

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT PROPERTIES OF THREE COMMON MEDICINAL HERBS

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1. Introduction

Chemical entities with one or more unpaired electrons in atomic or molecular orbitals are referred to be free radicals (**Halliwell and Gutteridge, 1999**). Free radicals have the ability to damage healthy cells in the body, stripping them of their structural integrity. They play a part in the aging process as well as degenerative illnesses of the aging process, including cancer, heart disease, cataracts, weakened immune systems, and brain dysfunction. In total, at least fifty different illnesses have been linked to the pathophysiology of free radicals.

Antioxidants are necessary to maintain optimal cellular and systemic health and well-being because they can neutralize free radicals before they attack cells. Two main mechanisms of action have been proposed for antioxidants: the first is a chain-breaking mechanism in which the primary antioxidants donate electrons to the free radicals in the system, such as lipid radicals; the second mechanism involves the removal of ROS (reactive oxygen species) and RNS (reactive nitrogen species) initiator by quenching chain initiator catalyst (**Sies 1997**). Antioxidants can be either enzymatic or nonenzymatic; examples of enzymatic antioxidants are catalase, superoxide dismutase, and glutathione, which catalyze the neutralization of many types of free radicals (**Jacob R.A, 1995**), while the nonenzymatic antioxidants include Vitamin C, selenium, vitamin E, carotenoids, and polyphenols. Antioxidants are shown to be essential in preventing heart disease, cancer, DNA deterioration, lung illness, and neurological disorders (**Percival M., 1998**). This evidence is growing.

The medicinal use of plants as antioxidants to lessen oxidative tissue damage has garnered attention recently. Rich in phenolic compounds such as flavonoids, plants, herbs, and spices have been shown to possess antioxidant qualities that help them fight cancer and have anti-inflammatory, antiallergenic, antiviral, and antiaging effects (**Aqil et al., 2006**). In India, people from diverse ethnic backgrounds and geographical locations have their own unique cultures, customs, and medical knowledge. Throughout human history, plants have been used to treat illnesses and have also served as a catalyst for the development of novel, side-effect-free pharmaceuticals. Previous research on antioxidant activity in plants primarily concentrated on vitamin content; however, phytochemicals that have been linked to increased levels of antioxidant activity have been the subject of extensive investigation to improve disease conditions. As a result, an appropriate technique was required to measure an agent's antioxidant capacity using in vitro antioxidant assays that call for a small amount of the material.

A study was conducted on three common plants, namely *Euphorbia hirta* Linn. (Leaves), *Centella asiatica* L. (Leaves), and *Calendula officinalis* Linn. (Flower). An aromatic, upright annual herb of the Asteraceae family is called *Calendula officinalis* Linn. (Thulukka samanthi in Tamil). According to **Parente et al. (2012)**, it is frequently used to treat minor wounds, skin infections, burns, bee stings, sunburns, and other conditions. Appearing in the Apiaceae family, *Centella asiatica* L. (Tamil name: vallarai). As per **Vijayashalini and Abirami (2018)**, it treats skin infections

and illnesses, inflammation, and persistent ulcers. The Euphorbiaceae family includes *Euphorbia hirta* Linn. (Tamil name: Amman pachcharisi). Conjunctivitis, wounds, and sores are treated with it as an antiseptic. The herb is known for its analgesic properties, which can be used to relieve colic, rheumatism, intense headaches, and toothaches (Kumar and others, 2010). The current investigation therefore sought to assess the antioxidant and phytochemical screening capabilities.

2. Material and Methods

2.1. Collection of Plant Material

The plant materials utilized in this study were gathered in and around Trichy. They included *Calendula officinalis* Linn. (Flower), *Centella asiatica* L. (Leaves), and *Euphorbia hirta* Linn. (Leaves) (Voucher number: BISH0000619230; FRI50032; 11077). Rev. Fr. Dr. John Britto, the taxonomist and director of the Rapinart Herbarium at St. Joseph College in Trichy, identified and verified the materials.

2.2. Preparation of Aqueous Extracts

After being shade dried, the leaves of *Euphorbia hirta* Linn., *Centella asiatica* L., and *Calendula officinalis* Linn. were coarsely powdered using an electrical blender. A mixture of 200g of each plant material and 1.2 litre of water was prepared. Subsequently, it was reduced to one-third by boiling and filtered. Dryness was reached by evaporating the filtrate. Additional research was done on the extracted paste form.

