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ORIGINAL ARTICLE **OPEN ACCESS**

Assessment of Anti-Diabetic and Anti-Oxidant Activity of *Coleus aromaticus* Benth. and *Coleus forskohlii* (Wild.) Briq and Its Phytochemical Analysis

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ABSTRACT
Coleus aromaticus Benth.and Coleus forskohlii (Wild.) Briq.are medicinally important plants widely used for multiple medicinal purposes as antispasmodic, stimulant and stomachic, treatment of headache, fever, epilepsy and dyspepsia and to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis. Therefore, the fresh leaves of C. aromaticus Benth. and roots of C. forskohlii (Wild.) Briq were collected from Thiruvannamalai District, Tamil Nadu, India and analysed for the presence of phytochemical compounds, antioxidant and antidiabetic activities after the extraction of powder sample with chloroform, petroleum ether and ethanol by cold extraction method in the present study. The results for the preliminary phytochemical analysis found with phenolics, saponins, alkaloids, terpenoids, flavonoids tannins, reducing sugars and protiens with higher to moderate level. The quantity of flavonoids and phenols also were assayed and found with 615 ug GAE/g of phenol and 435 ug QE/g of flavonoids and 1182 ug GAE/g of phenol and 529 ug QE/g of flavonoids of ethanonolic extracts of C. aromaticus and C. forskholli respectively. Further, the crude extracts from the plants were tested for it anti-diabetic and antioxidant activity which are found with moderate to higher level. The crude sample of ethanolic extracts from both C. aromatics and C, forskholli were separated with Thin layer chromatography and observed. From the present study it is concluded that the above plant species found with potential bio-compounds that exhibited good antioxidant and antidiabetic activity and may be a good source for natural drug preparation.
Keywords: *Phytochemical analysis, Coleus aromaticus Benth., Coleus forskolli (Wild.) Briq., Anti-oxidant activity, DPPH assay, antidiabetic activity, Glycosilation assay, phenols, flavonoids, TLC.*

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INTRODUCTION

According to Indian *Materia Medica*, about 2000 drugs of natural origin were reported to be medicinally significant and all of them are derived from different traditional system and folklore practices. For centuries, people have been using plants for their therapeutic values and today about 85,000 plants have been documented for therapeutic use globally [36]. The importance of plants as a source of medicine remains of greater relevance even with the current global shift to obtain drugs from plant sources, as a result of which attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy [1]. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer. [11]. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases [44]. Free radicals and reactive oxygen species formation in living cells play a vital role in the origin of life and biological evaluation [10]. India has about 8000 species of medicinal plants among which about 1000 plants have been used in the traditional system of medicine like Ayurveda, Unani and Siddha, in which tribals use 7500 plants species for medicinal purposes [32].
The major diseases occurred in human are diabetic, cancer, Parkinson disease, neurological disorders etc. which are life threatening and drugs available are very expensive. Diabetes mellitus is a major endocrine

disorder affecting nearly 10% of population all over the world. According to the world ethnobotanical information reports, almost 800 plants may possess antidiabetic potential. Traditional and indigenous methods have been employed in order to prevent diabetes mellitus in India since ancient times. Currently available drugs fail to maintain tight glycemic control and are having with various side effects. Therefore, a need to develop newer methods and drugs of plant origin with less side effects is essential.The antioxidant activity of polyphenols is mainly due to their redox properties, which allow them to act as reducing agents [32]. The antioxidant properties of many medicinally important plants were reported for their antioxidant potential by various researchers in laboratory assay method. [19, 27, 39, 33, 30].

Coleus aromaticus(Benth), belonging to the Family Lamiaceae is grown as a household herb in Tamilnadu is a succulent herb with aromatic leaves and it is also popularly known as "Indian Oregano"[8]. The leaves are mainly used for the treatment of stomach disorder, asthma, epilepsy and renal diseases, these are reported to have antioxidant and anti-microbial properties. [6]. *Coleusforskohlii*(Wild.) Briq.is also the most important species of the Coleus Herit genus [21] which is a perennial rootstalk. Forskolin is a compound from *C. forskohlii* has a unique property of activating almost all hormone sensitive adenylatecyclase enzymes in a biological system [12] and is reported to be useful in the treatment of congestive heart failure, glaucoma, asthma and certain type of cancers [4]and anti-inflammatory property [5].

