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Determination of the antioxidant content in selected medicinal plants of Kalyan and Kolhapur

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Abstract
The aim of this research is to qualitatively screen medicinal plants for the presence of various phytochemicals and to develop a simple spectrophotometric method for the quantitative analysis of flavonoids, phenolic content Ascorbic acid in Tulsi leaves, Tulsi stems, ajwain leaves, clove leaves, ginger rhizome and fenugreek seeds. This study provides an overview of flavonoids, phenolic content, and ascorbic acid in medicinal plants. The contents were determined by the proposed spectrophotometric method, and the obtained results were compared with the Reference method. Qualitative analysis of medicinal plants was performed, and it was observed that alkaloids, Flavonoids, tannins, phenolic compounds, proteins, oils, and fats were present in all plant extracts. TFC, TPC, and AA were determined by using a UV-Vis spectrophotometer. All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation. Tulsi and Ajwain are found to be rich in flavonoids, whereas ginger and fenugreek are rich in phenols. Ascorbic acid is an essential antioxidant present in all medicinal plants. Fenugreek is rich in all antioxidants. The proposed method is simple, rapid, sensitive, and obeys Beer-Lambert's Law.

Keywords*: Medicinal plants, phytochemicals, flavonoids, phenolic content, Ascorbic acid, visible spectrophotometer.*

1. Introduction
From ancient times, Medicinal plants have always used for traditional medicine. Plants can protect themselves from microorganisms, harmful insects, and adverse environmental changes by producing certain chemicals or secondary metabolites that are non-nutritive. In India, phytochemicals are also used in cosmetics, health and hygiene, fragrance, and food supplements (Bansal Funchal 2021). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants that are responsible for providing color, flavour, and aroma to fruits and vegetables. They also protect plants against invasion, multiple disease, and infection. Phytochemical screening is critical for identifying new sources of therapeutically and industrially important compounds (Saxena Malta 2013). The main phytochemical components present in medicinal plants are flavonoids, ascorbic acid, tannic acid, alkaloids, steroids, saponins, etc. they can be derived from the bark, stem, leaves, and seeds of medicinal plants. Given this background, the present study aimed to conduct a preliminary phytochemical analysis of the water extracts in Tulsi, ajwain, clove, fenugreek, and ginger (Agidew misganaw 2022). Among the herbs used in ayurveda, tulsi is an aromatic herb belonging to the basil family. Phytochemicals in tulsi have strong antioxidant properties. Thus, they help protect us from diseases. Tulsi stems and leaves have high levels of antioxidant properties (Cohen marc

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Maurice 2014). Ajwain is widely distributed and mainly cultivated in various parts of India and mainly grown in the month of October and November and should be harvested in month of May and June. Mainly brown seeds are used for medicine purposes (Praveena Panda 2020). Ajwain is an annual herb of the family Apiaceae, and both leaves and seeds are consumed by humans. The high level of phenols and flavonoids in Ajwain leaves influence their antioxidant activity. Clove is one of the most useful spices used over the years (Novi karoko 2023). Clove belongs to the family Myrtaceae and is rich in many phytochemicals such as flavonoids and tannic acid (Esther bela 2021). Ginger belongs to the Zingiberaceae family. Ginger plants provide nutritional value to our daily lives. The antioxidant properties of ginger are significant and can be used as a preventive agent against many diseases. Many bioactive compounds in ginger, such as phenolic and terpene compounds, have been identified (Mahmood Metham 2017). Fenugreek is an annual leguminous Bentham belonging to the Fabaceae family, and it has been used to heal wounds, aid digestion, and provide other health benefits to humans. Fenugreek seeds are the most important and useful part of plants. It is an herbal medicine used for the cure of diabetes. The biological and pharmacological actions of fenugreek are mostly attributed to the variety of its bioactive chemical constituents (Wang tian yang 2017). Flavonoids are a class of polyphenol secondary metabolites commonly found in plants. Flavonoids are also a broad class of compounds broadly found in nature. They are believed to have bioactive effects, including anticancer, antiviral, anti-inflammatory, and anti-aging effects. They have a basic structure of C6-C3-C6 with different substitution patterns to produce a series of subclass compounds. It has been reported that more than 10,000 classes of flavonoids have been found in kingdom plantae (Ekalu Babiche 2020). Natural phenolic compounds are plant secondary metabolites that hold an aromatic ring bearing at least one hydroxyl group. Phenolic compounds are good electron donors and have powerful biological activity. Phenolics are the largest group of phytochemicals that account for most of the antioxidant activity in plants. They also possess a wide spectrum of biochemical activities, such as antioxidant activity, and the ability to modify gene expression (Sulaiman CT 2012). Ascorbic acid is the most powerful antioxidant and plays an important role in oxidative reduction reactions. Ascorbic acid is a water-soluble nutrient. It is found in most medicinal plants. It is essential for humans (Jadhao 2016). The aim and objective of this research is to develop simple spectrophotometric method for the determination of phytochemicals present in different medicinal plants (Gupta VK 2006).

