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Monitoring antioxidant and antityrosinase activity of clove aromatic flower buds

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Abstract

The aim of the study is to investigate the *In vitro* antioxidant activity, total phenol and flavonoid content of clove bud (*Syzygium aromaticum*) and compared with that of oil extracted from clove bud oil. Antioxidant potential was evaluated using DPPH radical scavenging and Hydrogen peroxide scavenging assays. The total phenolic content was measured by using folin-Ciocalteu method while total flavonoid content was determined using $AlCl_3$ calorimetric method in both clove bud extracts and clove bud oil. The anti-melanogenic effect of extracts of clove bud and clove bud oil was also examined by using Tyrosinase as a substrate. Clove bud oil showed (87.2±0.83%) DPPH radical scavenging as compared to methanolic extract of clove bud which was (82.9±3.1%) at 200 µg/ml concentration. However, under the same condition, the standard Ascorbic acid showed (91.4±3.4%) inhibition at same concentration. In addition, clove bud oil and clove bud had an effective H_2O_2 Scavenging activity and was compared with the potential of synthetic antioxidant BHT. The present study showed that clove bud and clove bud oil contains many health nutrients and phytochemicals which are very useful for human health. Due to the presence of total phytochemicals such as phenols and flavonoids, the clove bud showed efficient antioxidant activity. The clove bud oil is much effective due to its capability to reduce free radicals as compared to clove bud.

Keywords: Clove bud, clove bud extracts, clove bud oil, phenols, flavonoids, antioxidant and antityrosinase activity, HPLC

1. Introduction

Certain diseases particularly cancer may be caused due to oxidative damage resulting from the excess production of free radical. A balance is necessary between antioxidants and free radicals for a proper physiological function. Free radicals induce damages and alter many biomolecules like lipids, proteins, and DNA. These damages can cause a large number of human diseases which particularly include cancer and aging. Antioxidants are known to very important in protecting the body against the damages caused due to free radicals. Some antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have recently been reported that they can cause damage and can be dangerous to human health, so there is a need, focus and search for effective, nontoxic natural compounds with antioxidant activities^[1].

In response to the greater demand, need and popularity for medicinal plants, a large number of different conservation groups are focusing and recommending the cultivation of wild medicinal plants. Medicinal plants and all its constituents possess very effective therapeutic potential. A large number of medicinal plants including different herbs and spices were reported to have strong antioxidant capacity^[2]. Antioxidant compounds provide protection against oxidative stress which is caused due to free radical. The antioxidant capacity of plant polyphenols are associated to different assays, such as free radical scavenging capacity, donating of hydrogen, singlet oxygen inhibition and metal ion chelation. So the antioxidant potential of plant depends upon the total phenolic contents present in it^[3].

Cloves are mostly known due to its usage in Ayurveda. Cloves are also used as medicines in china and as an herb in western countries. Cloves have a carminative effect that either helps in the expulsion of gas or also prevents its formation in the gastrointestinal tract. Clove is also known to be much effective to overcome the symptoms of diarrhoea, gastric irritability and vomiting^[4]. Essential oil of clove is also much effective against pain caused due to dental problem and it relief pain. So it acts as an anodyne in the dentistry industry^[5]. In addition, the cloves are also known for its activity against mutagens^[6].

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Cloves have a high content of flavonoids which make them effective anti-inflammatory compound [7]. Clove is known to be the best antioxidant among spices and very effective in protecting the body against damages caused by free radicals [8]. It is also reported that clove has antiulcerogenic effects [9]. The aim of this study is to investigate and compare the nutritional value and antioxidant potential of clove (*Syzygium aromaticum*) aromatic flower buds and clove bud oil.

2. Materials and Methods

Sample collection

Different types of dried clove buds (*Syzygium aromaticum*) were purchased from the local market of Rawalpindi/Islamabad regions. Collected samples were saved in fine plastic bags.

Sample preparation

The dried clove buds were further subjected to oven drying then was ground to powdered form using a laboratory grinder. The sample was saved in fine plastic bags and stored in refrigerator at 4 °C for further analysis.