Currently available drugs in the market is fail to maintain tight glycemic control over time and are associated with various side effects. Therefore, there is a need to develop newer treatment strategies such as hypoglycemic agents of plant origin as they are known to have fewer adverse effects. Hence, the present study is planned to investigate on the effect of antioxidant and antidiabetic activity of *Coleus aromaticus Benth.*. (Leaves) and *Coleus forskohlii*(Wild.)Briq (roots) by using different solvents like Ethanol, Chloroform and Petroleum Ether. This study is done through *in vitro* antioxidant and antidiabetic assays and the bioactive compounds were separated by Thin Layer Chromatography (TLC).

MATERIAL AND METHODS

Chemicals and Glassware

Analytical grade chemicals supplied from Loba, Hi-Media, S.D. Fine Chemicals, E. Merck, Qualigens and Sigma Chemicals (U.S.A.) were used in the present investigation. The glassware and glass apparatus used in the present study were from Borosil, Corning and Tarsonc ompany.

Sterilization

Dried glassware and culture media were sterilized in an autoclave at 121^{o} C temperature for 15 min at 15 lb/sq inch pressure.

Plant collection and extraction

Fresh leaves of *Coleus aromaticus Benth.*. and roots of *Coleus forskohlii* (Wild.) Briq were collected from the field of Thiruvannamalai District, Tamil Nadu, India (Fig. 1 and Fig. 2). The leaves and roots were washed with tap water, followed by distilled water to remove soil and other wastes. Then the samples were shade dried and ground into coarse powder by using mixer grinder. From the above sample, 50g of dried leaves and root powder samples were extracted in 250 ml of three different solvents such as chloroform, petroleum ether and ethanol under shaking condition for 24hr. The extracts were filtered through Whatmann No. 1 filter paper and the process was repeated until the solvent becomes colorless. The combined samples of solvent extract were condensed by using of vacuum evaporator and the the samples were labeled and kept in the refrigerator for further study.

Fig. 1 *C. aromaticus* leaves

Fig. 2 *C.forskohlii* roots

ANTIOXIDANT ACTIVITY ASSAYS

DPPH assay: **(2, 2-diphenyl-1-picrylhydrazyl)**

The Radical Scavenging Activity of different extracts was determined by using DPPH assay according to Chang et. Al., (2008)[9]with small modification. The decrease of the absorption at 517nm of the DPPH solution after the addition of the antioxidant was measured in a cuvette containing 2.960 µl of 0.1 m Methanolic DPPH solution mixed with 40 µl of 20 to 200 µg/mL of plant extract. Ascorbic acid and Butylatedhydroxytoluene (BHT) were used as references.

Phosphomolybdenum assay

The antioxidant activity of plant samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al*) (31). An aliquot of 100 µl of sample solution was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM Sodium phosphate and 4 mM Ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The result was expressed as percentage of inhibition.

Reducing power assay

Reducing power assay, the reducing power of the extracts was evaluated according to Oyaizu, 1986 (29) . Different amounts of aqueous extracts were perched in aqueous solvent and diverse with 2.5 ml of 0.2M phosphate buffer (pH 6.6), and 2.5 ml of 1% K3Fe (CN)6. This mixture was incubated at 50o C for 20 min, 2.5 ml of 10% TCA was added to the blend and centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was assorted with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increase in absorbance of the reaction mixture indicates increased reducing power.

Nitric oxide scavenging assay

The nitric oxide scavenging assay for antioxidant acidity of plant samples was carried out after the method of Kumar et. Al., (2008) [23]. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthylethylenediaminehydrochloride was observed at 550nm on spectrophotometer with ascorbic acid as a standard.

Formula: % inhibition = [O.D.of control - O.D. of Test/O.D. of control] X 100

Qualitative phytochemical analysis

Test for Phenolic compounds

Ferric chloride test [26]**:** The extract (50 mg) was dissolved in 5 ml of distilled water. To this few drop of neutral 5% ferric chloride solution was added. A dark green color indicated the presence of phenol.

Test for Glycosides - Borntrager's test[14]

About 50mg of extract was hydrolysed with 5ml of concentrated hydrochloric acid for two hours on a water bath and filtered. To 2ml of filtrate hydrosylate, 3ml of chloroform was added and shaken. Then the chloroform layer was separated and 10% ammonia solution was added to it. Appearance of a pink, red or violet colour in the ammoniacal (lower) phase indicated the presence of glycosides.

Test for Terpenoides - Salkowi test [38]

About 0.5g of the extract was added in 2ml of chloroform and concentrated sulphuric acid (3ml) and was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Test for Flavonoids - Sodium hydroxide test

About 0.5g of extract was dissolved in 5ml of distilled water and filtered. To 2ml of filtrate few quantities of each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids [14].

