioactive compounds from plants species which are either undiscovered or identified.(Ch Ramesh et al.2010).

In this study, six mushroom species were chosen for invitro screening namely *Lycoperdon perlatum, Cantharellus cibarius, Clavaria vermiculris, Ramaria Formosa, Marasmius oreades and Pleurotus pulmonavirus* (Ch Ramesh et al., 2010)

By making methanol extract with the help of the dried mushroom powder (100mg) with added solvent (methanol) at 25degree centigrade and constant stirring for 24 hours at 150rpm and filtration by whatman filter paper4, further analysis of various photochemical was taken place with the help of different methods which includes calorimetric assay (for phenolic compounds), flavonoids and ascorbic acid concentrations were determined by using a combination of solvents like methanol, ethanol, potassium acetate and aluminum nitrate.

Absorbance of the final solutions was taken in each case in spectrophotometer at different wavelengths specifically 765nm in *calorimeter assay* and 415nm in phenol concentration (Ch Ramesh et al., 2010).

Antimicrobial activity was also checked by using two gram positive bacteria that are *Staphylococcus aureus* and *Bacillus subtilis* while two gram negative species that are Escherichia coli and Pseudomonas aeruginosa (Ch. Ramesh et al., 2010).

Bunium persicum more commonly known as black cumin is part of Parsley family (Apiaceae), which comprises of 434 genera and is home to many other economically and medicinally important plants like *Corriandrum sativum*, *Petroselinum crispum, Foeniculum vulgare* and *Cuminum cymmium* to name a few. This herbaceous perennial plant has a limited distribution and is native to west Asia. It is found in the countries like Afghanistan, India, Iran, Pakistan, Tajikstan, and Turkmenistan.

The plant seeds have been reported to show anticonvulsant, antiasthma, antiemetic and hypoglycemic properties. However, economic plantation of *Bunium persicum* faces two major challenges namely long juvenile period and dormant seeds (Gul et al., 2012).

Seeds of Bunium persicum has been identified to contain 7-14% of essential oil and some researchers have successfully developed or applied different methods for extraction of these oils. Feyzi et al., (2017) successfully applied Instant Controlled Pressure Drop (DIC) method for essential oil extraction from Bunium persicum. However, they found this method to be best as a selective method despite its ectraction efficiencybeing higher than more commonly used such as hydrodistillation (HD) and ultra-sound-assisted extraction (UAE).

In a study by Mortazavin et al., (2010) the researchers presented extraction using Superheated Water (SWE) as an effective and fast method. However, this method again is best suited as a selective method and extracts only oxygenated components.

Various researchers have analysed the essential oils of Bunium persicum. Cuminaldeyde, ϒ-terpene, P-cymene, cuminyl alchohol and limonene were identified to be major constitutents by Foroumadi et al., (2011) by GC/MS. Cuminaldehyde, Gamma –terpene, Trans – 3- caren-2-ol, Acetic acid and 1.38-P methatriene were reported as main components by Amin et al., (2011). They used hydrodistillation technique for the analysis. Diphenylpicrylhydrazil (DPPH) assay, β-carotene bleaching and ammonium thiocyanate methods were used by Sharififar et al., (2010) for evaluating essential oil and antioxidant activity of *Bunium persicum*.

Ali et al., (2014) reported the potential therapeutic effect of *Bunium persicum* against ROS-induced hem toxicity. The researchers identified the antitoxic effect of *Bunium persicum* plant diferential extracts against leukemia blood induced hematotoxicity and antioxidative properties against free radicals.

(Fariba Sharififar et al., 2010) evaluated different extracts and essential oil of the *Bunium persicum* for anti-oxidant activity by using different three methods including DPPH assay, β

carotene bleaching and ammonium thiocyanate methods in which they got to know that *Bunium persicum* has high antioxidant activity with IC_{50} = 23.4 ± 1.6 and 45.7 ± 3.6 μgmL^{-1} in DPPH assay (Fariba Sharififar et al., 2010).

Rationale

The north Indian state of Himachal Pradesh is home to plethora of medicinal plants. However, there is a lack of studies on the nutraceutical and phytochemical aspects of these traditionally used plants by different native cultural and traditional communities of the region. They have been using these plants both as food and for medicinal purposes. Therefore, there is an urgent need to conduct nutraceutical analysis and identify other bioactive compounds in these plants. This would serve two major purposes

1). Recognize the commercial viability of these plants if grown on commercial (large) scale.

2). Create awareness among the masses about these underrated plants.

Objectives

1). To carry out phytochemical screening and nutraceutical analysis in *Morchella esculenta* and *Bunium persicum.*

Materials & Methods

Plant Selection

The selection of plant species chosen for this particular project were based on the following factors:

1). Thorough review of available literature on the medicinal and traditional plants of Himachal Pradesh.

2). Identification of research gaps found through literature review.

3). Availability of plant samples at the time of conduct of project lab work.

Plant Collection & Identification

The plant samples for *Morchella esculenta* were collected from wild Kinnaur region of Himachal Pradesh with the knowledge of local people, who helped us identify the plant and also guided us through correct measures for the concerned plant collection.

Since, the project required larger amount of the sample. The remaining sample was bought from a local shop in Shimla, Himachal Pradesh.