2.3. Preliminary Phytochemical Screening

Using standard procedures, alkaloids, glycosides, saponins, tannins, terpenoids, phenolic compounds, protein, and carbohydrates were tested for various phytoconstituents in the dry powder and aqueous extract of *Calendula officinalis* Linn. (Flower), *Centella asiatica* L. (Leaves), and *Euphorbia hirta* Linn. (Leaves) (Brindha et al., 1981).

2.4. In vitro Antioxidant Activity

The DPPH radical scavenging assay (Brand Williams et al., 2000), the ABTS radical scavenging assay (Robert Re et al., 1999), the reducing power assay (Manmohan Sngal et al., 2011), and the nitric oxide radical inhibition assay (Sreejayan and Rao, 1996) were used to assess the free radical scavenging activity of the aqueous extract of *Calendula officinalis* Linn. (Flower), *Centella asiatica* L. (Leaves), and *Euphorbia hirta* Linn. (Leaves).

2.4.1. DPPH radical scavenging assay (Brand williums et al., 2000)

DPPH was used to assess the chosen plants ability to scavenge free radicals. A 200µM DPPH solution was made with 95% methanol. Five test tubes containing 100 and 1000 µg/ml of the stock plant extract solution were used. Using a spectrophotometer, the absorbance was measured at 517 nm after 10 minutes of incubation of 0.5 ml of freshly prepared DPPH solution with the test drug. The reference was standard ascorbic acid.

Calculation

% scavenging of the DPPH free radical was measured using following equation

2.4.2. ABTS radical scavenging assay (Robert Re et al., 1999)

Different concentrations of standard ascorbic acid and plant extract (ranging from 50 to 250 µg) were added to a set of test tubes. Phosphate buffer was used to create a 2.5 ml volume with various concentrations. For the Control, two milliliters of phosphate buffer were added. Then, each test tube received 0.3 milliliter of ABTS solution. At 734 nm, the solution was immediately read.

Calculation:

The percentage scavenging activity of plant extract on ABTS

$$I (\%) = (A_0 - A_1) / A_0 \times 100$$

2.4.3. Reducing power assay (ManmohanSinghalet al., 2011)

1 ml of varying concentrations (100-500 µg/ml) of aqueous plant extracts was mixed with 2.5 ml phosphate buffer and 2.5 ml of potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Aliquots of 2.5 ml of trichloroacetic acid were added to the mixture, which was then centrifuged at 3000 rpm for 10min. The upper layer of the solution (2.5 ml) was mixed with 0.5ml of freshly prepared ferric chloride solution was added and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicates increase in reducing power.

2.4.4. Nitric oxide radical inhibition assay (Sreejayan and Rao, 1996)

Aqueous plant extracts at varying concentrations were incubated with sodium nitro prusside solution, with ascorbic acid serving as the standard. Ascorbic acid was added to 5 ml of 0.025M phosphate buffer and various concentrations of plant extracts are present in the volume. The tubes spent three hours being incubated at 25°C. While sodium nitro prusside and buffer were used in equal amounts, the test compounds were not used in the control experiment. Griess reagent (0.5ml) was combined with 0.5ml of the reaction mixture after three hours. A 546 nm absorbance measurement was made.

Calculation:

$$\text{NO}_2 \text{ Scavenging activity (\%)} = (A_{\text{Control}} - A_{\text{Test}}) / A_{\text{Control}} \times 100$$

3.Result and Discussion

Biologically active naturally occurring chemical substances called phytochemicals are present in plants. These compounds also give plants their flavor, color, and aroma. Their primary function is to shield the plants from disease and harm from biotic and abiotic stresses. These plant compounds, also known as phytochemicals, are created to shield plant cells from environmental dangers like UV radiation, pathogenic invasion, drought, stress, and pollution. Presently, they play a crucial role in preserving human health and preventing disease, which eventually improves quality of life.

There is a wide-ranging dietary phytochemical that are found and obtained from edible fungi, vegetables, fruits, nuts, whole grains, seeds, herbs, spices and legumes. They are accumulated in various plant portions such as in the seeds, roots, stems, leaves, flowers, and fruits.