Mayer's test

Solvent free extract (50mg) was stirred with 2 ml of dilute hydrocholoric acid (1mL HCL + 1mL H_2o) and filtered. The filtrate was tested carefully with various alkaloidal reagents as follow:

Mayer's test: To a 2 mL of filtrate, a drop or two of Mayer's reagent was added by the sides of the test tube. A white creamy precipitate indicated the test as positive.

Mayer's reagent: Mercuric chloride (0.135g) was dissolved in 6mL of water and potassium chloride (0.5g) was dissolved in 1mL of water. The two solutions were mixed and made up to 10mL with water.

Test for Tannins - Neutral Ferric Chloride [14]

About 0.5g of the extract was boiled in 10ml of water in test tube and then filtered. A few drops of 1% ferric chloride were added and observed for blue-green, green or brownish green precipitate indicates the presence of tannins.

Test for Reducing Sugars - Fehling's test [34] The extract(100mg) was dissolved in 5ml of water and filtered. About 1 ml of filtrate was boiled on water bath with 1ml each of Fehling's solution I and II. A red precipitate indicates the presence of reducing sugars.

Test for Saponin - Foam test[22]

The extract(50mg) was diluted with 5ml of distilled water .The suspension was shaken in a graduated cylinder for 15mins.A 2cm layer thick of foam indicates the presence of saponins.

Test for Proteins - Biuret test [16]**:**

The extract(100mg) was dissolved in 10mL of distilled water and filtered through Whatmann No.1 filter paper and the filtrate was collected. To 2mL of filtrate, one drop 2% of copper sulphate solution, 1mL of ethanol(95%) was added, followed by excess of potassium hydroxide pellets(1pellet).Pink color in the ethanolic layer indicates the presence of proteins.

Quantitative Phytochemical analysis

Determination of Flavanoids - Aluminium chloride test [42]

To 1ml of varying concentrations of extract, 3 ml of methanol, 0.2ml of 1 M potassium acetate, 0.2ml of10% aluminium chloride and 5.6ml of distilled water was added and left at room temperature for 30 minutes. Absorbance of the mixture was read at415 nm using UV–VIS spectrophotometer. Calibration curve was prepared using Quercetin as standard.

Determination of Phenolic compound – Folin Ciocaltaeu's method [28]

The total phenol content of the extract was measured at 765 nm by Folin-Ciocalteu reagent [28]. The dilute methanolic extract (0.5 ml of 1:10 g ml-1) and orgallic acid (standard phenolic compound) was mixed with 5ml of Folin-Ciocalteu reagent (1:10 diluted with distilled water) and 4ml of aqueous sodium carbonate (1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by spectrophotometer at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg l-1 solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg per gm of dry mass), which is a common reference compound.

Thin Layer Chromatography

The plant extract was loaded on pre-coated silica plates which were then developed using the solvents methanol, chloroform in the ratio of 0.75:9.25. The spots were identified both in the UV light, far light and in the iodine chamber. Then R_f value was calculated as the ratio of distance travelled by the solute to the distance travelled by the solvent.

Bioautography[13]

The extract which showed DPPH inhibition of more than 90% was examined by thin layer chromatography (TLC) bioautography. The plant extract was applied to pre - coated thin layer chromatography sheet and run with the developing solvent mixture then allow it for dry. After drying the plates, dipped in a 0.2% DPPH reagent in methanol or ethanol and were left for 30 minutes at room temperature. The plates were observed under white light. Antioxidant activity was confirmed when the DPPH purple color changed to yellow.

Statistical Analysis

All the tests in the present study were performed in triplicate to avoid the experimental errors. The data obtained were analysed statistically and expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Samples

Extracts of the *Coleus aromaticus* and *Coleusforskohlii* was obtained from coarse powder of leaves and roots. **Fig. 3** shows the dried coarse powder of leaves collected in glass petriplate. The dried powder was extracted with different kinds of polar and non-polar solvents such as chloroform, ethyl acetate and methanol. These extracted samples were diluted with respective solvents and used to perform various *in vitro* antidiabetic and antioxidant assys.

