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PHYTOCHEMICAL SCREENING AND BIOLOICAL STUDIES OF *Tetrataenium nepalense* SEED EXTRACTS

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Abstract: *Tetrataenium nepalense* is a wild herb used traditionally to treat fever, common cold, cough etc. This plant takes three years to mature. The study investigates for antimicrobial, antifungal and antioxidant properties of *Tetrataenium nepalense* extract. This plant was extracted by maceration using ethanol. Then the percentage yield of extract was calculated. This plant was choose since it has historically been used to cure variety of ailments. The microscopic characteristics, extractive values and ash value of plant powder was done while UV spectrophotometry was used for the quantitative analysis, color change was the basis for the qualitative phytochemical screening. The extract contained phytochemicals such as alkaloids, steroids, quininone, coumarin, flavonoids, terpenoids, and carbohydrates, according to a preliminary phytochemical screening. The phenolic content was found to be 36.75 mg/g GAE and the flavonoid content was found to be 40.085 mg QE/g. The DPPH scavenging assay was carried out for antioxidant property and IC_{50} value was found to be 36.441379. During antibacterial screening, the extract was found to be effective against *Bacillus subtilis* and *Staphylococcus aureus*. This extract exhibited no antifungal action. For *S. aureus*, the minimum bactericidal concentration (MBC) was found to be between 25 and 50 mg/ml, while for *Bacillius subtilis*, it was found to be between 0.78125 and 1.5625 mg/ml. With its high quantity of total phenolic and total flavonoid content, the plant exhibits significant antioxidant activity. It also works well against gram-positive bacteria, which aids in the creation of narrow spectrum antibiotics. Therefore, this could be a potential lead molecule for antibiotics. Further research in *Tetrataenium nepalense* may lead to the discovery of the new molecule.

Index Terms: Tetrataenium nepalense, antibiotics, antioxidant, MBC

INTRODUCTION

The production of synthetic, pharmacopoeial, and non-pharmacopoeial medications can all benefit from the abundance of components found in medicinal plants. *Tetrataenium nepalense* is one of the genera of the *Apiaceae* family that has above 120 species and 10 of them are documented in Nepal, among them one of which is *Tetrataenium nepalense*, commonly called as **Chimphing** and is very common in Nepal. Some other common names are hogweed, cow-parsnip, bhotey jeera, phaakee, bhutakesh, budo aushaadhi, trunag, tunak etc. However it is also found in Sikkim and Darjeeling of India. The majority of studies on *Tetrataenium* species have shown that they are edible, particularly as medicinal herbs and to a lesser extent as food.

Many ethnic and tribal groups who live in the high hills and mountains of eastern and central Nepal have been using the seeds as a species for eons. In addition to their seeds, other plant parts have also been found to contain various phytochemicals, so are used in traditional medicine to treat conditions like typhoid, influenza, flatulence, inflammation, nausea and vomiting, diarrhea, and convulsions. ***Ambu, G et al in 2020***. Additionally, it is used to treat a number of serious illnesses, including Parkinson's, Alzheimer's, liver, and heart conditions.

Tetrataenium has a variety of coumarin and furanocoumarin types, which are thought to be the main metabolites and are involved in different biological activities, including anti-inflammatory, anti-microbial, anti-neoplastic, anti-oxidant and anti-fungal properties. This plant's tannins are said to have anti-diarrheal properties. *Tetrataenium* species have been suggested as a possible source of therapeutic chemicals that do not necessarily have negative side effects.

This study aims for Phytochemical and Biological activities of *Tetrataenium nepalense*. Also carried out to study the macroscopic and microscopic features of Tetrataenium nepalense, perform phytochemical screening of extract, to calculate the extract's *Total Phenolic Content* and *Total Flavonoid Content*, assess the extract's antibacterial activity, and assess the extract's antioxidant effect.

Taxonomical Classification

.Kingdom : Plantae
Division : Angiosperm
Order : Apiales
Family : Apiaceae
Genus : *Tetrataenium*
Species : *nepalense*

NEED OF THE STUDY.

The plant, *Tetrataenium nepalense* is a traditional plant possessing medicinal value and is of therapeutic importance. It has been discovered that this plant possesses stronger antioxidant, antifungal, and antibacterial properties. Therefore a systematic research and development work should be undertaken for the conservation of *Tetrataenium nepalense* and development of products for their better economics and therapeutic utilization.