Extraction of clove bud oil

The powdered clove bud sample was first weighed and 12 grams of the sample was transferred into a filter paper extraction thimble. The thimble was placed in the loading chamber and extracted for about 6 hr in soxhlet apparatus with 125 ml *n*-hexane which was taken in the 250 ml reflux flask. Colour change was observed as the oil transferred in to the solvent. After extraction with Soxhlet apparatus, solvent was removed using rotary vacuum evaporator at 45 °C [10].

Proximate analysis of clove buds

Clove bud samples (*Syzygium aromaticum*) will be subjected for proximate analysis. Moisture content, dry matter content, total ash, crude protein and crude fat content of clove bud sample were determined according to AOAC methods [11].

Phytochemical screening

Preparation of plant extracts

Extracts of sample was prepared with *n*-hexane, ethanol and methanol solvents using maceration method with increasing polarity [12].

Qualitative determination of phytochemicals

The clove bud and clove bud oil were subjected to qualitative test for the detection of Alkaloids, flavonoids, phenols, tannins, saponins, terpenoids and glycosides.

Test for Glycosides

Keller-Killani test was used for the detection of glycosides [13].

Test for Alkaloids

To detect alkaloids, 5 ml of the extracts filtrate was diluted with of 5 ml of 2% HCl. HCl was saturated firstly with sodium chloride. The solution containing sample was then filtered and Wagner's reagent was added to 2-3 ml of filtrate. Orange brown precipitates were formed [13].

Test for Flavonoids

To detect flavonoids, alkaline reagent test was used. Firstly, 2-3 ml of the extract filtrate was taken which was treated with 10% ammonium hydroxide solution. Appearance of yellow colour indicated the presence of flavonoids [14].

Test for phenols

Firstly, extracts were taken in test tubes. 5 ml of distilled water was then poured in each test tube and shake it well until the extracts were completely dissolved. After that few drops of already prepared 5% FeCl₃ were added to the test tube. Appearance of dark green color indicated the presence of phenolic compounds [14].

Test for Saponins

The extracts were boiled for 15 minutes and allowed to cool down. After cooling it was shaken vigorously. Froth formation indicated the presence of saponins [12].

Test for tannins

For the detection of tannins, firstly 5 ml of the extracts filtrate was boiled for 5 minutes along with 5ml solution of 45% ethanol. The mixture was then allowed to cool at room temperature and was filtered using filter paper. 1 ml of the filtrate was then taken in a test tube and diluted with 2 ml of distilled water. Few drops of ferric chloride were then added to the solution. A greenish to black color appeared, indicates the presence of Tannins [15].

Test for Terpenoids

The extracts were firstly mixed with 3 ml of chloroform in the test tube. 3 ml of concentrated H₂SO₄ was then added to the test tube with continuous shaking. Appearance of reddish brown colour indicated the presence of terpenoids in the sample [15].

Quantitative determination of phytochemicals

Determination of total Flavonoids

The amount of total flavonoids in all extracts was measured according to method reported [16] with slight modification. Quercetin was used as a standard. 4 mg of each extract and quercetin was dissolved in 1 ml of Dimethyl sulfoxide separately to make a 4 mg/ml stock solution. Then 0.5 ml of each aliquot was put in to the test tubes and 1.5 ml of methanol was added. 0.1 ml of 10% AlCl₃ solution and 1 M potassium acetate was then added to each test tube. Total volume was made up to 5 ml by adding distilled water. Distilled water was added to make up the total volume up to 5 ml. The mixture was then incubated in dark for at least 30 min and the absorbance was measured against the blank (sample was replaced with methanol) at 430 nm with spectrophotometer. Various concentrations of standard quercetin solution were used to make a standard calibration curve. The flavonoid content was expressed in mg/g extracts as quercetin equivalent using a standard curve of quercetin and the process was repeated in triplicate.

Estimation of total Phenols

Total phenol content (polyphenols) in plant extracts was generally determined by Folin-Ciocalteu method. 1 mg of each extract and gallic acid was dissolved in 1 ml of DMSO to make a 1 mg/ml stock solution. Then 0.1 ml of each aliquot was put in to the test tubes. Then 2 ml of diluted folin-ciocalteu reagent (1:10) and 1 ml of 20% sodium carbonate was added to each test tube. After incubation at room temperature for 45 min, the absorbance was measured against the blank (sample was replaced with methanol) at 725 nm with spectrophotometer. Various concentrations of standard Gallic acid solution were used to make a standard calibration curve. The content of total phenolic compounds expressed as Gallic acid equivalent (GAE) mg/g of extracts and the process was repeated in triplicate [17].