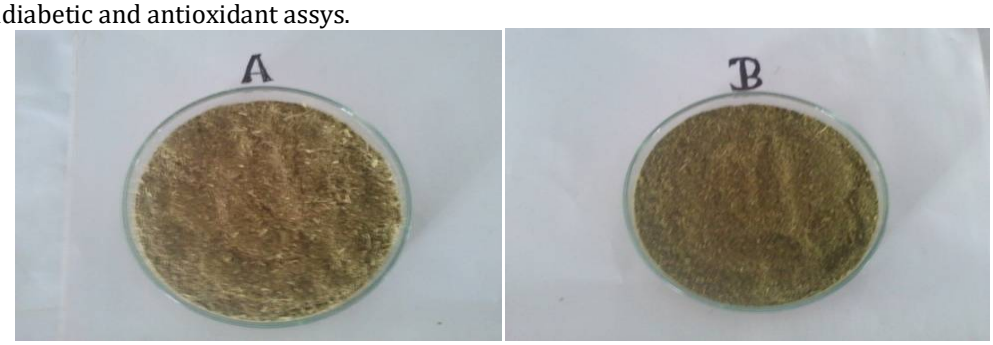


Fig 3: Powdered leaves of *Coleus aromaticus (A)* and roots of *coleus forskohlii (B)*

Inhibition of α-amylase activity:

Alpha amylase is an enzyme that hydrolyses alpha-bonds of alpha linked polysaccharide such as starch to yield high levels of glucose and maltose. Alpha amylase inhibitors bind to alpha- bond of polysaccharide and prevent break down of polysaccharide into mono and disaccharide. *In vitro* inhibitory assay of α-amylase was performed using the Ethanol, Petroleum Ether and Hexane extracts of *Coleus aromaticus* and C*oleus forskohlii*.

The result suggest that Ethanolic extract of *Coleus aromaticus* shows significant inhibitory activity when compare with Chloroform and Petroleum Ether extract with inhibition percentage of 15 – 52%, 16 – 44% and 11 – 38% respectively (concentration 25 - 100µg/ml) (**Table 1**).

Table 1:Glucose Assay Alpha-amylase - Comparison of Ethanol, Chloroform and Petroleum ether extract of *Coleus aromaticus*

S.No	Concentration µg/ml	Percentage of Inhibition- *coleus aromaticus*		
		Ethanol	Chloroform	Petroleum ether
1	25	15.33	16.59	11.83
2	50	36.76	36.38	28.3
3	75	43.27	44.44	34.8
4	100	52.52	44.89	38.97

The result for glucose assay shows that Ethanolic extract of *Coleus forshkolli* shows significant inhibitory activity when compare with Chloroform and Petroleum Ether extract with inhibition percentage of 7 – 40%, 2 – 19% and 10 – 36% respectively (**Table 2**).

Table 2: Glucose Assay Alpha-amylase - Comparison of Ethanol, Chloroform and Petroleum ether extract of *Coleus forshkolli*

S.no	Concentration µg/ml	Percentage of Inhibition- *Coleus forshkolli*		
		Ethanol	Chloroform	Petroleum ether
1	25	7.53	2.58	10.98
2	50	8.54	5.02	22.09
3	75	18.34	9.61	29.01
4	100	40.70	19.22	36.66

The results for non-enzymatic glycosylation of haemoglobin show that Petroleum Ether extract of *Coleus aromaticus* shows significant inhibitory activity when compare with Chloroform and Ethanol extract with inhibition percentage of 7 – 58 % ,11 – 28 % and 5 – 34% respectively (concentration 250 - 1250µg/ml) (**Table 3, Graph 3**).

Table 3: Non Enzymatic Glycosylation of Haemoglobin - *Coleus aromaticus*

S.no	Concentration µg/ml	Percentage of Inhibition- *Coleus aromaticus*		
		Ethanol	Chloroform	Petroleum ether
1	250	5.47	11.13	7.14
2	500	8.60	18.33	11.36
3	750	12.38	22.92	23.15
4	1000	22.47	24.74	43.06
5	1250	34.28	28.24	58.73

The results for non-enzymatic glycosylation of haemoglobin assay for *C.forshkolli* show that Petroleum Ether extract shows significant inhibitory activity as 46.7% to 88.10% when compare with Chloroform and Ethanol extract with inhibition percentage of 7.43 – 43.71 % and 11.86 – 66.66% respectively (concentration 250 - 1250µg/ml) (**Table 4**).