2. Methods

2.1 Plant collection

As per Table 1, Fresh samples of Tulsi leaves, Tulsi stems, Ajwain leaves, Clove leaves, Ginger rhizome, and Fenugreek seeds were purchased and collected from a nursery at Kalyan and Kolhapur.

2.2 Aim & Objective

The aim of the research is to screen medicinal plants for the presence of various anti-oxidants qualitatively and few of them quantitatively.

The objective of this study is to develop simple spectrophotometric method for the quantitative analysis of flavonoids and phenolic content and ascorbic acid in Tulsi leaves, Tulsi stems, Ajwain leaves, Clove leaves, Ginger rhizome, and Fenugreek seeds.

2.3 Apparatus

All glassware used for the experimental purpose was made of Pyrex or Borosil glass. The burette, pipette, and standard flasks were calibrated using the method described by Vogel.

2.4 Instrument

Absorption measurements were performed on a visible spectrophotometer (LMSPV320, LABMAN) using 1-cm-matched glass cells. The spectrophotometer was calibrated by measuring the absorption spectra of potassium chromate in potassium hydroxide solution and that of potassium permanganate in sulfuric acid.

2.5 Chemicals

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Quercetin, 0.5% Boric acid, 5% NaOH, Tannic acid, 0.3% p-Aminophenol, Cr (VI), 0.2% Piperidine, Ascorbic acid, 0.2% Potassium hydrogen phthalate, 0.1M HCL, Ammonium ferrous sulphate,Peroxide, Distilled water. All chemicals and reagents used in the present study were analytical grade.

3. Preparation of plant extracts

Plant samples were washed with distilled water, dried in a microwave oven at 900 W at 525 degrees for 3 min, and grounded into powder. One gram of each sample in 100 ml of distilled water was further refluxed for 2.5 hr in distilled water to obtain an aqueous extract, which was used for qualitative and quantitative analysis of phytochemicals.

Table 1 Fresh Samples Collection

Sr. No	Family	Common Name	Photograph	Part used	Biological Activity
1	Ocimum Sanctum	Tulsi Sacred Basil		Leaf and Stem	Stomach, Ringworm, and other cutaneous diseases
2	Apisceae	Ajwain		Leaf	Anti-microbial, anti-viral Properties
3	Myrtaceae	Clove		Leaf	Major antimicrobial component
4	Zingiber Officinale	Ginger		Rhizome	Antidiabetic, antinausea, and antiemetic activities
5	Fabaceae	Fenugreek		Seeds	Anti-oxidative, Anti-viral.

4. Qualitative determination of phytochemicals

The individual extracts were subjected to qualitative phytochemical screening for the presence of some chemical constituents. The plant extract using the standard biochemical methods as described below:

4.1 Alkaloids

Wagner test: 1 ml of plant extract was treated with Wagner's reagent; formation of Brown reddish precipitate indicates presence of alkaloids.

4.2 Carbohydrate

Iodine test: 2 ml of plant extract were treated with 5 drops iodine solution the blue color indicates the presence of carbohydrates.

4.3 Glycosides

Legal's test: To the plant extract, 1 ml of pyridine and a few drops of freshly prepared sodium nitroprusside solution were added; the appearance of pink to red color indicates the presence of glycosides.

4.4 Flavonoids

Zn test: 2ml plant extract was treated with Zn dust and conc. HCL formation of red color indicates presence of flavonoids.

4.5 Saponin

5ml plant extract was mixed with 20 ml of distilled water and agitated in a graduated cylinder for 15 min to form foam, indicating saponin.

4.6 Terpenoids

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Salkowski test: 5ml plant extract was mixed with 2ml chloroform and concentrated sulfuric acid was added carefully to form a layer. A reddish-brown coloration of the interface was formed indicating the presence of terpenoids.