Quantification of Tannins

For the quantitative determination of tannins, firstly the extraction was carried out according to the method reported by [18]. Folin-Ciocalteu method (FAO/IAEA, 2000) was used for the analysis of tannins. In this assay, 800 mL of the sample were put into a test tube and mixed with the same volume of Folin-Ciocalteu (Sigma-Aldrich) reagent, shaken and left for 5 min. Then this solution was diluted with 5 mL of distilled water and analyzed in a UV-Visible spectrophotometer at 725 nm for the determination of total tannins and at 480 nm for hydrolysable tannins. The obtained absorbance values were analyzed against the standard curves prepared with tannic and gallic acid for total phenols and hydrolysable tannins, respectively. The process was repeated in triplicate and results were expressed in mg Tannic acid equivalent per gram.

Determination of Saponins

Total amount of saponins in sample was evaluated according to the method reported by Okwu and Josiah [19]. The samples were ground. 20 g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90 °C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage.

Determination of antioxidant activity

Antioxidant activity of the tested compounds was determined by using the DPPH and Hydrogen peroxide scavenging methods.

Diphenyl Picrylhydrazyl (DPPH) Radical-Scavenging Activity

The free radical scavenging activity of the extract was determined according to method reported by Khan [20] with slight modification. In 2, 2'-diphenyl-1-picrylhydrazyl free radical is a delocalized structure. The function of antioxidant compounds is to reduce this free radical by donating hydrogen. This reduction will result in the colour change from purple. Different concentration of extracts methanol, ethanol, n-hexane and clove bud oil was prepared from the 4 mg/ml stock solution of each. 2 ml of 60 µM solution of DPPH (2, 2'-diphenyl-1-picrylhydrazyl) in methanol was added to 0.1 ml of each sample solution. The reaction mixture was shaken thoroughly and then was incubated for 40 min at 28 °C. DPPH radical scavenging activity was evaluated by measuring the absorbance at 517 nm. The antioxidant activity was evaluated using a formula and was expressed as percentage inhibition of DPPH. The control contained all reagents except the extract. All tests were performed in triplicate and the means were calculated.

$$\text{Scavenging capacity percentage} = \frac{A_c - A_s}{A_c} \times 100$$

Where

A_c = Absorbance of control
 A_s = Absorbance of sample

Hydrogen Peroxide Scavenging

Hydrogen peroxide is free radical which is cytotoxic and cause damage to the cell. In this antioxidant assay, such as phenolics scavenge hydrogen peroxide by donating their electron to free radical and convert it into reduce form. The ability of the extracts to scavenge hydrogen peroxide was determined according to reported by Nabavi. First of all 0.2 M of phosphate buffer (pH 7.4) was prepared and then 40 mM hydrogen peroxide was prepared in this phosphate buffer. Dilution of different concentrations of sample extracts and standard were prepared. To different dilutions of sample extracts (3 mg/ml) in distilled water, 2 ml hydrogen peroxide and 1 ml of phosphate buffer solution was added. The absorbance of H₂O₂ was measured against the blank solution containing phosphate buffer without hydrogen peroxide after 10 minutes at 230 nm using a spectrophotometer. The antioxidant activity was evaluated using a formula and was expressed as percentage inhibition of H₂O₂ radicals. All tests were performed in triplicate and the means were calculated [21, 22].

$$\text{Scavenging capacity percentage} = \frac{A_c - A_s}{A_c} \times 100$$

Where

A_c = Absorbance of control
 A_s = Absorbance of sample

Determination of antityrosinase activity

Antityrosinase activity of clove bud extracts and syzigium aromaticum bud oil was determined according to the method explained by Rangkadilok with slight modification [23]. 4 mg/ml solution of the sample extracts and standard was prepared in DMSO. 2mM solution of L-tyrosine solution was prepared in 0.1 M phosphate buffer (pH 6.8). 1 ml of the of the sample and standard was taken in separate test tubes and 1 ml of the L-tyrosinase solution (2 mM) was added. The solution was then incubated for 10 min at room temperature. After that, 200 µl of tyrosinase enzyme (333 U/ml in phosphate buffer (pH 6.8)) was added and was mixed carefully. The absorbance was measured at 490 nm after 20 min of incubation at room temperature using a spectrophotometer. The percentage inhibition of the enzyme tyrosinase was determined by using a formula.