Table 1a. Preliminary Phytochemical analysis of *Calendula officinalis* Linn.

S.No	Test	Drug Powder	Aqueous Extract
1	Saponin	-	-
2	Tannin	+	+
3	Sterol	+	+
4	Terpenoids	+	+
5	Flavonoid	+	+
6	Coumarin	+	-
7	Quinines	-	+
9	Alkaloids	+	+
10	Glycosides	+	+
11	Sugar	-	-
12	Phenols	+	+

Table 1a shows the results of the preliminary phytochemical analysis of *Calendula Officinalis* Linn. It was discovered that the medicinal powder and watery extract of *Calendula Officinalis* Linn. contained sterols, alkaloids, phenols, glycosides, flavonoids, and terpenoids. The antioxidant properties of *Calendula Officinalis* Linn. may have been caused by the presence of flavonoids, terpenoids, and phenols.

Table 1b. Preliminary Phytochemical analysis of *Centella asiatica* Linn.

S.No	Test	Drug Powder	Aqueous Extract
1	Saponin	+	+
2	Tannin	-	-
3	Sterol	+	+
4	Terpenoids	-	-
5	Flavonoid	+	+
6	Coumarin	+	-
7	Quinines	-	+
9	Alkaloids	+	+
10	Glycosides	+	+
11	Sugar	-	-
12	Phenols	+	+

Result obtained for qualitative screening of phytochemicals in the leaves of *C. asiatica* L. and its bioactivity are presented in **Table 1b**. The drug powder and aqueous extracts of *C. asiatica* L. showed the presence of major phytoconstituents such as phenol, alkaloids, flavonoids, sterol and saponin which may be responsible for pharmacological actions and medicinal properties.

Table 1c. Preliminary Phytochemical analysis of *Euphorbia hirta* Linn.

S.No	Test	Drug Powder	Aqueous Extract
1	Saponin	-	-
2	Tannin	+	+
3	Sterol	+	+
4	Terpenoids	+	+
5	Flavonoid	+	+
6	Coumarin	+	-
7	Quinines	-	+
9	Alkaloids	+	+
10	Glycosides	+	+
11	Sugar	-	-
12	Phenols	+	+

The results of the phytochemical screening of drug powder and aqueous extract of leaves of *Euphorbia hirta* Linn. showed the presence of alkaloids,phenol, Flavonoids,terpenoids,sterol and tannin. The presence of these compounds suggests that *Euphorbia hirta* Linn. having various medicinal properties which can be used in the treatment of various diseases.

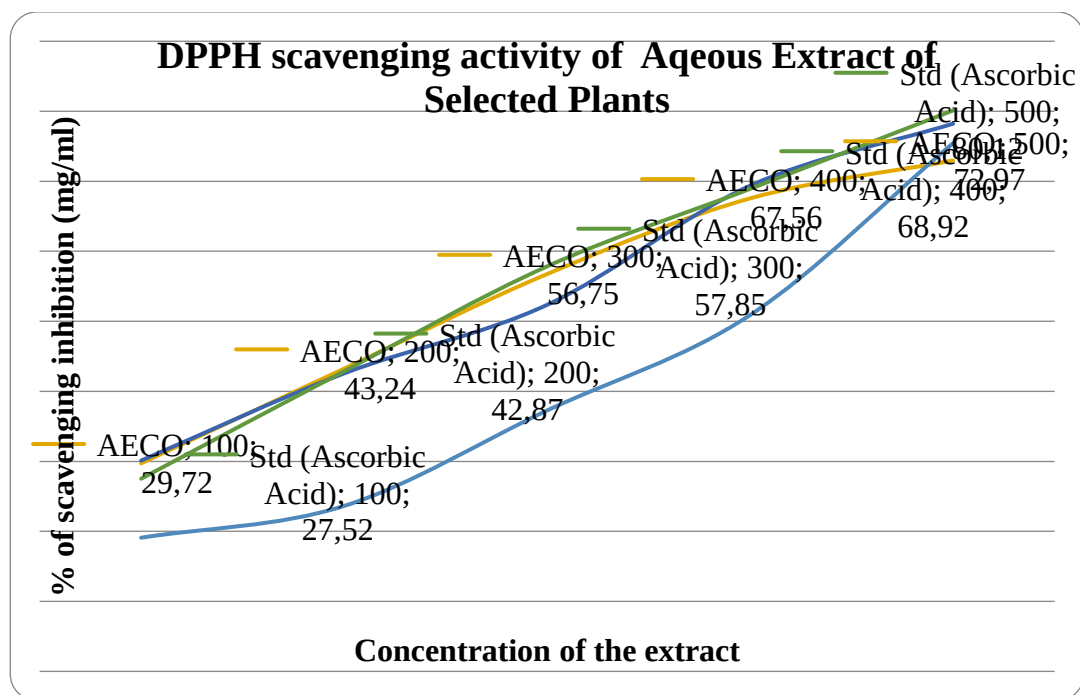
Based on the preliminary phytochemical results, alkaloids,phenol, glycosides and flavonoids was found in both drug powder and aqueous extract of *C. officinalis* , *Centella asiatica* L. and *Euphorbia hirta* Linn. The saponin was totally absent in *C. officinalis* and *Euphorbia hirta* Linn.(Table 1a,b and c).