4.7 Tannin

4ml plant extract was treated with 4ml $FeCL_3$ formation of green color indicating presence of tannin.

4.8 Phenolic

Ferric Chloride test: Plant Extract was treated with 4 drops of Alcoholic $FeCl_3$ solution. Formation of a bluish black color indicates the presence of phenol.

4.9 Amino acid

Ninhydrin test: To the 2ml plant extract, ninhydrin reagent was boiled for a few minutes. The formation of blue color indicates the presence of amino acid.

4.10 Proteins

Xanthoproteic test: plant extract was treated with few drops of concentrated HNO_3 formation of yellow indicates the presence of proteins.

4.11 Oils & Fats

A small quantity of plant extract was pressed between two filter papers separately. Oily appearance of the filter paper indicated the presence of oils & fats.

4.12 Coumarins

3 ml of 10% NaOH was added to 2 ml of aqueous plant extract, and formation of yellow color indicates presence of coumarins.

4.13 Phlobatannins

Deposition of red ppt when aqueous extract of each plant sample was boiled with 1% Aq. HCl was taken as evidence for the presence of phlobatannins (R.S. Sawant 2013)

5. Quantitative determination of phytochemicals

5.1 Determination of the total flavonoid content

To determine the total flavonoid content (TFC), a standard solution of Quercetin was prepared by dissolving 10 mg of quercetin in 10 ml of methanol. An aliquot of the solution containing 2-160 µg of quercetin was transferred into a series of 10 mL flasks. A volume of 1 mL of 0.5% boric acid solution followed by 0.5 mL of 5% NaOH was added. The contents were gently shaken until a yellow color appeared and were topped up to the mark with distilled water. The absorbance of the color solution was measured at 420 nm against the corresponding reagent blank. The plant sample was determined using 1 ml of Solution. The results of the proposed method were compared with the reference method of the aluminium chloride colorimetric method for flavonoids (Shazia tabacum 2016).

5.2 Determination of the total phenolic content

A 50ppm standard stock solution of tannic acid was prepared for the determination of the total phenolic content (TPC) of the sample. Different concentrations of solutions containing 2-20µg of tannic acid were prepared. A volume of 0.5 mL of 0.2% piperidine was added, and 0.2 mL of 0.3% p-aminophenol with 1 mL of Cr (VI)1mL was added. The mixture was gently shaken, and a buffer was added to it. (Buffer pH=3), (0.2% Potassium hydrogen phthalate, 0.1M HCL). The contents were up to the mark with distilled water, and absorbance was measured at 480 nm against the blank. The plant sample was determined using 1 ml of each solution. Tannic acid was determined using p-aminophenol and chromium acid at a pH of 3.0. The colored species originate from the involvement of p-aminophenol with chromium acid in tannic acid in the formation of charged transfer complexes. P-aminophenol and chromium acid are necessary for maximum color development. The results of the proposed method were compared with those of the reference Folin-Ciocalteu reagent method (Kavitha Chandran CI 2016).

5.3 Determination of ascorbic acid

To determine the ascorbic acid content of the sample, the calibration curve was plotted in the range of 2-200 ug/ml of ascorbic acid, and the absorbance of the complexes was measured at 400 nm. Ascorbic acid was used as the standard to plot the calibration curve. A standard solution of ascorbic acid was prepared by dissolving 10 mg of ascorbic acid in 10 ml of distilled water. A volume of 1 mL of 10% ammonium ferrous sulphate was added. The mixture was gently shaken, and 0.5 mL of peroxide was added. The contents were kept up

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to the mark with distilled water. The assay of each plant material was performed using 1 ml of each extract stock solution. Ammonium ferrous sulphate undergoes an oxidation reaction with ascorbic acid in the presence of peroxide. Adding peroxide gives a dark yellow color to the solution. The results of the above proposed method were compared with the reference method of oxidation - reduction method of iodine. (Rahman khan 2006).

6. Results

Since ancient times, medicinal plants have been attributed to the presence of secondary plant metabolites such as flavonoids, phenols, tannins, and ascorbic acid, and they have played a significant role worldwide in ayurveda. Medicinal plant samples were collected, washed, and refluxed with water extract for further analysis of antioxidants.