MATERIALS

Collection: In October 2022, the plant materials were gathered from Illam, Nepal. Tetrataenium nepalense, the plant that was obtained, was identified by the National Herbarium and Plant Laboratory, Godavari- Lalitpur.

Plant sample were collected and shade dried and grinded into coarse powder using electric grinder. 40 g of powdered sample was soaked in 100 ml 80% ethanol each in two different conical flask and allowed to macerate in a mechanical shaker for 24 hours at room temperature. All laboratory activities, with the exception of biological activity, were carried out at the NMCAL laboratory in Lainchour; biological activity was carried out at the Banaspati Vivag laboratory in Thapathali.

RESEARCH METHODOLOGY

After collection and drying, extraction process was done. Then the list of tests carried in laboratory is given below:

3.1 Physiochemical analysis

The physiochemical analysis is performed to obtain following:-

1. Total Ash value.
2. Acid insoluble ash.
3. Water soluble ash.
4. Water soluble extractive value.
5. Water soluble extractive value.
6. Alcohol soluble extractive value.
7. Alcohol soluble extractive value.
8. Loss on Drying.

3.2 Phytochemical screening:

3.2.1 Qualitative phytochemical screening: *Tetrataenium nepalense* extracts' primary chemical components were determined by analyzing color responses with various reagent. The extract was subjected for following test

- **a.** Detection of Tannin: *Ferric chloride test;*
- **b.** Detection of Fats and Fixed oil : *Saponification Test;*
- **c.** Detection of Carbohydrate: *Fehling's test; Molisch's test*
- **d.** Detection of Terpenoids : *Salkowski test*
- **e.** Detection of Alkaloids : *Mayer's test;.Wagner's test;. Dragendroff's test:*
- **f.** Detection of glycosides: Cardiac glycosides *(Keller-killani test),* Anthraquinone glycosides *test,*
- **g.** Detection of flavonoids: *Shinoda test; Alkaline reagent test*
- **h.** Detection of saponin: *Foam test;*
- **i.** Detection of amino acids: *Millon's test;*
- **j.** Detection of protein: *Xanthoproteic test;*
- **k.** Detection of quinines
- **l.** Detection of steroids
- **m.** Detection of coumarin

3.2.2. Quantitative phytochemical screening

Total Phenolic Content (TPC)

"TPC is an assay commonly applied to evaluate the quantity of phenolic compounds which are present in plant extracts. Gallic acid is mostly used as a standard for measurement of total phenolic compounds of various plants since this compound stands for the simplest form of phenolic acid" ***Molole et al in 2022***. The results of assay were presented using ***Gallic acid equivalent***. Color changes were then shown depending on the various forms of phenolic compounds present in the plant material. The changes in the color of the solution containing extracts in the presence of ***Folin-ciocalteu*** were measured using a spectrophotometer.

Reaction mechanism of Folin-ciocalteu reagent: **"**The Folin-ciocalteu reagent which is a mixture of tungstates and molybdates works on the mechanism of oxidation-reduction reaction. The method strongly relies on the reduction of mixture hetero polyphosphotungsates-molybdates by the phenolic compound which results in the formation of blue coloured chromogen." ***Maria Pérez et. Al in 2015***

"The phenolic compound reacts with Folin-Ciocalteu reagent only under basic conditions adjusted by sodium carbonate solution. Under basic conditions it has been observed that the phenolic compound undergoes dissociation to form a phenolate

anion which reduces the Folin-ciocalteu reagent i.e. the mixture of tungstates and molybdates rendering a blue coloured solution. The color intensity of the formed blue chrom2ogen can be measured by the absorbance readings using a spectrophotometer" ***Maria Pérez et. Al in 2015***

Sample preparation

Plant extracts of 100µg/mL is prepared by dissolving 0.01g in 100 ml, 50% DMSO solution.