In Vitro Antioxidant Activity

Owing to the complexity of phytoconstituents, the antioxidant activity of herbal drugs or plant extracts cannot be assessed by a single assay; thus, the study must use widely recognized assays to assess the antioxidant activity of plant extracts. Numerous techniques for assessing antioxidant activity and elucidating the mechanism of antioxidants have been developed. The assays most frequently used to assess the antioxidant activity of plant extracts are DPPH assay, ABTS, Nitric oxide, superoxide anion hydroxyl radical, and AAPH scavenging. Therefore, the goal of the current study is to demonstrate the chosen plants' capacity for antioxidants.

Table 2: Effect of aqueous extracts of *calendula officinalis* linn., *Centella asiatica* l. and *euphorbia Hirta* linn. on DPPH scavenging activity

Concentration of plant extract and standard (µg)	DPPH Radical scavenging Assay(%)			
	AECO	AECA	AEEH	Std (Ascorbic Acid)
100	29.72	19.09	30.11	27.52
200	43.24	23.63	42.56	42.87
300	56.75	37.27	52.35	57.85
400	67.56	50.9	69.37	68.92
500	72.97	75.45	78.23	80.12

**Figure 1.** Effect of aqueous extracts of *calendula officinalis* linn., *centella asiatica* l. and *euphorbia hirta* linn. on dpph scavenging activity

DPPH is a relatively stable free radical with a nitrogen center that can readily take on an electron or a hydrogen radical to transform into a stable diamagnetic molecule. The concentration of the plant extract was raised to enhance this activity. The foundation of the DPPH antioxidant assay is the ability of the stable free radical 1,1,di phenyl -2 picryl hydrazyl to become decolorized when antioxidants are present. An odd electron found in the DPPH radical is what causes the absorbance at 517 nm as well as the noticeable deep purple color. Changes in absorbance provide a quantitative measure of the decolorization of DPPH, which occurs when it accepts an electron donated by an

antioxidant compound. The ability of substances or plant extracts to function as free radical scavengers has been extensively tested using this kind of reactivity. In the current study, the DPPH radical scavenging activity of aqueous extracts of *Calendula officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. was studied and the levels are given in **Table 2 and figure 1**. The DPPH free radical scavenging ability, the aqueous extracts of those plants showed strong radical scavenging activity with percentage of 72.97% -AECO, AECA- 75.45 %, AEEH-78.23% at higher concentration (500 µg/ml). The order of scavenging activity was maximum in *Euphorbia hirta* Linn. followed by and *C. officinalis*, *Centella asiatica* L. The values are also comparable with standard ascorbic acid at the same concentration (**Table 1a & Fig.1a**). This result of the present study suggested that the *C. officinalis*, *asiatica* L. and *Euphorbia hirta* Linn. contain flavonoids that can donate electron/hydrogen easily.