6.1 Screening for the presence of phytochemicals in Tulsi leaves, stems, ajwain leaves, clove leaves, ginger rhizome, and fenugreek seeds

Table 2 Phytochemical Screening for the Samples

Phytochemical tests	Tulsi leaves	Tulsi stems	Ajwain leaves	Clove leaves	Ginger rhizome	Fenugreek seeds
Alkaloids	+	+	+	+	+	+
Carbohydrates	-	-	-	-	+	+
Glycosides	+		-	-	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	-	-	-	-
Terpenoids	+	-	+	-	+	+
Tannin	+	+	+	+	+	+
Phenolics	+	+	+	+	+	+
Amino acid	+	+	-	+	+	+
Proteins	+	+	+	+	+	+
Oils and Fats	+	+	+	+	+	+
Coumarins	-	+	+	+	-	-
Phlobatannins	-	-	-	-	-	-

+ Indicates presence of phytochemicals; - Indicates absence of phytochemicals

6.2 Quantitative analysis

Table 3 LOD and LOQ Value Analysis

Phytochemicals	UV (λ_{max})	Linear regression	R^2	LOD ($\mu g\ mL^{-1}$)	LOQ ($\mu g\ mL^{-1}$)
Quercetin	420	y=0.0101x+0.1778	0.9883	1.056	3.201
Tannic Acid	480	y=0.0233x+0.4449	0.9983	13.02	39.515
Ascorbic Acid	400	y=0.0539x+0.028	0.9959	11.76	35.659

The total flavonoid content in the examined plant extracts using boric acid with NaOH was determined from the regression equation of the calibration curve (y=0.0101x+0.1778, R^2=0.9883) respectively. The total phenolic content in the mentioned plant extracts using p-aminophenol with chromium acid at pH 3.0 was calculated from the regression equation of the calibration curve (y=0.0233x+0.4449, R^2=0.9983) respectively. The content of ascorbic acid in the above selected plant extracts using oxidation reactions with ammonium ferrous sulphate was examined from the regression

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equation of the calibration curve ($y=0.0539x+0.028$, $R^2=0.9959$) respectively.

The results of the proposed method for **Kalyan** plant extract are compared with those of the reference method:

Table 4 Quantitative Analysis in reference with Kalyan Plant Extract

Sr.no	Plant sample	Quercetin (µg/mL)		Tannic acid (µg/mL)		Ascorbic acid (µg/mL)	
		Proposed	Reference	Proposed	Reference	Proposed	Reference
1	Tulsi Leaves	88.66±0.152	88.65±0.942	32.07±0.065	32.82±11.08	16.47±0.030	16.21±0.451
2	Tulsi Stems	55.39±0.295	55.66±0.190	19.65±0.176	20.59±0.665	8.12±0.081	8.82±0.198
3	Ajwain Leaves	76.58±0.057	76.67±0.259	21.82±0.066	21.04±1.389	13.99±0.085	13.91±0.150
4	Clove Leaves	57.34±0.264	57.92±0.098	39.41±0.088	39.48±0.386	12.45±0.026	12.97±0.130
5	Ginger Rhizome	75.46±0.264	74.98±0.427	58.14±0.237	58.59±2.309	11.84±0.030	11.45±0.247
6	Fenugreek Seeds	92.06±0.202	92.16±0.484	39.49±0.066	39.26±0.665	13.81±0.011	13.78±0.198

The results of the proposed method for **Kolhapur** plant extract are compared with those of the reference method:

Table 5 Quantitative Analysis in reference with Kolhapur Plant Extract

Sr. no	Plant sample	Quercetin (µg/mL)		Tannic acid (µg/mL)		Ascorbic acid (µg/mL)	
		Proposed	Reference	Proposed	Reference	Proposed	Reference
1	Tulsi Leaves	94.04±0.345	94.47±0.707	10.56±0.066	10.82±0.381	19.23±0.055	19.55±2.241
2	Tulsi Stems	52.55±0.640	52.66±0.259	15.73±0.051	15.75±1.154	13.86±0.045	13.78±0.198
3	Ajwain Leaves	91.4±0.173	91.82±0.098	20.11±0.409	20.59±0.665	14.37±0.035	14.8±2.523
4	Clove Leaves	51.56±0.404	51.48±0.931	30.61±0.995	30.59±1.154	16.52±0.011	16.21±0.451
5	Ginger Rhizome	71.13±0.115	71.48±0.098	43.28±0.092	43.48±3.357	16.46±0.011	16.68±2.571
6	Fenugreek Seeds	93.44±0.057	94.08±1.283	39.56±0.085	39.26±0.665	13.82±0.025	13.35±0.219

Table 6 Analytical Methods for the Proposed Methods for TFC, TPC and AA

Antioxidants	Beers Range µg/mL	Molar Absorptivity $Lmol^{-1}\ cm^{-1}$	Sandel's Sensitivity $\mu g.cm^{-2}$	Atomic Weight amu
TFC (Quercetin)	6-160	0.00513	215.80×10^{-3}	302.23 g/mol
TPC (Tannic acid)	2-20	0.18920	6.958×10^{-3}	1701.22 g/mol
AA (Ascorbic acid)	20-120	0.01914	97.769×10^{-3}	176.12 g/mol

7. Discussion

All plant materials were extracted from different parts of the plants using aqueous (water) as solvent.