Procedure

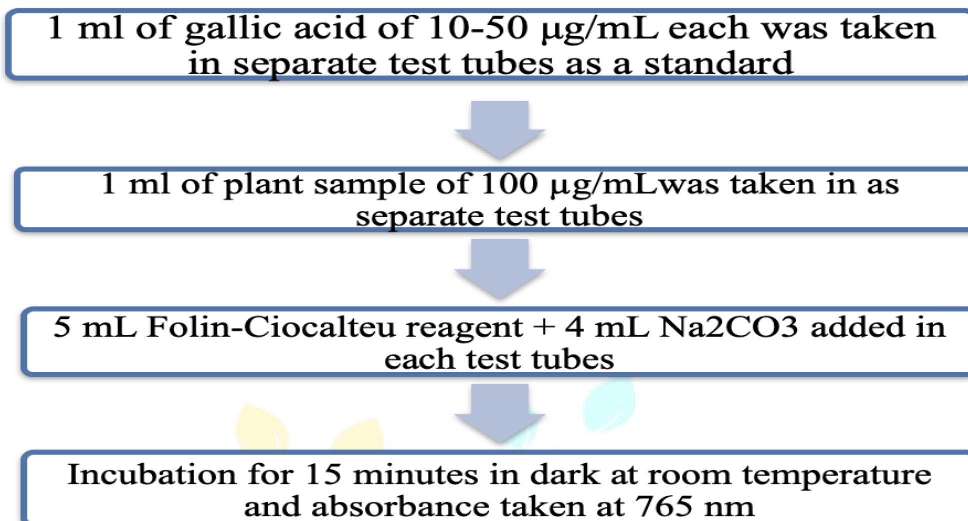


Figure 1: Procedure for study of Total Phenolic Content

"The total phenolic content was determined using Folin-ciocalteu reagent spectrophotometrically" ***Maria Pérez et. Al, 2023***. The absorbance was taken at 765 nm using a UV spectrometer. Gallic acid was used for constructing the standard curve (10-50 µg/mL) and the total phenolic compound concentration in the extract was expressed as milligram of gallic acid equivalent per gram of dry weight (mg GAE/g) of the extract using gallic acid standard curve.

Total Flavonoid Content (TFC)

Aluminum chloride colorimetric techniques were used. Total flavonoid concentration of extract was ascertained. The extract was combined with aluminum chloride and methanol, and then potassium acetate and distilled water were added. After the combination was let to stand for thirty minutes, a UV-visible spectrophotometer was used to measure the absorbance at 415 nm. The total flavonoid component content in the extract will be represented as milligrams of quercetin equivalent per gram of dry weight (mg QE/g) of extract. Quercetin was utilized to generate the standard curves.

Preparation of sample: Plant extracts of 100µg/mL concentration were prepared by dissolving 0.01g in 100 ml of 50% DMSO.

Procedure

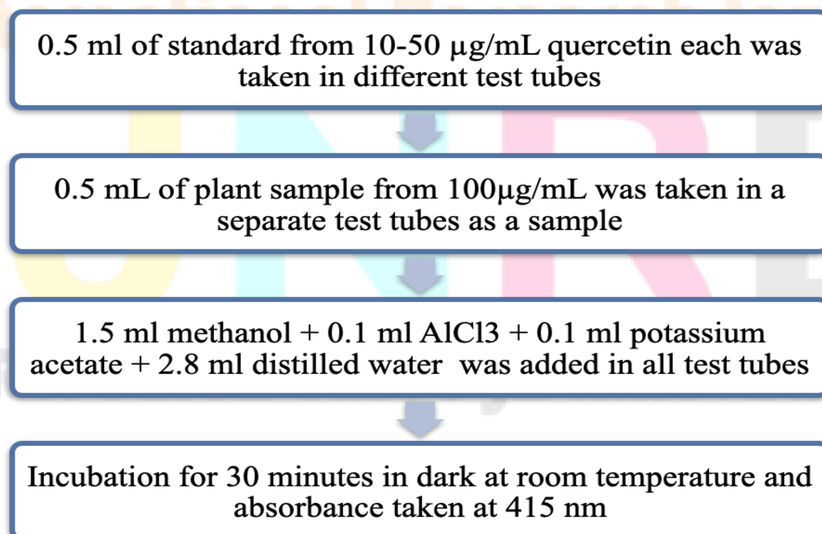
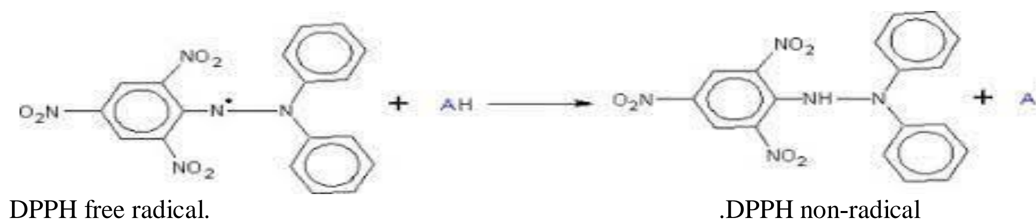