The plant samples for *Bunium persicum*, collected from Upper Himachal regions like **Rekong Peo** were provided to us by our project supervisor Dr. Hemant Sood.

Cleaning of Plant

The plant samples obtained were washed thoroughly under tap water for removal of dirt and other dust particles.

Drying

The plant samples for *Morchella esculenta* were first sun dried for 2 days.

The plant samples (seeds) for *Bunium persicum* were also sun dried for 2 days.

Choice of Solvent

Methanol was chosen as the solvent for the preparation of the extract. The choice of solvent was based on literature review. Also, methanol has been identified to have highest extraction yield (). Additionally, methanol has a boiling point of 64.7°C so we require lower temperature for solvent evaporation, while ensuring thermoliable components of plants are not damaged.

Extract Preparation

- ***Morchella esculenta***

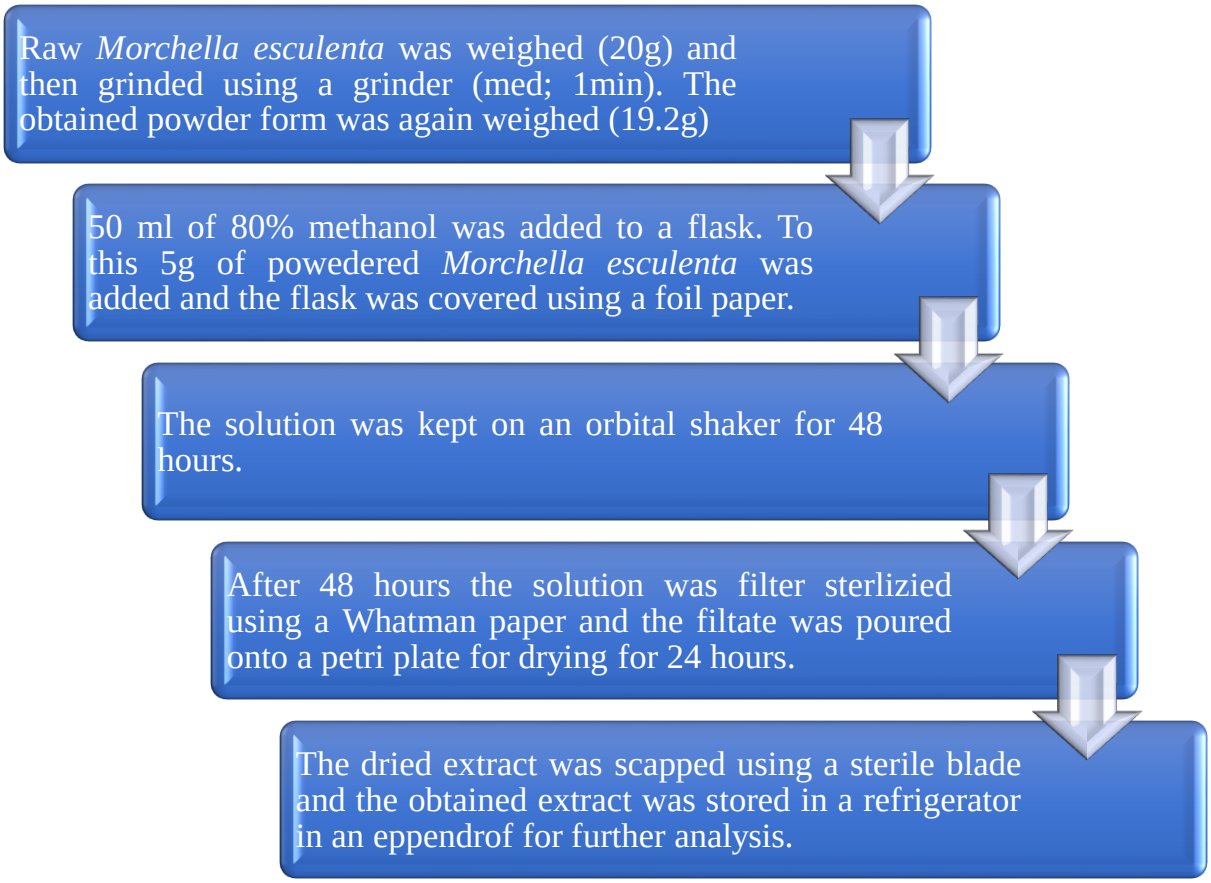


Figure 4.1 **Incubation on shaking incubator for extract preparation.**

- ***Bunium persicum***

Raw *Bunium Persicum* was weighed (20g) and then grinded using a grinder (med; 1min). The obtained powder form was again weighed (19.2g)

50 ml of 80% methanol was added to a flask. To this 5g of powedered *Bunium Perscium* was added and the flask was covered using a foil paper.

The solution was kept on an orbital shaker for 48 hours.

After 48 hours the solution was filter sterlizied using a Whatman paper and the filtate was poured onto a petri plate for drying for 24 hours.

The dried extract was scapped using a sterile blade and the obtained extract was stored in a refrigerator in an eppendrof for further analysis.

[4.1] QUALITATIVE ANALYSIS:

Following tests were performed for both the species; *Morchella esculenta* and *Bunium persicum* to check the presence of some common phytochemicals. The tests were performed in triplicates to ensure credibility.