$$\% \text{ inhibition of tyrosinase} = \frac{A_c - A_s}{A_c} \times 100$$

Where

A_c = Absorbance of control
 A_s = Absorbance of sample

Quantitative estimation of quercetin by hplc method

For the generation of quercetin, 2 mobile phases were used i.e. water and methanol.

Preparation of Standard

Standard was prepared according to the method reported by Gulati [24]. Quercetin was used as a standard. 100 mg of quercetin was transferred in 100 ml flask and dissolved in mobile phase. The flask was then refluxed for 10 mins and the stock solution (1000 µg/ml) was prepared by the addition of methanol to make the volume up to the mark. The stock solution was then filtered by passing it 3 working standard solution of Quercetin was prepared from suitable aliquots of stock solution were pipette out.

Sample Preparation

10 mg of the sample extracts were dissolved in 100 ml volumetric flask. Samples were dissolved in mobile phase (water: methanol)

Procedure

The HPLC analysis was carried out by C18V reversed phase column (4.6 nm x 250 nm). The column was packed with 5 µm diameter particles and the mobile phase used was water: methanol (30:70 v/v). The mobile phase was firstly deaerated and filtered using a membrane filter (0.45 µm). Quercetin was quantified by Pulsed amperometric detector (PAD) following RP-HPLC separation at 368 nm. Flow rate was adjusted at 0.5 ml/min and the injection volume was 20 µl. The chromatographic peaks of the analytes were obtained and confirmed by comparing the retention time with those of the standard which was used as a reference. Quantification of

quercetin in percentage was carried out by peak areas using standard method [25].

Statistical analysis

Each analysis was carried out using mean and standard deviation.

3. Results and Discussions

Proximate analysis of sample

Crude fiber content of clove buds is (7.98±0.07) which is less than the value (12±1.0) previously reported [26]. Crude protein content present in clove buds is (6.26±0.28) and this amount is higher than the (5.88±1.01) which was previously reported by Shafique [27]. Crude fat content of the sample is (3.54±0.08). Previously reported crude fat, ash and moisture content was (13.58±1.75), (4.62±0.45) and (9.67±1.20) respectively [27].

Table 1: Proximate analysis of Clove bud (*Syzygium aromaticum*)

Biochemical parameters	Percentage content (%)
Dry matter	88 ± 0.07
Moisture content	11.99 ± 0.2
Crude protein	6.26 ± 0.28
Crude fat	3.54 ± 0.08
Crude fiber	7.98 ± 0.07
Ash content	4.62 ± 0.02

Values are expressed as Mean ± SD after triplicate analysis

Values are expressed in percentage (w/w)

Qualitative and quantitative determination of Phytochemicals of clove buds

Highest amount of phenolic content was found in *n*-hexane (64.79±1.42) extract of clove buds followed by ethanol (57.08±0.89) and methanol (54.56±0.93) extract. Previously determined total phenolic content of Clove bud oil is 480 mg/g of gallic acid equivalents in the concentration of 1 mg/ml [28]. Abozid *et al.*, 2013 reported the total flavonoid and total phenolic content in ethanol extract of clove bud as (10.06 and 8.3 mg GAE/100 gm) respectively [29]. Total tannins percentage in the acetone extract of clove buds is (20.34±3.07 mg tannic acid equivalents per gram extract). Results are shown in Table 2 and 3 after qualitative and quantitative analysis.