Figure 2: Procedure for study of Total Flavoniod Content

PPH* (2, 2-Diphenyl -1- picrylhydrazyl *) free radical scavenging assay:

A test for scavenging free radicals, called DPPH, is used to measure the antioxidant activity. The reduction of DPPH in methanol solution in the presence of antioxidants that donate hydrogen is the basis of this technique, as demonstrated by the reaction that follows.



DPPH free radical. .DPPH non-radical

Figure 3: Reaction of DPPH assay

"0.1mM solution of DPPH in methanol was added to the solutions of each extract in methanol at different concentrations. After vigorous shaking of these mixtures, they were allowed to stand in the dark at room temperatures for 30 minutes. Then the absorbance was measured at 517nm using a UV- visible spectrophotometer. Lower absorbance values indicate higher free radical scavenging activity. The capability of scavenging DPPH free radical was calculated*." Medicinal Emmanuel Mfotie Njoya,, Chapter 31 plants, antioxidant potential, and cancer, Academic Press, 2021, Pages 349-357*

3.3 Antimicrobial Susceptibility Testing of plant extracts

"*Antimicrobial screening was performed by the Agar Well Diffusion method. The method is to evaluate the antimicrobial activity of plant extracts similar to disc diffusion method*." ***Balouiri M. et. Al 2016***.

Methods

In-vitro antimicrobial assay was carried out by Kirby Baur well diffusion method in a Muller *Hinton Agar plate* (MHA) for ethanol extract. The concentration of plant extracts used was 100 mg/ml and 200 mg/ml for standard (Ciprofloxacin and Amoxicillin) was 1 mg/ml. Test organisms taken Clinical isolates of active culture of standard strain of bacteria were obtained from Department of Microbiology, National College Kathmandu, Nepal. *S aureus, Bacillus subtilis, Staphylococcus epidermidis, Enterococcus faecalis* and *Shigella dysenteriae* was taken as Gram positive and, *E. coli*, *Klebsiella pneumoniae, Proteus vulgaries, Pseudomonas aeruginosa* and *S. typhii* were taken as Gram negative bacteria. Ciprofloxacillin and Amoxicillin taken as positive control: 50% Dimethyl Sulfoxide (DMSO) as negative control.

Preparation of working solution and control.

Firstly, 0.5 g and 1 g of extracts were weighed in a tube and dissolved in 50% DMSO and were subjected for sonication and vertexing. After complete dissolution the extracts were assayed for antimicrobial activity. 50% DMSO was prepared in distilled water for negative control.

Screening and evaluation of antibacterial activity

"The antibacterial activity of crude extracts of *Tetrataenium nepalense* was screened against test organisms by Agar Well Diffusion method. 3-4 hours old broth culture of respective bacteria standard to 0.5 Mc Farland were lawn cultured on MHA surfaces of plates in four quadrants by swabbing using a sterile cotton swab. Then the inoculated plates were allowed to diffuse for 5-10 minutes by closing the lid. Wells of 6 mm diameter were bored in the inoculated media using a flame sterilized borer and each well was labeled from the backside of the plate. Each well was filled with 50 µL of extract of composition 100 mglml and 200 mg/ml, positive control and negative control respectively with the help of micropipettes.

"*For positive controls, disc of Amoxicillin (10mcg/disc) and Ciprofloxacin (5mcg/disc) were used. The filled extracts and plates were left for 30 mins for diffusion at room temperature and were incubated at 37°C for 18-24 hours. After incubation the clear zone of inhibition was measured and evaluated.* ". ***Bishnu P. Marasini et. Al in 2015***

Determination of MBC:

MBC was determined by subculturing *Bacillus subtilis* and *Staphylococcus aureus* and incubating it overnight. Then the subculture was diluted in Mueller Hinton Agar broth to different concentrations. (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, 0.390625, 0.1953125 and 0 mg/ml by serial dilution. The highest dilution that yields no bacterial growth on solid medium was taken as MBC.