1). Terpenoids:

For terpenoids, 1 ml of test sample is taken in a test tube containing 2ml chloroform and to this we add 3ml of sulfuric acid (conc.). Formation of reddish brown layer indicates the presence of terpenoids

- 1ml + 2ml chloroform + 3ml H_2SO_4 ⟶ Reddish Brown layer

2). Alkaloids:

For alkaloids, 3 ml of test sample is taken in a test tube and to that 3 drops of Mayer's reagent is added. Red colour appearance indicates the presence of alkaloids.

- 3ml + few drops of Mayer's reagent ⟶ Red colour

3). Phenols:

For phenols, 1ml of test sample is taken in a test tube and 2ml of distilled water is added. Now 10% ferrous chloride is added to it. Blue green colour appearance indicates the presence of phenols.

- 1ml + 2ml dH_2O + 10% $FeCl_2$ ⟶ Blue green colour

4). Tannins:

For phenols, 1ml test sample is taken in a test tube to which 3% ferrous chloride is added. Brownish green colour indicates the presence of tannins.

- 1ml + 3% $FeCl_2$ ⟶ Brownish green colour

5). Saponins:

For saponins, 1ml test sample was taken in a test tube and 5ml of distilled water was added to it which leads to the froth formation. Now 3-4 drops of oil are added. Formation of emulsion indicates the presence of saponins.

- 1ml + 5ml dH_2O⟶ froth formation + 3-4 drops of oil ⟶emulsion formation

6). Sterols:

For sterols, 2ml pf test sample is taken in a test tube to which 2ml of sulfuric acid (conc.) is added. Red colour appearance indicates the presence of sterols.

- 2ml + 2ml H_2SO_4 ⟶ Red colour

7). Flavonoids:

For flavonoids, 2ml of test sample is taken in a test tube to which only few drops of sodium hydroxide is added which leads to yellow colour formation. Now few drops of hydrochloric acid is added to it and if the solution becomes colourless then it is an indication for the presence of flavonoids in the test.

- 2ml PE + few drops of NaOH ⟶ yellow colour + few drops of HCl ⟶ soln. turns colourless.

8). Carbohydrates:

For carbohydrates, 3ml of test sample is taken in a test tube to which only few drops of Molish reagent is added. Now few drops of sulfuric acid (conc.) is added. Formation of violet ring indicates the presence of carbohydrates.

- 3ml PE + few drops of Molish reagent + few drops of H_2SO_4 ⟶Violet ring formation

9). Proteins:

For proteins, 1ml of test sample (aqueous sol.) is taken in a test tube to which equal volume of 1% strong base like sodium or potassium hydroxide is added. Now few drops of aqueous copper sulphate is added. Purple colour appearance indicates presence of proteins.

1ml PE+ 1ml NaoH/ KOH + few drops $CuSo_4$ ⟶ Purple colour

[4.2] QUANTITATIVE ANALYSIS:

To quantify the phenolic and flavonoid content along with the carbohydrates and protein amount in both the plant samples the following tests were performed respectively as shown in **tables 4.2.1, 4.2.2, 4.2.3** and **4.2.4.**

1) Total Phenolic Content using Folin Ciocalteau method

- This includes the reduction of Folin reagent in the presence of phonics in the sample to form a blue chromophore of phosphotungstic and phosphomolybdenm complex which has a maximum absorbance at 650nm.
  - Five concentrations of control were made as shown in table 4.2.1 and gallic acid was used as a standard with concentration 1mg/ml methanol and 20% aqueous sodium carbonate solution was made for desired volume.

Table 4.2.1 Protocol of Folin-Ciocalteau method.

Reagents→ Test↓	**Gallic Acid (1mg/ml methanol)**	**Distilled Water**	**FC reagent**	**Na_2CO_3**
Control 1	100 µl	900 µl	150 µl	500 µl
Control 2	200 µl	800 µl		
Control 3	300 µl	700 µl		
Control 4	400 µl	600 µl		
Control 5	500 µl	500 µl		
Blank	0 µl	1000 µl		
Sample (Unknown)	0 (100 µl)	400 µl		

Sample concentration = 1mg/ml

2) Total Flavonoid Content using Aluminum Chloride Colorimetric assay.

- Aluminum chloride makes acid stable complex with Keto and hydroxyl groups of the flavonoids which has a maximum absorption at 415 nm.
  - Five control concentrations were made and Quercetin was used as a standard with concentration 1mg/ml of methanol, 10% aluminum chloride was used and 1M sodium acetate was used for which 0.82g of it was dissolved in 100ml of distilled water.

Table 4.2.2 Protocol of Aluminum chloride colorimetric assay.

Reagents→ Test↓	**Quercetin (1mg/ml methanol)**	**Methanol**	**$AlCl_3$**	**$C_2H_3NaO_2$**
Control 1	100 µl	900 µl	100 µl	100 µl
Control 2	200 µl	800 µl		
Control 3	300 µl	700 µl		
Control 4	400 µl	600 µl		
Control 5	500 µl	500 µl		
Blank	0 µl	1000 µl		
Sample (Unknown)	0 (100 µl)	400 µl		

Sample concentration = 1mg/ml

3). Anthrone method for carbohydrates Quantification.