As shown in Table 2, in the qualitative phytochemical investigation of the medicinal plants, the medicinal plant extracts had different

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phytochemicals. Alkaloids, flavonoids, tannins, phenolic compounds, proteins, oils, and fats were present in all six extracts.

7.1 For Quantitative determination

The three proposed methods are suitable for the determination of phytochemicals. TFC, TPC, and AA were determined by the spectrophotometric method. All analyses were performed in triplicate and the results are expressed as mean ± standard deviation. The LOD and LOQ values are shown in Table 3. The quantitative phytochemical composition of Tulsi leaves, Tulsi stems, ajwain leaves, clove leaves, ginger rhizome, and fenugreek seeds are shown in Tables 4 and 5. Beer's range, molar absorptivity, Sandel's sensitivity, and atomic weight are shown in Table 6. The above results show that Tulsi leaves, Tulsi stems, ajwain leaves, clove leaves, ginger rhizome, and fenugreek seeds contained flavonoids equivalent to Quercetin. Phenolic content as tannic acid equivalents and ascorbic acid Tulsi and ajwain are rich in flavonoids, whereas ginger is rich in phenols. Ascorbic acid is an essential antioxidant present in all medicinal plants. Fenugreek is rich in all antioxidants. Kolhapur samples are rich in antioxidants compared with Kalyan samples.

7.2 Graphs

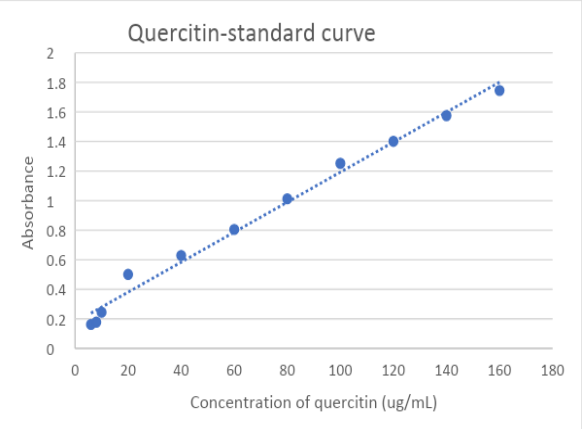

Figure 1 Standard Curve for Quercetin

Quercetin was used as a standard to determine flavonoid content. The molar absorptivity is 0.00513 L mol^{-1} cm^{-1}, and the Sandell's sensitivity is 215.80× 10^{-3} ^{3}µg.cm^{-2}. Figure 1 shows a straight-line calibration curve.

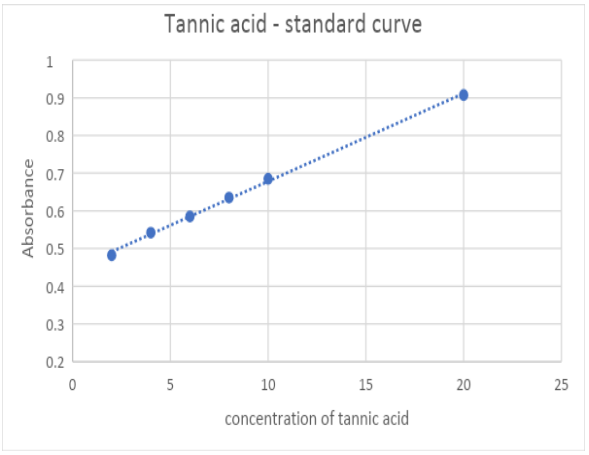

Figure 2 Standard Curve for Tannic Acid

Tannic acid was used as a standard to determine the total phenolic content. The molar absorptivity 0.18920 L mol^{-1} cm^{-1}, and the Sandell's sensitivity is 6.958 × 10^{-33}µg.cm^{-2}. Figure 2 shows straight-line calibration curve.