3.4 Antifungal screening

Antifungal activities were also assayed by agar well diffusion method in MHA.GMB. Test organisms taken were clinical isolates of active culture of standard strain of fungus, *Candida albicans*. Clotrimazole was taken as positive control: Dimethyl Sulfoxide (DMSO) as negative control the study as per WHO guideline 1991.

Preparation of working solution and control.

0.5g and 1g of extracts were weighed in a labeled tube and dissolved in 50% DMSO and were subjected for sonication and vertexing. The extracts were assayed for antifungal activity after complete dissolution. 50% DMSO was prepared in distilled water for negative control.

Screening and evaluation of antifungal activity:

Agar Well Diffusion Method is used for antifungal activity screening against test organism of crude extracts of *Tetrataenium nepalense*. "*3-4 hours old broth culture of fungus standard to 0.5 Mc Farland was lawn cultured on MHA.GMB surfaces of plates in four quadrants by swabbing using a sterile cotton swab. Then the inoculated plates were allowed to diffuse for 5-10 minutes by closing the lid. Wells of 6 mm diameter were bored in the inoculated media using a flame sterilized borer and each well was labeled from the backside of the plate. Each well was filled with 50 µL of different extracts (100mg/ml and 200mg/ml), positive control (Clotrimazole 5mcg/ disc) and negative control respectively with the help of micropipettes. The filled extracts and plates were left for 30 mins for diffusion at room temperature and were incubated at 28°C for 24 hours. After incubation the clear zone of inhibition was measured and evaluated* as per WHO guidelines". ***WHO, Guidelines for the Assessment of Herbal Medicines 1991***.

IV. RESULT AND DISCUSSION

This study was focused on screening of phytochemicals and finding out biological activities such as antioxidant and antimicrobial and antifungal activity of *Tetrataenium nepalense*. This chapter includes the results of organoleptic characters, fluorescence test, and extractive value, phyto-chemical screening and biological activities of the seed of the plants.

4.1 Organoleptic characteristics of powdered *Tetrataenium nepalense* seed

Table 1: Organoleptic charactersitcs of Tetrataenium nepalense

S. No.	Characteristics	Inference
1	Color	Yellowish - brown
2	Odor	Characteristics aromatic
3	Taste	Characteristics astringent
4	Texture	Coarse
5	Sand and other foreign matters	Absent

4.2 Fluorescence test

The powdered sample of the *Tetrataenium nepalense* was dissolved in different reagent and viewed from naked eyes and in UV-length of wavelength 365nm and 254nm

Table 2: Fluorescence test

S.N	Reagent	Visible	Short UV (365nm)	Long UV (254nm)
1	Powder	Yellowish-brown.	Dark-green.	Light-green.
2	Powder + H_2O	Light-brown.	Dark-green.	Light-green.
3	Powder + NaOH	Light-brown.	Dark-green.	Light-green.
4	Powder + Methanol	Light- brown.	Dark-green.	Light-green.
5	Powder + Ethanol	Light-brown.	Dark-green.	Light-green.
6	Powder + 1N NaOH in H_2O	Light-brown.	Dark-green.	Light-green.
7	Powder + Ammonia	Light-brown.	Black.	Light-green.
8	Powder + H_2SO_4	Light-brown.	Black.	Light-green.
9	Powder + HNO_3	Light-brown.	Dark-green.	Light-green.

4.3 Physiochemical analysis

Table 3: Physiochemical analysis of *Tetrataenium nepalense* seed extract

S.N.	Physiological parameter	Value
	Ash value	
a.	Total Ash.	6 %
b.	Acid insoluble ash.	0 %
c.	Water soluble ash.	0 %
	Extractive value	
a.	Alcohol extractive value.	7.2 %
b.	Water soluble extractive value.	12 %
	Loss on Drying.	12.75 %

There was no study on the physiochemical parameters of *Tetrataenium nepalense* extract of the seed. But in a study conducted by **Mukum 3 et al., 2019**, revealed that the loss on drying at 105℃ was found to be 9.87% w/w, total ash value 0.07% w/w, which differs from my study because of geographical variation.