- Hydrolyses of free carbohydrates form that is polysaccharides, monosaccharides and even bounded glycolipid and glycoproteins by the concentrated acid present in anthrone reagent to form component monosaccharide followed by their dehydration by the conc. acid to form pentose and hexoses which are condensed with two naphthole molecules from anthrone to form a blue-green complex which has a maximum absorbance at 630nm.
    - Five control concentrations were made and glucose was taken as standard in concentration 1mg/ml. Anthrone reagent with concentration 1mg/ml and volume 4ml was added to each test tube of controls, blank and test. OD was taken at 630nm after incubation in water bath for 8 minutes.

Table 4.2.3 Protocol of anthrone method for carbohydrates quantification.

Glucose conc. (mg/ml)	**Volume (µl)**	**Distilled water (µl)**	**Anthrone reagent (1mg/ml) (ml)**	**Incubation in water bath for 8 minutes.**	**OD at 630nm.**
0.2	20	980	4		
0.4	40	960	4		
0.6	60	940	4		
0.8	80	920	4		
1	100	900	4		
Blank	0	1000	4		
Sample	0	0	4		

Sample concentration = 1mg/ml

4.) Lowry's method for protein quantification.

- Subsequent redox reaction including oxidation of aromatic amino acids by alkaline copper sulphate and reduction of sodium potassium molybdate tungstate in Folin's reagent to form a purple color complex which has maximum absorbance at 660nm and represents the direct relationship of the color intensity with the concentration of aromatic amino acids present in the sample.
    - Five control concentrations were made for which BSA was used as a standard with concentration 1mg/ml. Distilled water was added and reagent A was used which included 2% sodium carbonate in dissolved 1N NaOH, 1% sodium potassium tartrate dissolved in water and 0.5% copper sulphate dissolved in distilled water all mixed together and FC reagent was used as a reagent B in 1:1 with distilled water.

Table 4.2.4 Protocol of Lowry's method for protein quantification.

BSA (µl)	**Distilled water (µl)**	**Reagent A (ml)**	**Incubation For 15 minutes.**	**FC reagent (ml)**	**Incubation for 40 minutes in dark.**
200	800	4.5		0.5	
400	600	4.5		0.5	
600	400	4.5		0.5	
800	200	4.5		0.5	
1000	0	4.5		0.5	
Blank	1000	4.5		0.5	
Sample	0	4.5		0.5	

Sample concentration = 1mg/ml

RESULTS

[5.1] Qualitative Analysis:

- Qualitative analysis is always done to check presence of certain specific bioactive compounds in the sample for further desired quantification. In this research qualitative tests for *Bunium persicum* and *Morchella esculenta* were done in which phenols, flavonoids, carbohydrates and proteins were found positive for both species as mentioned in table 5.1.1

Table 5.1.1 Results of qualitative analysis for *Morchella esculenta* **and** *Bunium persicum.*

Plant Species→ Phytochemical↓	**Morchella esculenta**	**Bunium persicum**
Alkaloids	-ve	+ve
Flavonoids	+ve	+ve
Phenol	+ve	+ve
Saponins	-ve	+ve
Sterol	+ve	+ve
Tannins	-ve	+ve
Terpenoids	-ve	-ve
Proteins	+ve	+ve
Carbohydrates	+ve	+ve

[5.2] Quantitative Analysis:

- The methanolic extract of both the species (*Bunium persicum* and *Morchella esculenta)* were evaluated for total phenolic and flavonoid content by using Folin- Ciocalteau method for phenolic compounds, aluminum chloride colorimetric assay for flavonoids along with carbohydrates and protein quantification was done using anthrone method for carbohydrates and lowry's method for protein quantification. The results of TPC for *Bunium persicum* and *Morchella esculenta* are shown in (Table 5.2.1, Fig 5.2.1 and Table 5.2.2, Fig 5.2.2), TFC results are shown in (Table 5.2.3, Fig 5.2.3 and Table 5.2.4, Fig 5.2.4). Results for carbohydrates in *Bunium persicum* and *Morchella esculenta* are shown in (Table 5.2.5, Fig 5.2.5) and for proteins the results of both species are shown in (Table 5.2.6, Fig 5.2.6) below.

- ***Bunium persicum***

Folin-Ciocalteau assay for determining TPC.