Table 4: **Non Enzymatic Glycosylation of Haemoglobin - *Coleus forshkolli***

S.no	Concentration(µg/ml)	Percentage of Inhibition- *Coleus forshkolli*		
		Ethanol	Chloroform	Petroleum ether
1	250	7.43	11.86	46.72
2	500	17.64	18.75	53.65
3	750	21.95	24.27	77.38
4	1000	35.44	47.82	78.16
5	1250	43.71	66.66	88.10

GLUCOSE UPTAKE BY YEAST CELLS:

Transport of glucose across yeast cell membrane was studied in an *in vitro* system comprising of yeast cells suspended in glucose solution of varying concentration in the presence of the extracts. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The inhibitory activity of Ethanol, Chloroform and Petroleum Ether extract of ***Coleus aromaticus*** and *Coleus forshkolli* were found and compared with the standard drug acarbose. Results showed that the individual extracts of *Coleusa romaticus* and *Coleus forshkolli* having comparable inhibitory activity with the standard drug which is presented in Table 5; and Table 6.

Table 5: Glucose uptake by Yeast Cells – *Coleus aromaticus*

S.No	concentration	Percentage of Glucose uptake by yeast cells		
		Ethanol Extract		
		5mM	10mM	25mM
1	250	9.94	7.24	10.26
2	500	24.32	20.05	20.37
3	750	30.72	37.38	26.56
4	1000	40.58	42.44	27.96
Chloroform Extract				
1	250	32.65	11.00	3.37
2	500	45.06	13.24	21.08
3	750	46.98	29.90	23.01
4	1000	48.32	36.09	26.54
Petroleum Ether Extract				
1	250	24.43	14.13	7.27
2	500	36.96	28.66	10.63
3	750	44.64	32.79	19.11
4	1000	47.06	35.43	21.89

Table 6: Glucose uptake by Yeast Cells – *Coleus forshkolli*

S.No	concentration	Percentage of Glucose uptake by yeast cells		
		Ethanol Extract		
		5mM	10mM	25mM
1	250	28.34	16.26	2.20
2	500	31.64	18.65	9.10
3	750	37.34	27.49	11.87
4	1000	42.38	30.04	22.65
Chloroform Extract				
1	250	3.91	1.79	1.18
2	500	13.38	4.92	3.46
3	750	18.93	10.07	4.20
4	1000	36.45	14.30	8.68
Petroleum Ether Extract				
1	250	44.94	10.38	11.43
2	500	51.48	19.14	15.22
3	750	55.38	25.61	20.42
4	1000	60.15	30.42	24.51

Radical scavenging activity (RSA) of *Coleus aromaticus* and *Coleus forshkolli*
Ethanol, Chloroform and Petroleum Ether extracts (DPPH assay)

From the dose dependent response curve of DPPH radical scavenging activity of leaf and root extracts of *Coleus aromaticus* and *Coleus forshkolli* were observed that the Ethanol extract had higher scavenging activity. In considering this, the Ethanol leaf extract was chosen for further study.

Table 7: DPPH radical scavenging activity - *Coleusa romaticus*

S.no	Concentration µg/ml	Percentage of inhibition		
		Ethanol	Chloroform	Petroleum Ether
1	100	10.2	5.0	5.0
2	200	46.5	23.1	12.4
3	300	60.5	29.9	18.0
4	400	69.7	37.1	24.1
5	500	76.1	61.3	38.9
6	600	78.3	65.0	45.6

DPPH radical scavenging activity of *Coleus forshkolli*

In *Coleus forshkolli, it* was observed that the Petroleum Ether extract had higher scavenging activity when compare with Ethanol and Chloroform. At a concentration of 600 µg/mL. The scavenging activity of Petroleum Ether, Ethanol and Chloroform extract reached 95%, 84% and 50% respectively. The above results were obtained from the the following data (**Table 8**).

Table 8: DPPH radical scavenging activity of *Coleus forshkolli*

S.no	Concentration µg/ml	Percentage of inhibition		
		Ethanol	Chloroform	Petroleum Ether
1	100	27.6	22.3	79
2	200	59	24.1	85.6
3	300	66.4	37	86.7
4	400	80.6	38.3	89.5
5	500	83.3	40.7	91.8
6	600	84.7	50.4	95

The results indicate that the Ethanol extract is more powerful antioxidant in the reduction of phosphomolybdenum complex when compare with Chloroform and Petroleum Ether extracts (**Table 9**).