4.4 Extraction:

Tetrataenium nepalense seed extracts were made by the maceration process using ethanol.. By this method, the percentage yield was found to be 36.86%. Ethanol was used as a solvent for extraction which is polar solvent and can rupture the cell membrane and extract the cellular components. Most phyto-constituents dissolve well in higher polarity solvents, which is why many biochemists choose them as a solvent for extracting a wide range of phyto-constituents.. The extractive value could have been increased if the time of extraction was increased.

4.5 Phytochemical screening

Table 4: Phytochemical screening of *Tetrataenium nepalense* seed extract.

S.N.	Phytochemical tests	Results
1	Alkaloids.	
	a. Mayer's test	+ ve
	b. Wagner's test	+ ve
	c. Dragendroff's test	+ ve
2	Glycosides	
	a. Anthraquinone test	- ve
	b. Keller-killani test	- ve
3	Phenols	
	a. Lead acetate test	+ ve
4	Flavonoids	
	a. Shinoda test	+ ve
	b. Alkaline reagent test	+ ve
5	Tannins	
	a. Ferric chloride test	- ve
6	Saponins	
	a. Foam test	- ve
7	Terpenoids	+ ve
8	Carbohydrates	
	a. Fehling's test	- ve
	b. Molisch's test	+ ve
9	Proteins	
	a. Xanthoproteic test	- ve
	b. Ninhydrin test	- ve
	c. Millon's test	- ve
10	Fats and fixed oils	+ ve
11	Steroids	+ ve
12	Quinone	+ ve
13	Coumarin	+ ve

The preliminary results from qualitative phytochemical screening of extracts of whole plant of *Tetrataenium nepalense* showed that the presence of bioactive compounds like alkaloids, phenols, flavonoids, tannins, coumarin, terpenoids, steroids, quinine, carbohydrates and proteins. The results was similar as in Shrestha ***et. Al 2015.***

We can identify the medicinally active plant secondary metabolites by qualitative analysis of the different bioactive compounds, which also makes quantitative estimate and qualitative separation of the metabolites easier. This technique might help scientists find and create new drugs. Quantitative Phytochemical Screening

4.6 Determination of TPC (Total Phenolic Content)

The content of total phenolic compounds was determined by the Folin-Ciocalteu reagent. Total phenolic compounds in extract and fractions were expressed in terms of micrograms of gallic acid equivalent per gram of dry weight (mg GAE/g of dry weight of extract) which is calculated from the regression equation of the calibration curve ($y = 0.008x + 0.429$, $R^2 = 0.9508$) and is expressed as gallic acid equivalents (GAE). Calibration curve is shown in figure.

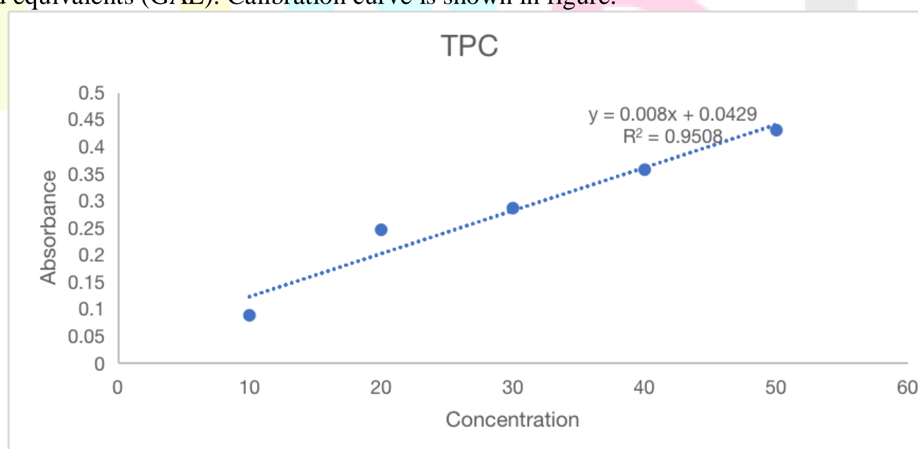


Figure 4: Calibration curve for TPC using gallic acid

4.7 Determination of Total Flavonoid Content:

Total flavonoid compounds was determined by aluminum chloride colorimetric method. Total flavonoid compounds in extract and fractions were expressed in terms of micrograms of quercetin equivalent per gram of dry weight (mg QE/g of dry weight of extract of fractions) which is calculated from the regression equation of the calibration curve ($y = 0.0117x + 0.1761, R^2 = 0.9973$) and is expressed as quercetin equivalents (QE). The ethanolic extract of Tetrataenium *nepalense* showed the TFC value of mg QE/g of extract.