Table 2: Qualitative analysis of different phytochemicals of clove bud and clove bud oil

S. No.	Phytochemicals	Clove bud	Clove bud oil
1	Alkaloids	+	+
2	Glycosides	+	+
3	Flavonoids	+	-
4	Phenols	+	+
5	Saponins	+	-
6	Tannins	+	-
7	Terpenoids	+	+

+ = Present, - = Absent, n = 3

Table 3: Total phenolic content and flavonoid content of clove bud clove bud oil

Sample extracts	Total phenolic content mg GAE/g extract	Total flavonoid content mg QE/g extract
Clove bud (<i>n</i> -hexane)	34.79±1.4	1.5±0.21
Clove bud (Methanol)	54.56±0.9	3.64±0.19
Clove bud (Ethanol)	57.08±0.8	4.70±0.31
Clove bud oil	71±0.87	

Values are expressed in mean ± SD after triplicate analysis n = 3

Table 4: Quantitative analysis of other phytochemicals in Clove bud

Phytochemical	Total content
Tannins (mg TAE/g extract)	20.34±3.07
Saponins (mg/g extract)	28.2±1.67

Values are expressed mean ± SD after triplicate analysis n = 3

Determination of antioxidant activity

Every antioxidant present in medicinal plants and herbs is differ in chemical nature from the other due to which they act differently and that's why we used 2 different assays to evaluate antioxidant activity.

DPPH radical scavenging assay

It was reported that ethanolic and *n*-hexane extract of *S. aromaticum* showed (66 to 87%) and (43 to 83%) DPPH radical scavenging as compared to synthetic antioxidants BHT which showed (64 to 95%) inhibition at different concentrations (50 to 400 µg/ml) [30]. Clove bud oil showed 85.2% inhibition at 200 µg/ml [31]. *S. aromaticum* oil shows highest antioxidant potential as compared to the extracts of *S. aromaticum* bud. Results are shown in Table 5.

H₂O₂ scavenging activity

Previously clove oil showed (22.9±2.3%) H₂O₂ inhibition as compared to BHT which showed (16.7±4.1%) inhibition at 15 µg/ml concentration [32]. Mainly phenols are responsible for scavenging hydrogen peroxide as *S. aromaticum* is rich in phenolic compounds so both the oil and bud showed significant activity as shown in Table 6.

Determination of Antityrosinase activity

The antityrosinase activity of syzygium aromaticum bud and syzygium aromaticum bud oil was evaluated and the values were compared with the standard kojic acid. Previously determined anti-tyrosinase IC₅₀ value of clove bud oil is 9.6±1.5 [33]. Results showed that antityrosinase IC₅₀ value of clove bud oil is 12.16±2.1 while the *n*-hexane extract of clove

bud oil showed nearest IC₅₀ value to that of clove bud oil. The results are shown in Table no 8.

Table 5: DPPH free radical scavenging activity of Clove bud and Clove bud oil

Test	DPPH radical scavenging activity at 517nm					
	25 ($\mu\text{g/ml}$)	50 ($\mu\text{g/ml}$)	100 ($\mu\text{g/ml}$)	150 ($\mu\text{g/ml}$)	200 ($\mu\text{g/ml}$)	250 ($\mu\text{g/ml}$)
Methanolic (Clove bud)	16.5 \pm 2.5	22.2 \pm 1.7	56 \pm 4.3	66.6 \pm 4	72.94 \pm 4.9	78.41 \pm 1.9
Ethanollic (Clove bud)	22.45 \pm 1.2	63.24 \pm 1.24	71.19 \pm 4.2	76.1 \pm 3.73	82.9 \pm 3.1	87.8 \pm 0.9
<i>n</i> -hexane (Clove bud)	33.19 \pm 2.6	35.14 \pm 2.1	43.92 \pm 3.7	55.98 \pm 4.8	64.28 \pm 1.2	69.87 \pm 0.7
(Clove bud oil)	42.33 \pm 0.4	50.9 \pm 0.02	65.13 \pm 0.04	80.1 \pm 0.1	87.2 \pm 0.83	89.15 \pm 0.8
Ascorbic acid	17.3 \pm 1.4	32.5 \pm 2	75.3 \pm 3.2	90.7 \pm 4.3	91.4 \pm 3.4	96 \pm 0.4