Table 9: Phosphomolybdenum assay - *Coleusa romaticus*

S.no	Concentration µg/ml	Reducing power		
		Ethanol	Chloroform	Petroleum Ether
1	50	0.171	0.092	0.147
2	100	0.310	0.161	0.208
3	150	0.315	0.171	0.307
4	200	0.358	0.264	0.319
5	250	0.381	0.317	0.336
6	300	0.385	0.418	0.388
7	350	0.576	0.473	0.402

The results indicate that the Chloroform extract is more powerful antioxidant in the reduction of phosphomolybdenum complex when compare with Petroleum Ether and Ethanol extracts (**Table 10**).

Table 10: Phosphomolybdenum assay – *Coleus forshkolli*

S.no	Concentration µg/ml	Reducing power		
		Ethanol	Chloroform	Petroleum Ether
1	50	0.039	0.04	0.028
2	100	0.119	0.281	0.053
3	150	0.127	0.478	0.231
4	200	0.155	0.736	0.291
5	250	0.164	1.258	0.410
6	300	0.198	1.474	0.423
7	350	0.241	1.823	0.456

Nitric oxide scavenging assay

The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the extracts of highest concentration (300 **µg/ml**) for *Coleus aromaticus*. The Nitric Oxide scavenging activity of Ethanol , Chloroform and Petroleum Ether extract reached 57%, 58% and 46% respectively. The above results were obtained from the the following data (**Table 11**).

Table 11: Nitric oxide scavenging activity of *Coleus aromaticus*

S.no	Concentration µg/ml	Percentage of inhibition		
		Ethanol	Chloroform	Petroleum Ether
1	50	8.0	0.6	6.9
2	100	36.0	12.9	15.2
3	150	44.0	20.0	37.9
4	200	49.3	39.1	39.3
5	250	52.7	51.1	44.8
6	300	57.3	58.4	46.9

Nitric oxide scavenging assay - *coleus forshkolli*

The scavenging of NO was found to increase in dose dependent manner. Maximum inhibition of NO was observed in the extracts of highest concentration (300 **µg/ml**) for *coleus forshkolli*. The Nitric Oxide scavenging activity of Ethanol , Chloroform and Petroleum Ether extract reached 83%, 64% and 53% respectively. The above results were obtained from the the following data (**Table 12**).

Table 12: Nitric oxide scavenging activity of *Coleus forshkolli*

S.no	Concentration µg/ml	Percentage of inhibition		
		Ethanol	Chloroform	Petroleum Ether
1	50	72.0	7.7	18.3
2	100	73.7	17.4	21.5
3	150	76.1	34.2	25.8
4	200	76.8	43.2	29.2
5	250	80.8	58.7	40.4
6	300	83.2	64.5	53.5

Fe^{3+} Reducing power assay

The antioxidant activity of Coleus *aromaticus* was evaluated using reducing power assay. The results of anti-oxidant screening were depicted in **Table 13.**The Ethanolic extracts of the plants showed increased ferric reducing power with the increased concentration compare with Chloroform and Petroleum Ether extracts.

Table 13: Fe^{3+} Reducing power assay – *Coleus aromaticus*

S.no	Concentration µg/ml	Absorbance		
		Ethanol	Chloroform	Petroleum Ether
1	50	0.117	0.049	0.118
2	100	0.189	0.055	0.163
3	150	0.210	0.087	0.175
4	200	0.228	0.142	0.189
5	250	0.321	0.158	0.277
6	300	0.336	0.185	0.288

The antioxidant activity of *Coleus forshkolli* was evaluated using reducing power assay. The results of anti-oxidant screening were depicted in **Table 14**. The Ethanolic extracts of the plants showed increased ferric reducing power with the increased concentration compare with Chloroform and Petroleum Ether extracts.

Table 14: Fe^{3+} Reducing power assay – *Coleus forshkolli*

S.no	Concentration µg/ml	Percentage of inhibition		
		Ethanol	Chloroform	Petroleum Ether
1	50	0.032	0.040	0.047
2	100	0.035	0.174	0.055
3	150	0.052	0.270	0.12
4	200	0.093	0.314	0.141
5	250	0.109	0.355	0.219
6	300	0.119	0.402	0.228

QUALITATIVE PATTERN OF PHYTOCHEMICAL COMPOUNDS

The phytochemical screening tests revealed the presence of various plant constituents present in the plants. Among the various phyto-constituents, Alkaloids and flavanoides were fairly present and phenolics, glycosides, tannins, saponins are moderate and no terpenoids and reducing sugars in *C.coleus*. Alkaloids, saponins, phenolics are fairly present, and terpenolids, flavanoids, tannins, reducing sugars found in moderate level and no glycosides in *C. forskholii*(**Table 15).**