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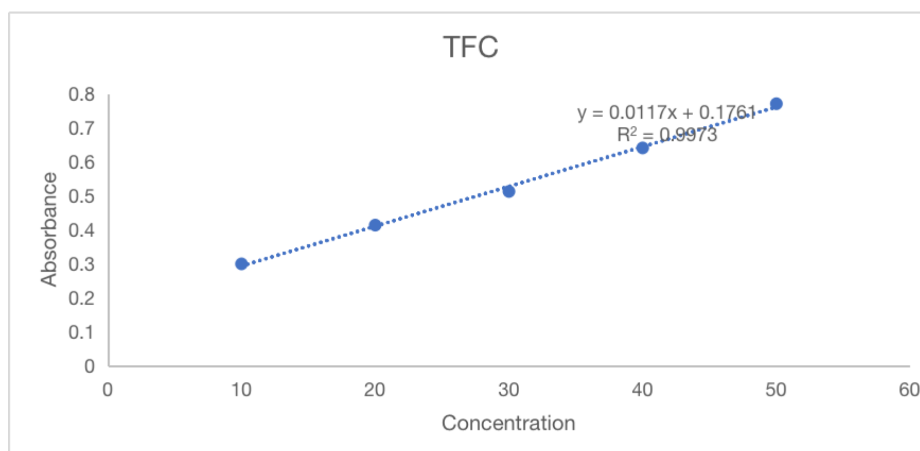

Figure 5: Calibration curve for total flavonoid content using Quercetin as standard

4.8 DPPH scavenging activity

"Measurements of free radical scavenging activity (DPPH) were carried out as per the methods given by ***Mehdi et al.,2016"***.

"This method is based on the reaction of DPPH that is characterized as a preformed stable free radical with a deep violet color and any substance that can donate a hydrogen atom to DPPH reduces it to a stable diamagnetic molecule".

Table 4: DPPH scavenging activity

S.N	Concentration µg/ml	Absorbance	% inhibition
1	20	0.891	48.01
2	40	0.850	50.40
3	60	0.808	52.85
4	80	0.750	56.22
5	100	0.692	59.6

The IC50 value was found to be 36.4413793 µg/mL.

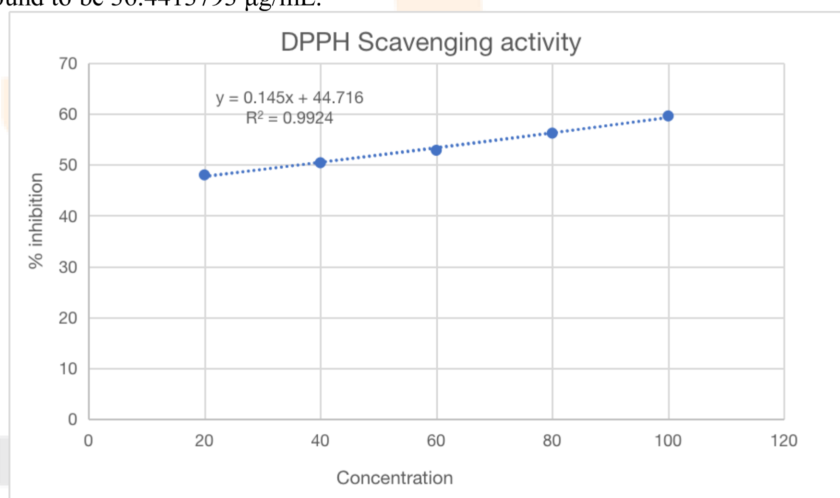

Figure 6 : Percentage inhibition of DPPH radical by ***Tetrataenium nepalense*** seed extract.