Values are in terms of mean \pm SD after triplicate analysis

Table 6: Free radical H₂O₂ scavenging activity of Clove bud and Clove bud oil

Test	Percentage inhibition of H ₂ O ₂					
	25 ($\mu\text{g/ml}$)	50 ($\mu\text{g/ml}$)	100 ($\mu\text{g/ml}$)	150 ($\mu\text{g/ml}$)	200 ($\mu\text{g/ml}$)	250 ($\mu\text{g/ml}$)
Methanolic (Clove bud)	18.8 \pm 0.45	25.82 \pm 0.8	35.7 \pm 1.1	48.3 \pm 0.12	59.2 \pm 1.5	71 \pm 0.57
Ethanollic (Clove bud)	19 \pm 1.02	33.7 \pm 0.6	43.6 \pm 0.5	54.8 \pm 0.7	66.1 \pm 0.1	78.1 \pm 1.08
<i>n</i> -hexane (Clove bud)	30.1 \pm 0.5	37.7 \pm 0.19	48.9 \pm 0.3	62.7 \pm 0.3	65 \pm 0.5	69.1 \pm 0.09
(Clove bud oil)	39.8 \pm 0.25	46.5 \pm 0.3	57.2 \pm 0.7	71.9 \pm 1.01	82.5 \pm 0.1	92.4 \pm 0.8
BHT	31.3 \pm 0.7	43 \pm 0.5	54.5 \pm 0.60	66.3 \pm 0.3	73.8 \pm 0.9	81.6 \pm 0.1

Values are in terms of mean \pm SD after triplicate analysis

Table 7: IC₅₀ value of different Clove bud and Clove bud oil using DPPH and H₂O₂ radical scavenging assays

Sample	IC ₅₀ value ($\mu\text{g/ml}$)	
	DPPH	H ₂ O ₂
Methanolic (Clove bud)	121.81 \pm 0.9	159.07 \pm 2.3
Ethanollic (Clove bud)	53.16 \pm 1.2	132.36 \pm 1.4
<i>n</i> -hexane (Clove bud)	126.88 \pm 0.45	116.40 \pm 2.02
(Clove bud oil)	41.28 \pm 0.23	65.67 \pm 0.37

Values are in terms of mean \pm SD after triplicate analysis

Table 8: Antityrosinase activity of Clove bud and Clove bud oil

Test	Tyrosinase Inhibition at 517nm					
	50 ($\mu\text{g/ml}$)	100 ($\mu\text{g/ml}$)	150 ($\mu\text{g/ml}$)	200 ($\mu\text{g/ml}$)	250 ($\mu\text{g/ml}$)	IC ₅₀ ($\mu\text{g/ml}$)
Methanolic (Clove bud)	49.7 \pm 0.3	59.5 \pm 0.39	60.3 \pm 2.3	62.5 \pm 1.72	69.81 \pm 2.1	30.1 \pm 0.5
Ethanollic (Clove bud)	49.5 \pm 0.9	52.4 \pm 1.6	54.7 \pm 1.07	57 \pm 2.07	62.8 \pm 3.1	65.3 \pm 1.6
<i>n</i> -hexane (Clove bud)	51.03 \pm 1.9	57.5 \pm 2.7	66.3 \pm 0.56	67.3 \pm 0.76	69.3 \pm 0.98	17.4 \pm 2.6
(Clove bud oil)	55.05 \pm 3.1	64.01 \pm 2.0	77.11 \pm 2.13	83.2 \pm 1.2	88.05 \pm 1.1	12.1 \pm 1.7
Kojic acid	52.7 \pm 2.2	63.3 \pm 0.45	73.3 \pm 0.56	79.2 \pm 0.34	83.5 \pm 1.06	18.3 \pm 1.3

All values are in terms of mean \pm SD after triplicate analysis

Quantification of Quercetin by HPLC

HPLC spectra showed peak at 5.317 for the standard quercetin. The mobile phase used was methanol:water. Both the sample extracts i.e methanolic and ethanolic extracts showed peak at the same retention time for quercetin as compared to that of the standard retention time. This similarity shows the specificity of this method. By comparing the area of sample and standard, the amount of quercetin was calculated in $\mu\text{g/ml}$ from the value of standard Quercetin ($R^2 = 0.8732$) shown in figure 1. Results are shown in table 9 and 10.