Table 15: Qualitative Phytochemical analysis of *Coleus aromaticus* and *Coleus forshkolli*

S.no	Phytochemicals	Results	
		C. aromaticus	***C. forshkolli***
1	phenolic compound	+	++
2	Glycosides	+	_
3	Terpenoids	_	+
4	Flavonoids	++	+
5	Tannins	+	+
6	Reducing sugars	_	+
7	Saponins	+	++
8	Alkalodis	++	++

QUANTITATIVE SCREENING

Detection of flavonoid

The results revealed that the flavonoid was present in significant amount when compared to other constituents. In the extract, the quantity of flavonoid was determined to be 529.17 QE/g and 434.90QE/g in *C. Aromaticus and C. forshkolli* respectively (Table 16).

Detection of phenolic compound

For the *quantitative* analysis of phenolic compounds, it was found that the phenolic compound was fairly low in amount when compared to other constituents. The data obtained was equivalent to the standard Gallic acid and the amount of phenolic compound present was found to be 1182.54 GAE/g, 615.27 GAE/g in *C. aromaticus and C. forshkolli* respectively (Table 16).

Table 16: Quantitative phytochemical estimation

S.No.	Name of the Plants	Phenol	Flavonoid
1	***C.aromaticus***	**1182.54** GAE/g	**529.17** QE/g
2	***C.forshkolli***	**615.27** GAE/g	**434.90** QE/g

SEPARATION OF COMPOUNDS BY THIN LAYER CHROMATOGRAPHY

The solvent system used for chromatogram in the ratio 2:3 (Ethyl Acetate : Toluene v/v), shows better separation of compounds and it is most distinct and clear. This solvent system chosen for further separation and identification of anti-diabetic compounds present in the leaves of *C. aromaticus* and roots of *C. forshkolli*. Under the influence of UV, 5 and 7 compounds were recognized and the Rf value (retention factor) were calculated. Iodine was used as a reaction reagent for the compounds present in the chromatogram and 5 and 7 compounds were recognized in both the species respectively. The Rf values of compounds from *C. aromaticus* were calculated and found as 0.23, 0.40, 0.61, 0.80 and 0.91 as red colour in UV light and brown colour in iodine vapour (**Table 17 and Fig. 4**). The Rf values of the compounds separated from *C. forshkolli* were calculated and found as 0.11, 0.20, 0.28, 0.48, 0.57, 0.74 and 0.88 appeared as red colour in UV light and light brown in iodine vapour (**Table 18 and Fig. 5**).

Table 17: Compounds separated from *Coleus aromaticus* in TLC

S.No	Ratio	Rf value UV long/UV short	Iodine
1	2:3	0.11	-
2	2:3	0.20	-
3	2:3	0.28	-
4	2:3	0.48	-
5	2:3	0.57	-
6	2:3	0.74	-
7	2:3	0.88	-

Fig 4: Sample spots separated from *Coleus aromaticus* in TLC

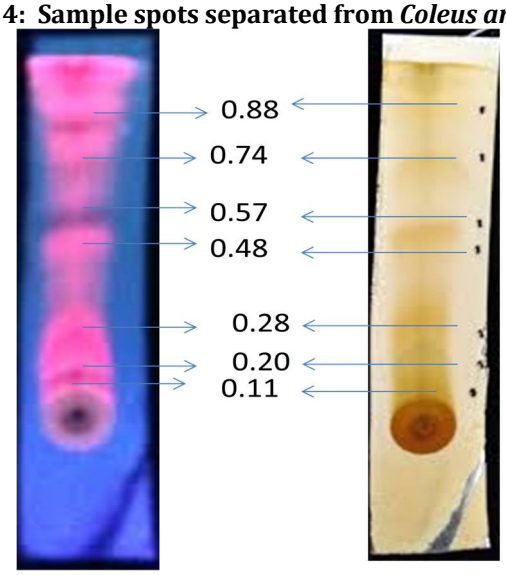
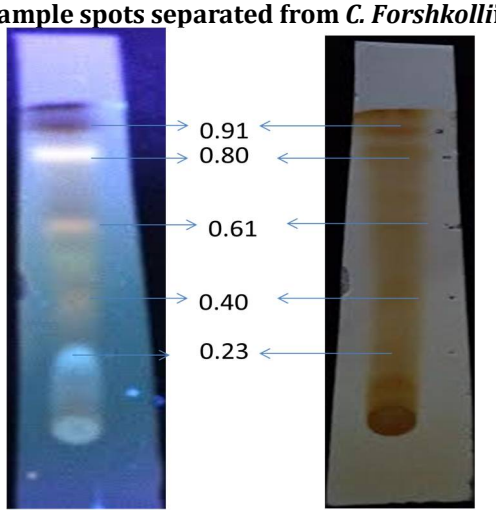