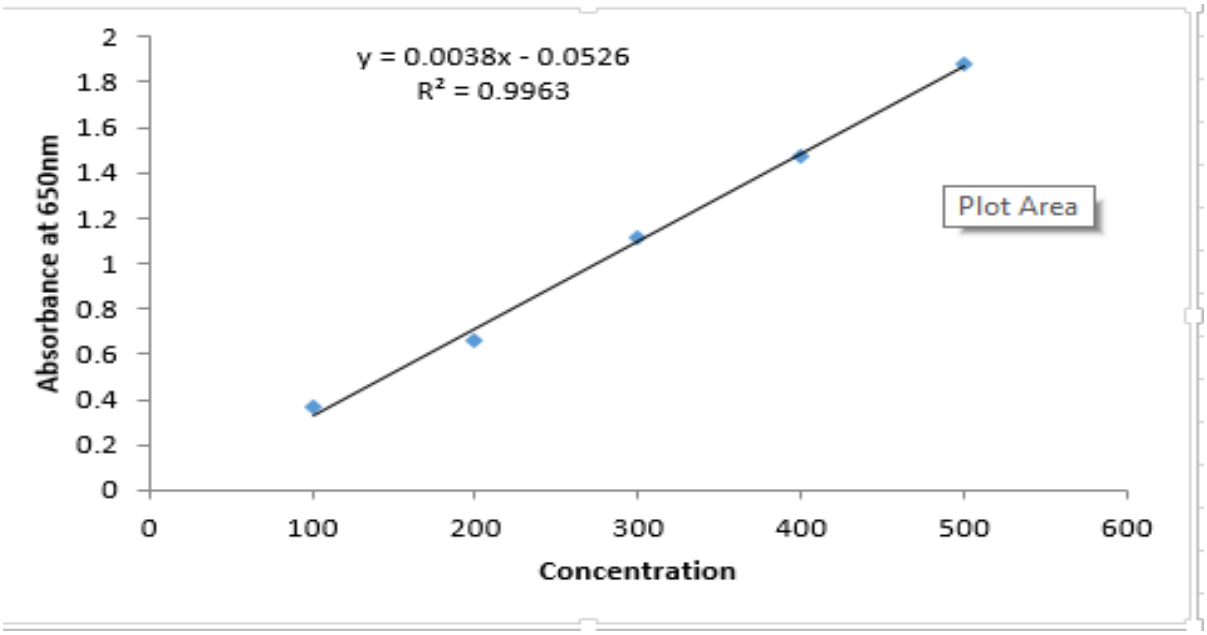
- To quantify the amount of phenol present in the sample of *Bunium persicum* the Total Phenolic Content test was performed using Folin Ciocalteau method.

Table 5.2.1 Observations of absorbance at 650 nm.

Concentration (µl)	Absorbance			Mean	SD
100	0.365	0.369	0.371	0.368333	0.00305505
200	0.655	0.655	0.652	0.657333	0.006806859
300	1.12	1.119	1.115	1.118	0.002645751
400	1.495	1.453	1.462	1.47	0.022113344
500	1.898	1.878	1.868	1.881333	0.015275252
Sample				0.3	

- Graph showing Standard plot of concentration against Absorbance along with the straight line equation and R^2 value =0.9963 stating that the readings are fine.

Figure 5.2.1 Standard plot for TPC of *Bunium persicum.*



X= (0.3+0.0526)/0.0038

X= 217.368 microgram gallic acid equivalent/ mg of plant extract.

Note: 'X' is the amount of test compound present in the sample.

Aluminum chloride colorimetric assay for TFC.

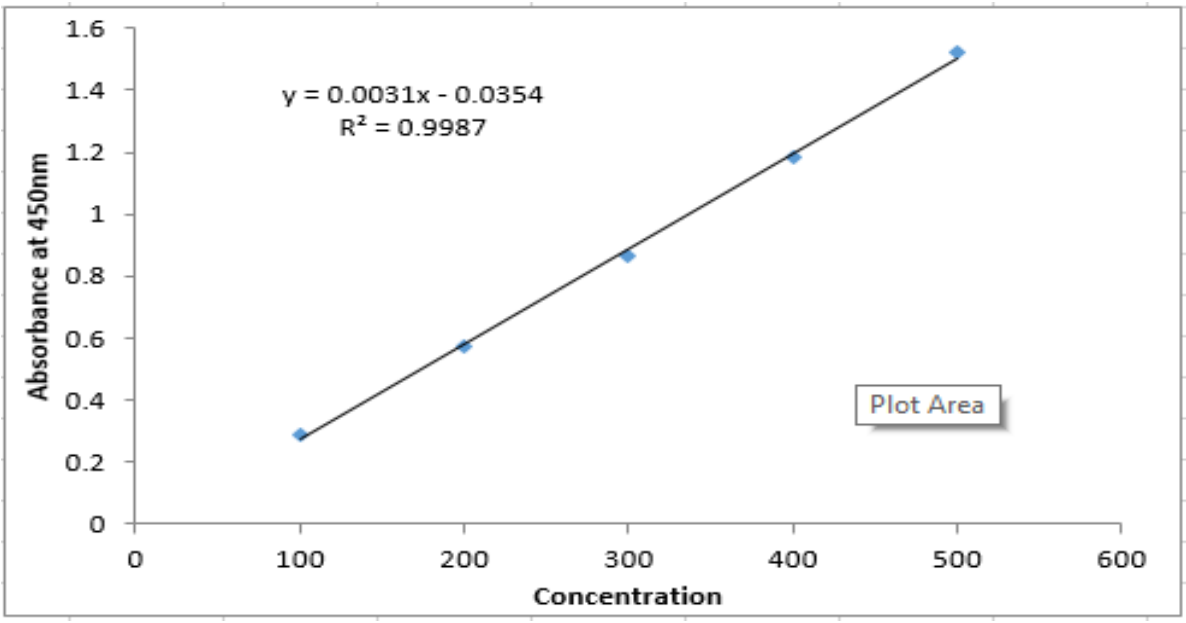
- To quantify the amount of Flavonoid in the sample of *Bunium persicum* the Total Phenolic Content test was performed using Aluminum Chloride Colorimetric assay.

Table 5.2.2 Observations of absorbance at 450 nm.