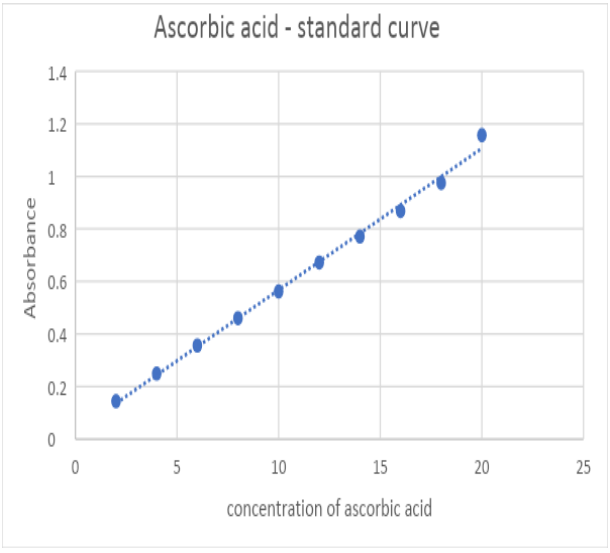

Figure 3 Standard Curve for Ascorbic Acid

Ascorbic acid was used as the standard for determining ascorbic acid levels. The molar absorptivity is 0.01914 Lmol^{-1} $cm^{-1,}$ and Sandell's sensitivity is 97.769×10^{-3}µg.cm^{-2}. Figure 3 shows a straight-line calibration curve.

7.3 Characteristics

- **Spectral Characteristics**

The optimum wavelength of maximum absorption ($Ë_{max}$) of tannic acid flavonoid and ascorbic acid was scanned on a spectrophotometer in the wavelength region from 340 to 900 nm against a

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blank. The maximum wavelengths shown were 480 nm for tannic acid, 420 nm for flavonoids, and 400 nm for Ascorbic acid.

- **Optical characteristics**

Tannic acid, flavonoid and ascorbic acid adhered to the Beer-Lambert law. Beer's range for tannic acid obtained was 2-20 µg/ml, for flavonoids 6-160 µg/ml, and for ascorbic acid 2-120 µg/ml. A calibration curve was constructed by measuring the absorbance at an appropriate wavelength of a set of solutions containing varying amounts of tannic acid, flavonoids and ascorbic acid a specified amount of reagents against suitable blank. Beer- Lambert's Law plots are recorded graphically.

7.4 Statistical analysis

The adherence to Beer's Law was studied by measuring the absorbance values of solutions varying in phytochemical concentration. A straight-line graph was obtained by plotting absorbance against concentration of TFC, TPC and AA. The values obtained are in triplicate and the results are expressed as mean ± standard deviation. The molar absorptivity and Sandel's sensitivity in TFC, TPC, AA were found to be 0.00513, 0.18920, 0.01914 L mol^{-1} cm^{-1} and 215.80× 10^{-3}, 6.958 × 10^{-3}, 97.769 × 10^{-3} respectively. The limit of detection and limit of quantification in TFC, TPC, AA were found to be 1.056, 13.02, 11.76 µg mL^{-1} and 3.201, 39.515, 35.659 µg mL^{-1} respectively. The data was statistically analysed by using MS excel. Regression statistics were used to calculate LOD & LOQ. The values obtained are compared with the values obtained by the reference method.

Conclusions

The proposed method is simple, rapid, and obeys Beer-Lambert's law. The proposed method has advantages in terms of Saving time and sensitivity. In determination of phytochemicals, Validity of beers is achieved in a low range, which is sensitive for analysis and with an effective molar absorption coefficient. The present study involves the qualitative and quantitative study of total flavonoid content, total phenolic content, and ascorbic acid of Tulsi stems, Ajwain leaves, clove leaves, fenugreek seeds, and ginger rhizomes, which act as a useful source of ayurveda and improve human health because of the presence of these phytochemicals, which can be powerful antioxidant agents. Hence, the proposed method can be used to determine antioxidants.

List of Abbreviations

TFC: Total Flavonoid content; TPC: Total Phenolic content; AA: Ascorbic acid; max: Maximum; ppm: Parts per million; LOD: Limit of Detection; LOQ: Limit of Quantification.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All the data analyzed during the current study are available online in different forms such as journals and articles

Competing interests

The authors declare no competing interests.

Funding

Not applicable

Authors contributions

Reviewed and approved the final version of manuscript for publication.

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