The IC50 values are negatively related to the antioxidant activity, as it expresses the amount of antioxidant needed to decrease its radical concentration by 50%. The lower IC50 value represents the higher antioxidant activity of the tested sample. In my current study, the quantitative phyto-chemical analysis showed that the extracts showed the presence of phenolic content and flavonoid content which supports the high antioxidant activity because the plant polyphenols are the potent antioxidants. *Mehdi et al in 2016* conducted a study in *Tetrataenium lasiopetalum* flower extract where significant antioxidant activity was found with an IC50 value of 170µg/mL, which is not similar to my study. This may be due to the use of different species and also from different geography.

4.9 Antibacterial testing

Table 5: The ethanolic extracts of Tetrataenium nepalense exhibited inhibitory effect against S. aureus and Bacillus subtilis, according to the results of the antimicrobial activity investigation.

S N	Organism	Standard ZOI (mm)	ZOI of 10% Extract	ZOI of 20% Extract
1.	*B. subtilis*	Ciprofloxacin (39.54)	0.00	8.20
2.	*S. epidermidis*	Amoxicillin (12.32)	0.00	0.00
3.	*S. aureus*	Amoxicillin(35.12)	8.12	8.60
4.	*P. vulgaris*	Ciprofloxacin(34.24)	0.00	0.00
5.	*E. faecalis*	Ciprofloxacin(21.8)	0.00	0.00
6.	*E. coli*	Ciprofloxacin(31.94)	0.00	0.00
7.	*S. typhii*	Ciprofloxacin(33.52)	0.00	0.00
8.	*S. dysenteriae*	Ciprofloxacin(33.44)	0.00	0.00
9.	*P.aureginosa*	Ciprofloxacin(33.62)	0.00	0.00
10.	*K. pneumoniae*	Ciprofloxacin(18.58)	0.00	0.00

Minimum bactericidal concentration: The MBC value of ethanolic extract of *Tetrataenium nepalense* against *B. subtilis* and *S. aureus* was found to be in between 0.78125 - 1.5625 mg/ml and 25 - 50 mg/ml respectively

4.10 Antifungal activity

Table 4: The result of the antifungal activity study of ethanolic extracts of *Tetrataenium nepalense* seed extracts.

S.N	Name of orgaaanism	Standard ZOI(mm)	ZOI of 10% extract	ZOI of 20% extract
1.	*C. albicans*	Clotrimazole (16.36)	0.00	0.00

The ethanolic extract of *Tetrataenium nepalense* seed showed no inhibitory activity against the given organism, *Candida albicans.*

CONCLUSION AND RECOMMENDATIONS

Tetrataenium nepalense plant ethanolic extract was discovered to contain many phytochemicals, including alkaloids, quinine, steroids, coumarin, phenols, terpenoids, tannins, flavonoids, and carbohydrates. The plant may be a useful source of antioxidants because of its high levels of total flavonoid and total phenolic contents. While none of the Gram-negative bacteria exhibited any inhibition, the ethanolic plant extract shown inhibitory action against the Gram positive bacteria. This indicates that the plant consists of chemical with antibacterial properties which could be a potential source for production of drugs with a narrow spectrum of therapeutic activity. The extract has shown significant percentage inhibition in DPPH free radical scavenging activity**.**

The findings show that *Tetrataenium nepalense* has high-quality natural compounds with therapeutic value that function as antioxidants and antibacterial agents. The plant's traditional claim is supported by the scientific investigation of this study. To identify and describe the active phytochemical substances that are responsible for the reported activities, as well as to comprehend the mechanism underlying them, more study and research are necessary.

Recommendations: Minimum effective concentration (MIC) of plant extract was not performed due to the presence of color in the plant extract. If MIC was conducted then it would help in providing the ability to precisely determine the concentration required to inhibit the growth of pathogenic organisms.

Animal testing should be conducted for better understanding of its therapeutic and toxic effect on man.

GC-MS could also be performed which helps in development of new pharmaceuticals and analysis of their purity.

From the above study it is not possible to point to the exact phytoconstituent responsible for antimicrobial activity. Hence further study should be conducted for structural elucidation of chemical constituents.

Due to the time limit it was not possible to conduct animal testing. So on further study, determination of anti-inflammatory activities, anti-diarrhoeal activity, anti-alzheimer's and anti-parkinsonism effects and cytotoxic effects can be evaluated.

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