Concentration (µl)	Absorbance			Mean	SD
100	0.284	0.29	0.298	0.290666667	0.007023769
200	0.564	0.58	0.575	0.573	0.008185353
300	0.867	0.875	0.861	0.867666667	0.007023769
400	1.12	1.25	1.19	1.186666667	0.065064071
500	1.45	1.53	1.59	1.523333333	0.070237692
Sample				0.079	

- Graph showing Standard plot of concentration against Absorbance along with the straight line equation and R^2 value =0.9987 stating that the readings are fine.

Figure 5.2.2 Standard plot for TFC of *Bunium persicum*.



X= (0.079+0.0354)/0.0031

X= 36.903microgram quercetin equivalent/mg of plant extract.

- ***Morchella esculenta***

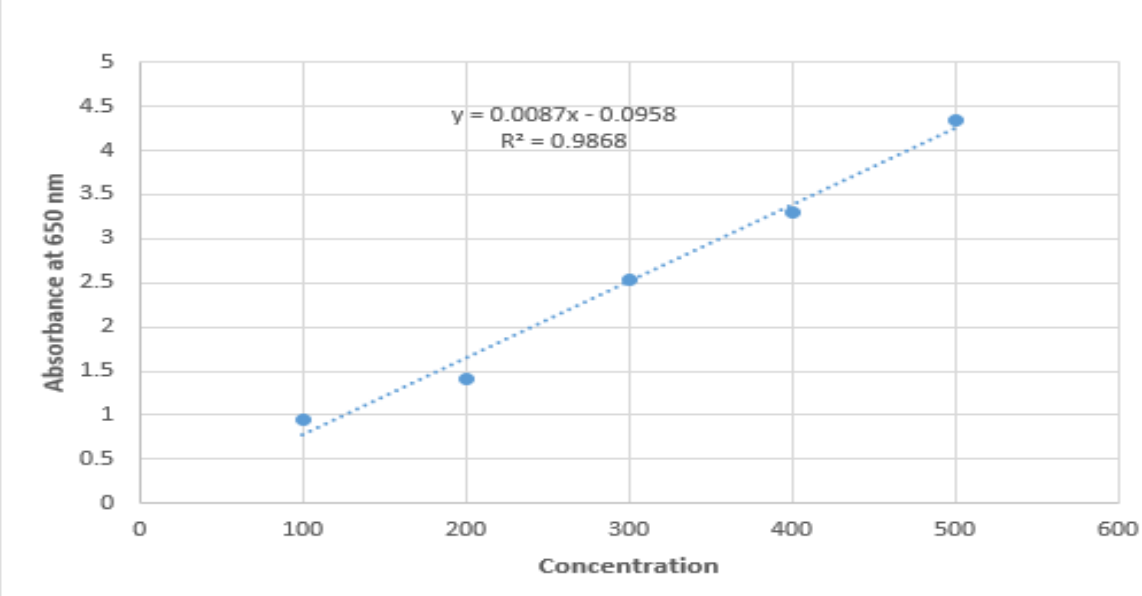
Folin-Ciocalteau assay for determining TPC.

Table 5.2.3 Observations of absorbance at 650 nm.

Concentration (µl)	Absorbance			Mean	SD
100	0.921	0.937	0.978	0.945333333	0.029399546
200	1.432	1.454	1.349	1.411666667	0.055374483
300	2.394	2.786	2.454	2.544666667	0.211142922
400	3.053	3.332	3.487	3.290666667	0.219932565
500	4.428	4.251	4.354	4.344333333	0.088895069
Sample				1.99	

- Graph showing Standard plot of concentration against Absorbance along with the straight line equation and R^2 value =0.9868 stating that the readings are fine.

Figure 5.2.3 **Standard plot for TPC of *Morchella esculenta.***



X = (1.99+0.0958)/0.0087

X = 239.747 microgram gallic acid equivalent/ mg of plant extract.

Note: 'X' is the amount of test compound present in the sample.

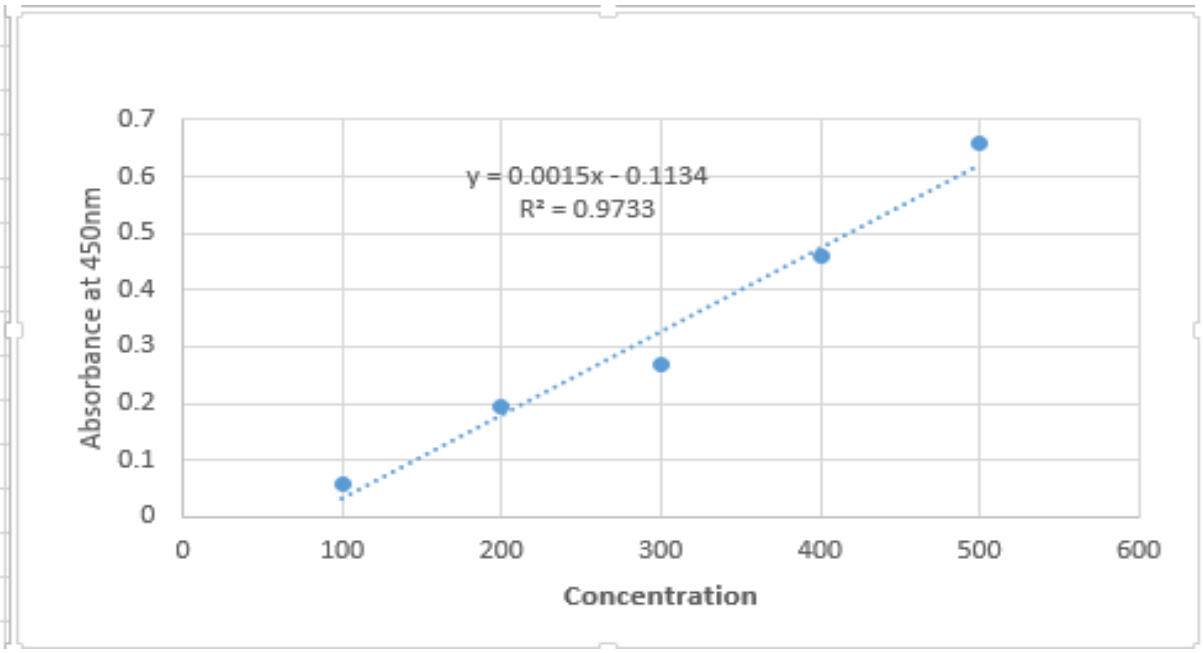
Aluminum chloride colorimetric assay for TFC.