Table 3. Effect of Aqueous Extracts Of *Calendula Officinalis* Linn., *Centella Asiatica* L. And *Euphorbia Hirta* Linn. on Abts Radical Scavenging Activity

Concentration of plant extract and standard (µg)	ABTS Radical scavenging Assay(%)			
	AECO	AECA	AEEH	Std (Ascorbic Acid)
100	29.56	15.62	22.31	29.32
200	43.91	31.25	32.07	42.37
300	58.26	37.5	46.7	56.25
400	72.6	46.87	49.14	70.3
500	82.6	65.62	66.2	82.1

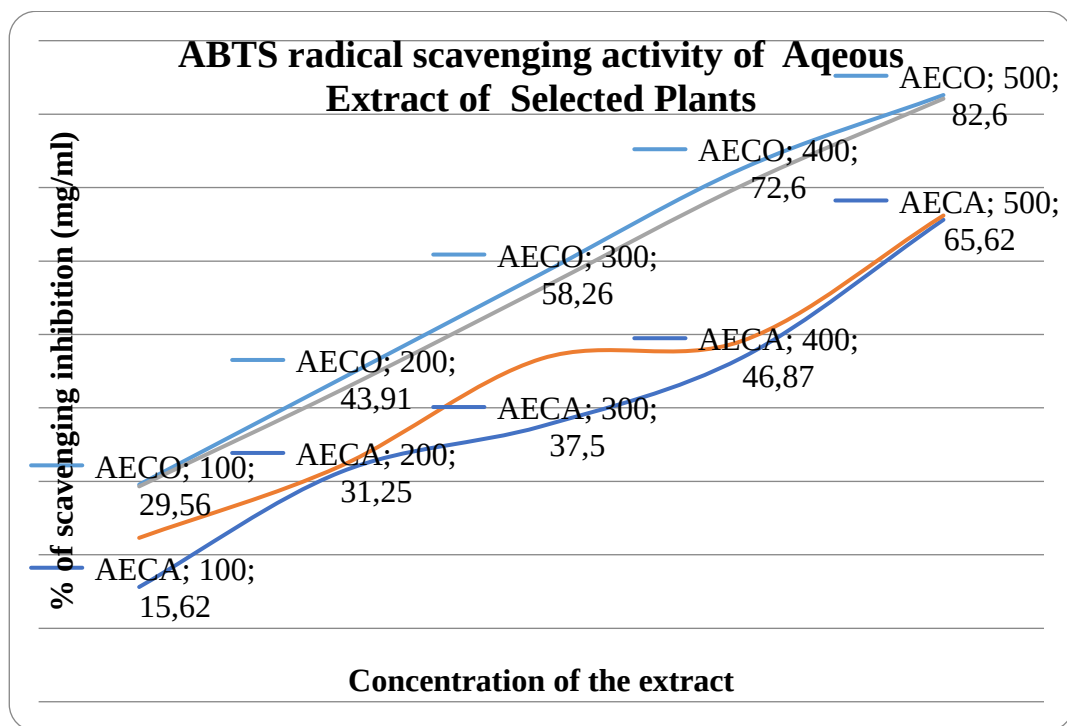


Figure 2. EFFECT OF AQUEOUS EXTRACTS OF *Calendula officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. ON ABTS RADICAL SCAVENGING ACTIVITY