Table 18: Compounds separated from *Coleus forshkolli* in TLC

S.No	Ratio	Rf value UV long/UV short	Iodine
1	2:3	0.23	-
2	2:3	0.40	-
3	2:3	0.61	-
4	2:3	0.80	-
5	2:3	0.91	-

Fig 5: Sample spots separated from *C. Forshkolli*in TLC



DISCUSSION

Plants considered to be medicinally important are having various phytochemicals, many of which are reported to be biologically active compounds and are responsible for exhibiting diverse pharmacological

activities [18]. Researchers from various parts of the world have reported that phytochemicals including alkaloids, glycosides, terpenoids, saponins, phenols and steroids possess enormous antioxidant and free radical scavenging activities [33, 15, 2]. Among the phytochemicals, Phenolics were the main antioxidant components and their total contents were directly proportional to their antioxidant activity [24]. Flavonoids and tannins also were considered to be the most promising polyphenolic compounds [41] and have antioxidant activity in vitro and in vivo [17, 3, 7] reported that the flavonoids are highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various other free radicals implicated in several diseases. The presence of high level phenolics and flavonoids from the medicinally important plant *C. forskohlli* and the higher antioxidant activity of the plant root extracts supports the present findings and are responsible for antioxidant activity. Similarly, phenolics conferring oxidative stress tolerance on plants. Crude extracts of fruits, herbs, vegetable s, cereals and other plant materials rich in phenolics are increasingly being used in the food industry for their antioxidative properties and health benefits.

The present study reveals that tubers of *C.forskohlii* may contain rich in reductone, which could react with free radicals to stabilize and block radical chain reactions. This is an agreement with previous report; the reducing properties are commonly related to reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [20]. The isolation and separation of few biocompounds from the root extracts of *C. forskohlii* indicate the presence of active compound in the plant which can play an important role in the antioxidant and antidiabetic (hypoglycemic actions) activity. The leaf extracts of *C. forskholii* showed significantly high amounts of total polyphenols (23.46 mg g-1 fw), flavones and flavonols (250.8 µg g-1 fw) and high antioxidant activity (12.29 mM g-1fw) were reported by Rasineni et al., [35]. The ethanolic extracts of the leaves of *Vitexnegundo*, rhizomes of *Acorus calamus*, leaves of *Euphorbia hirta* and roots of *Coleus forskohlii* were used in the aboave study found the flavonoids, terpenoids, saponins, tannins and reducing sugars.

The results of the present study showed the presence of phenols, flavonoids in major amounts and other compounds like terpenoids, saponins, tannins and glycosides in small amount. The higher number of phenols and flavonoids may be responsible phytochemicals which might be representing for the potential of *C. forshkolli*. There are many scientific reports on *Coleus* species and the compounds are isolated and in treatment for various ailements. However, the antimicrobial and antidiabetic compounds are seldom produced from the above plants. To conclude, the plant species *C. aromaticus* and *C. forshkolli* are potential in scavenging free radicals, Fe3+ Reducing power, Nitric Oxide scavenging activity and in chelating metal ions and also hypoglycemic activity. Hence, these plants could be considered as an efficient source of therapeutic drugs against free radical induced cancer and also anti-diabetic disease.

CONCLUSION

The present study reveals on the in vitro anti-diabetic effect of *C. aromaticus* and *C. forshkolli* provides promising results for the utilization of the extracts of the plants as a formulation for the drug to treat diabetes. The leaf and root extracts of *C. aromaticus* and *C. forshkolli* are exhibited higher phenolics and flavonoid content, which have significant antioxidant and free radical scavenging activities. Moreover, *C. aromaticus*and *C. forshkolli* root extract exerted potent antioxidant activity through its reducing power ability. The antioxidant activity `of herbal extracts is of considerable interest in the food industry with significant biological potential to be used as alternatives to synthetic and conventional food preservation systems. Thepresent findings indicate that the leaf and root extract of above plants could be a source of natural antioxidants for use in various purposes. Further studies are required to isolate the biologically active elements of *C. aromaticus* and *C. forshkolli* and ascertain in vivo antioxidant and antidiabetic model to exact mode of action.

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