Table 5.2.4 **Observations of absorbance at 450 nm.**

Concentration (µl)	Absorbance			Mean	SD
100	0.055	0.057	0.059	0.057	0.002
200	0.189	0.195	0.197	0.193666667	0.004163332
300	0.212	0.271	0.318	0.267	0.053113087
400	0.402	0.489	0.491	0.460666667	0.050816664
500	0.634	0.657	0.683	0.658	0.024515301
Sample				0.19	

- Graph showing Standard plot of concentration against Absorbance along with the straight line equation and R^2 value =0.9733 stating that the readings are fine.

Figure 5.2.4 Standard plot for TFC of *Morchella esculenta.*



X= (0.19+0.1134)/0.0015

X= 202.2667 microgram quercetin equivalent/mg of plant extract.

Note: 'X' is the amount of test compound present in the sample.

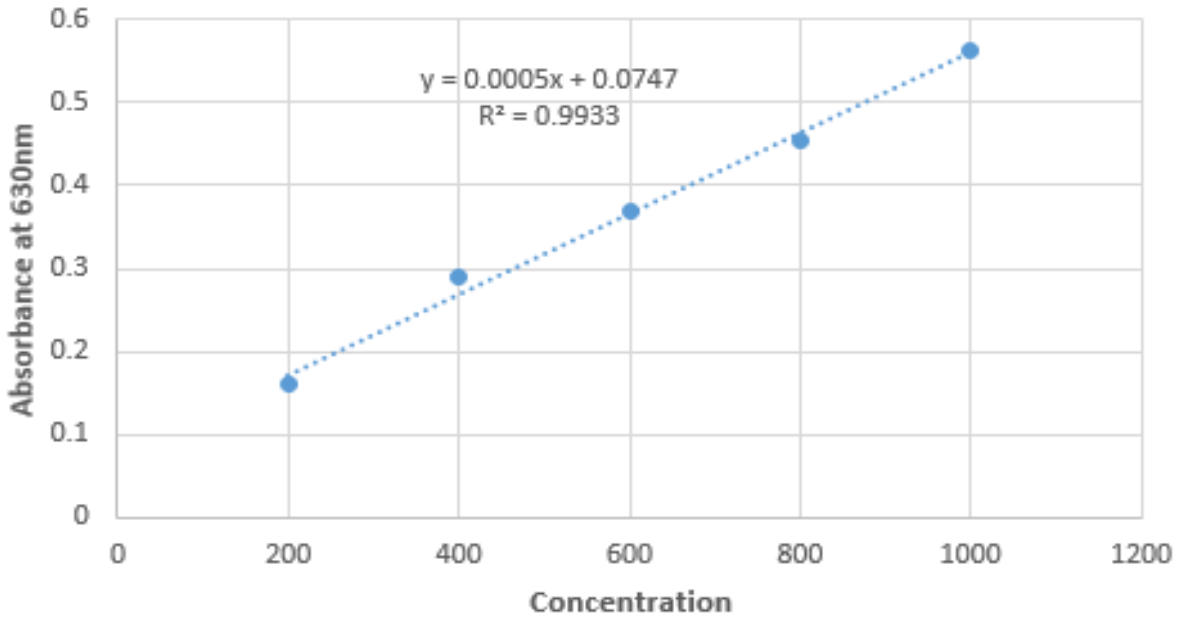
Anthrone method for Carbohydrate quantification.

Table 5.2.5 Observation of absorbance at 630 nm.

Concentration (µl)	Absorbance			Mean	SD
200	0.169	0.189	0.121	0.159666667	0.03494758
400	0.288	0.291	0.287	0.288666667	0.002081666
600	0.367	0.375	0.363	0.368333333	0.006110101

800	0.441	0.467	0.454	0.454	0.013
1000	0.597	0.543	0.552	0.564	0.028930952
Sample (BP)				0.41966	
Sample (ME)				0.692	

Figure 5.2.5 Standard plot for Carbohydrate Quantification.