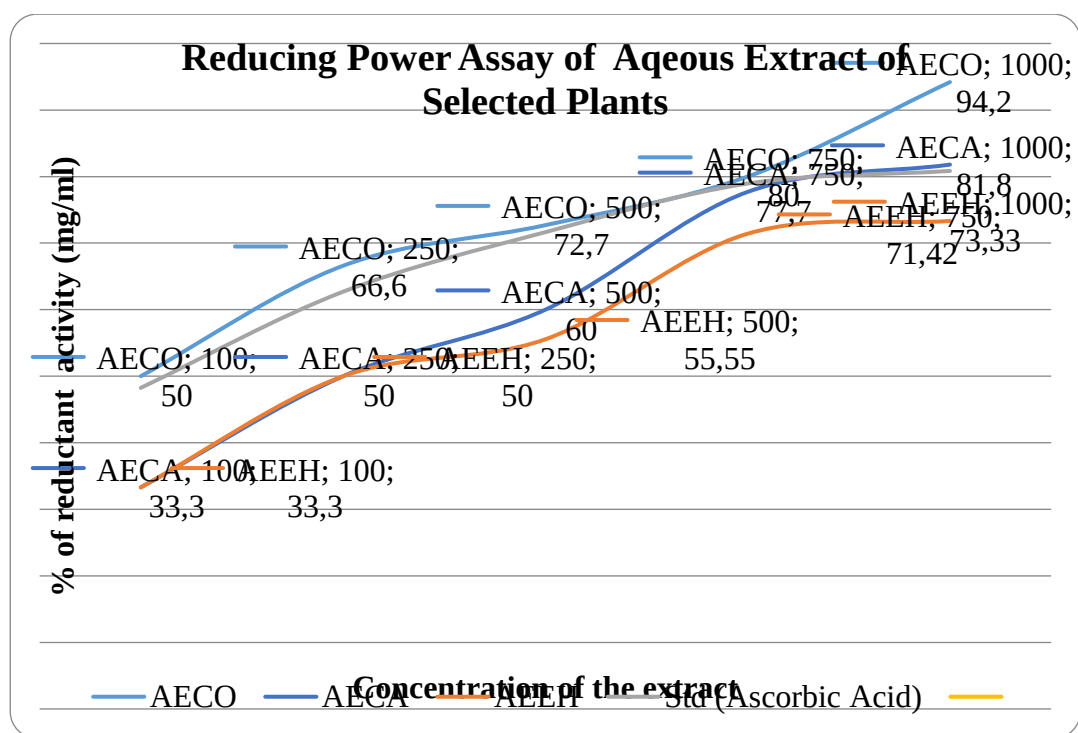
Compared to other antioxidant assays, the ABTS assay is more suitable for evaluating the antiradical capacity of both hydrophilic and lipophilic antioxidants because it can be used in both organic and aqueous solvent systems. The ABTS radical cation can be reduced by antioxidants, which is the basis for this method (Pellegrini *et al.*, 1999). The basis of the ABTS assay is the way antioxidants inhibit the radical cation, ABTS⁺, which has a distinctive wavelength of 734 nm. The method's basic idea is that ABTS and potassium persulphate react to form the blue-green chromogen known as ABTS radical cation (ABTS⁺). The color radical is changed into a colorless state when an antioxidant reductant is present (Sreejayan and Rao, 1996). Aqueous extracts of *Euphorbia hirta* Linn, *Centella asiatica* L., and *C. officinalis* Linn. were used in this study.

From the table 3 and Fig.2, The observed ABTS⁺ cation radical showed a significant increase, which could be attributed to scavenging activity. of *C. officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. and the inhibition is dependent on the concentration of plant extracts. The maximum activity was noted at the higher concentration of (500 µg) aqueous extracts of the selected plants. Among the four plants tested, the aqueous extracts of *C. officinalis* has better ABTS⁺ cation radical scavenging activity. The aqueous extracts of *C. officinalis*, *Centella asiatica* L. and *Euphorbia hirta* Linn. at concentration of 500 µg exhibited a maximum inhibition of ABTS⁺ cation formation at a percentage of 82.60%, 65.62%, 66.20% respectively.

Certain chemical compounds have the ability to inhibit the potassium persulfate activity, which in turn reduces the amount of ABTS^{•+} produced. According to the aforementioned findings, flavonoids with greater ability to lower free radicals (ABTS^{•+}) are present in the aqueous and ethanol extracts of *calendula officinalis* Linn., *Centella asiatica* L., and *Euphorbia hirta* Linn. These extracts have the highest antioxidant activity.

Table 4. EFFECT OF AQUEOUS EXTRACTS OF *Calendula officinalis* Linn., *Centella asiatica* L. And *Euphorbia hirta* Linn. ON REDUCING POWER ASSAY

Concentration of extract and standard (µg)	Reducing Power Assay(%)			
	AECO	AECA	AEEH	Std (Ascorbic Acid)
100	50	33.3	33.3	48.25
250	66.6	50	50	62.7
500	72.7	60	55.55	71.65
750	80	77.7	71.42	78.95
1000	94.2	81.8	73.33	80.85

**Figure 3.** EFFECT OF AQUEOUS EXTRACTS OF *Calendula officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. ON REDUCING POWER ASSAY