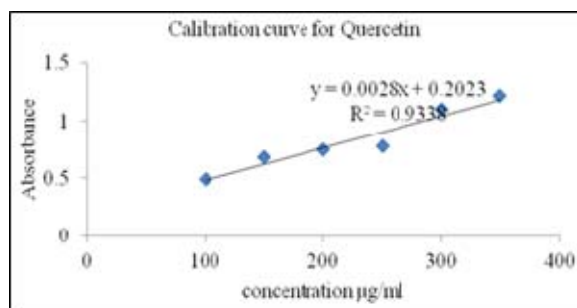


Fig 1: Calibration curve for Quercetin Standard (HPLC)

Table 9: Quercetin quantification in Methanolic extract of clove bud

ID no	Ret. Time	Area	Height	Conc. $\mu\text{g/ml}$
1.	5.317	1084755	30719	172.72

Table 10: Quercetin quantification in Ethanolic extract of clove bud

ID no	Ret. Time	Area	Height	Conc. $\mu\text{g/ml}$
1.	5.317	1302251	41542	191.05

4. Discussion

Proximate analysis of clove bud (*Syzygium aromaticum*) gives us an idea about the dry matter, moisture content, crude protein, crude fiber, crude fat and total ash content which is presented in Table no 1. Clove bud is much importance in cure for many diseases due to the presence of above primary metabolites. Fiber is very effective in the digestion of food so that digestive system works properly. For normal functioning of the digestive system, the optimum concentration of fiber is very important. Fats are generally insoluble in water and soluble in organic solvents. Protein is very important biomolecule and found in every cell in the human body. After water, protein is known to be the most abundant substance in the human body. The presence of all these biomolecules

enhances the medicinal potential of clove buds and makes them effective against many diseases. Due to these biochemical they play a vital role in animal body.

Screening of phytochemicals from clove buds reveals the presence of flavonoids, alkaloids, glycosides, terpenoids, phenols, saponins and tannins as shown in Table no 2. All phytochemicals have their own specificity and functions. Determination of quantity of phenols, flavonoids, tannins and saponins was also carried out. Variable amounts of total phenolic content are found in all extracts of clove buds. Ethanolic extract of clove buds possesses a high concentration of flavonoids than the methanolic and *n*-hexane extract. Tannins play an important role as an antimicrobial agent and this role was explained many times. Antioxidant occurs naturally are very important due to their use in food as well as in the pharmaceutical industry as they have the ability to scavenge free radicals. The antioxidant potential makes medicinal plants and herbs most important as compared to synthetic drugs. So, in order to study the antioxidant potential, different assays are used.

Among all assays, the best and most commonly used assay is the DPPH radical scavenging assay. This method does not require too much time and is the most approachable method. In DPPH, there is the presence of an electron which is the basis of purple colour of DPPH. This electron shows maximum absorption at 517nm. When we add antioxidant such as phenol or flavonoid, these have the ability to give hydrogen to free radical. The DPPH will then be a reduced structure due to lose pairing of hydrogen with it and colour change occurs [34]. Reduction will results in colour change from purple to yellow. Hydrogen peroxide itself is a free radical which is cytotoxic and can cause damage to the cell. It can produce hydroxyl radicals in the cell which can damage cell very badly. So phytochemicals present in plants have the potential to scavenge this free radical by reducing its electrons.

Tyrosinase enzyme is involved in the synthesis of melanin and plays an important role in pigmentation of skin. Tyrosinase is copper associated enzyme and present in plants and animal tissues for the production of melanin and many other pigments from tyrosine by the oxidation process. Certain tyrosinase inhibitors are used in cosmetics industry for whitening of the skin and their potential to suppress melanin production such as Kojic acid.

In summary, clove bud oil displayed a potent antioxidant and antityrosinase activity as compared to the other extracts of clove bud. Further more, the antioxidant activity depends upon the total phenolic and flavonoid content.

5. Conclusion

The present study showed that clove bud and clove bud oil contains many healthnutrients and phytochemicals which are very useful for human health. Due to the presence of total phytochemicals such as phenols and flavonoids, the clove bud showed efficient antioxidant activity. The clove bud oil is much effective due to its capability to reduce free radicals as compared to clove bud. Clove bud showed much effective antityrosinase activity so that it will be much effective if used in skin whitening creams. The present study showed that clove bud and its oil is very important herb spice due to its medicinal properties and health benefits.

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