For *Bunium persicum*

Average sample concentration (ASC), **Y** = **0.41966**

Therefore, **X= (0.41966-0.0747)/0.0005**

X= 689.92/100 = 6.89%

For *Morchella esculenta*

Average sample concentration (ASC), **Y** = **0.692**

Therefore, **X= (0.692-0.0747)/0.0005**

X= 1234.6/100 = 1.234%

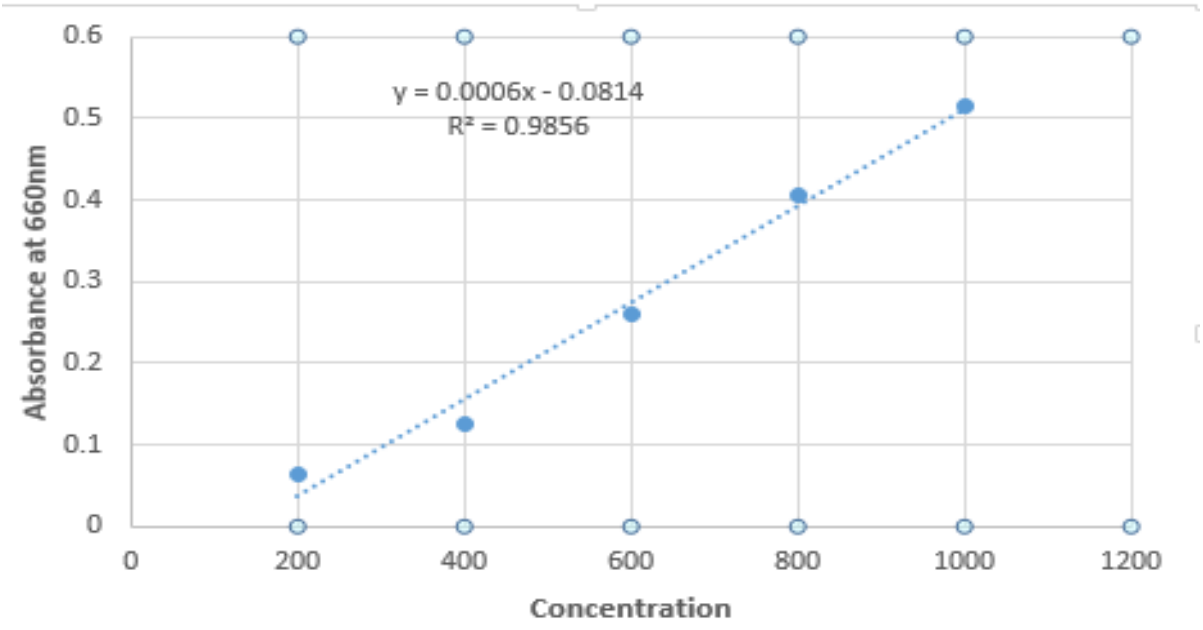
Note: 'X' is the amount of test compound present in the sample and ASC is the average absorbance value of the sample.

➢ Lowry's method for Protein quantification.

Table 5.2.6 Observations of absorbance at 660 nm.

Concentration (µl)	Absorbance			Mean	SD
200	0.072	0.063	0.055	0.063333333	0.008504901
400	0.121	0.128	0.126	0.125	0.003605551
600	0.267	0.242	0.271	0.26	0.015716234
800	0.410	0.390	0.421	0.407	0.015716234
1000	0.502	0.489	0.553	0.514666667	0.033827996
Sample (BP)				0.164	
Sample (ME)				0.271	

Figure 5.2.6 Standard plot for Protein quantification by Lowry's method.



For *Bunium persicum*

Average sample concentration (ASC), **Y** = **0.164**

Therefore, **X= (0.164+0.0814)/0.0006**

X= 409/100= 4.09%

For *Morchella esculenta*

Average Sample concentration (ASC), **Y** = **0.271**

Therefore, **X= (0.271+0.0814)/0.0006**

X= 587.333/100=5.87%

Note: 'X' is the amount of test compound present in the sample and ASC is the average absorbance value of the sample.

DISCUSSION

The qualitative testing on the species *Morchella esculenta* and *Bunium persicum* indicated the presence of "Flavonoids, Phenols and Sterols" in *Morchella esculenta* whereas "Alkaloids, Flavonoids, Phenol, Saponins, Sterol, Tannins" in *Bunium persicum* out of which quantification of only Phenols and Flavonoids was done. The results indicated that **239.747 microgram gallic acid equivalent/ mg of plant extract** and **202.2667 microgram quercetin equivalent/mg of plant extract** was present in *Morchella esculenta* and **217.368 microgram gallic acid equivalent/ mg of plant extract** and **36.903microgram quercetin equivalent/mg of plant extract** was found in *Bunium persicum*.

CONCLUSION

This research reports the significant presence of two major phytochemicals that are phenolic compounds and flavonoids in *Morchella esculentum* and *Bunium persicum* and also the significant amount of carbohydrates and proteins in reference to their nutraceutical aspects. Therefore these two plant species should be shown to the world commercially by developing and introducing good cultivation methods for these species so that these could be prevented and grown for their medicinal as well as consumption utilization.

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[1] Divyam, H. Sood. "The traditional underutilized plants of Himachal Pradesh" in *International conference on Sustainable Asia's Problems and Prospects by Shri Shankararcharya Institute of Professional Management and Technology*, Raipur, Chhatisgarh, March 15, 2021. (Oral ppt).

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