The reduction of the Fe^{3+} -ferricyanide complex to the ferrous (Fe^{2+}) ions creates a chromogenic complex, which is measured at 700 nm of the resulting blue-green colored solution. This absorbance is proportional to the amount of Fe^{2+} in the mixture. The reducing power assay measures the electron donating capacity of reducing agents, i.e. antioxidants. The ferric reducing antioxidant power of the aqueous extracts of *Euphorbia hirta* Linn., *Centella asiatica* L., and *C. officinalis* was assessed; the results are shown in Table 4 and Figure 3. The current investigation

shows that certain plant extracts have the capacity to lower ferric ions. For *C. officinalis*, *Centella asiatica* L., and *Euphorbia hirta* Linn., the ferric reducing antioxidant value measured at 1000 µg was regarded as 100%. It could be found that aqueous extracts of all the selected plants exhibited highest activity and the values are 94.2% (*C. officinalis*) followed by 81.8% (*Centella asiatica* L.) and 73.33% (*Euphorbia hirta* Linn.).

From the above results, it was suggested that the aqueous extracts of *C. officinalis*, *Centella asiatica* L. and *Euphorbia hirta* Linn. have significant antioxidant activity which could be attributed to the presence of flavanoids and phenolic content.

Table 5. EFFECT OF AQUEOUS EXTRACTS OF *Calendula officinalis* Linn., *Centella asiatica* L. and *Euphorbia hirta* Linn. ON NITRIC OXIDE INHIBITION ASSAY

Concentration of plant extract and standard (µg)	NO Inhibition assay (%)			
	AECO	AECA	AEEH	Std (Ascorbic Acid)
100	27.85	25.7	27.25	28.02
200	30.38	28.23	29.78	30.4
300	38.87	36.72	38.27	39.45
400	50.36	48.21	49.76	50.7
500	79.4	77.25	78.8	79.25

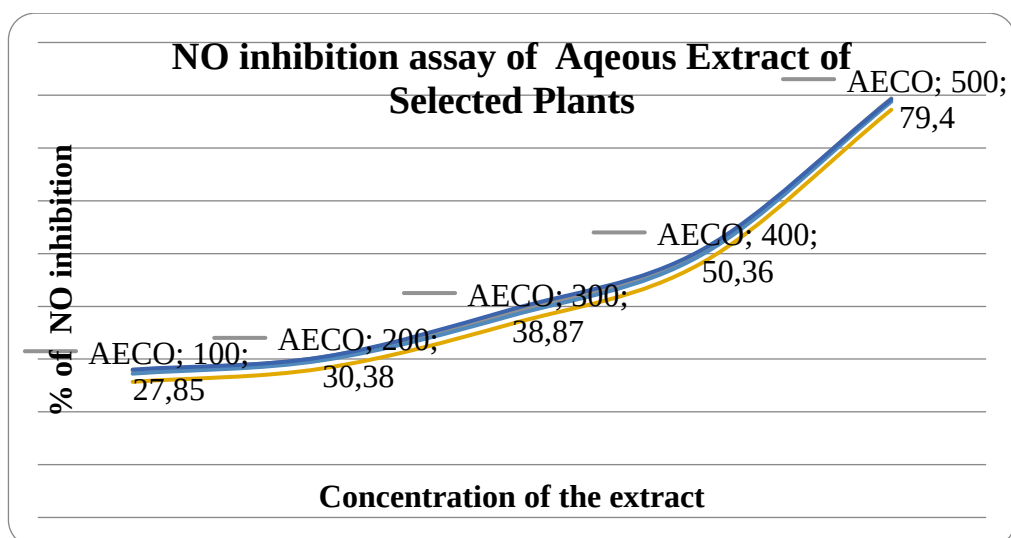


Figure 4. EFFECT OF AQUEOUS EXTRACTS OF *Calendula officinalis* Linn., *Centella asiatica* L. And *Euphorbia hirta* Linn. ON NITRIC OXIDE INHIBITION ASSAY

Strong pleiotropic inhibitors of physiological processes, including neuronal signaling, smooth muscle relaxation, platelet aggregation inhibition, and cell-mediated toxicity regulation, are exhibited by nitric oxide (NO). As an effector molecule in various biological systems, such as neuronal messenger, vasodilatation, and antimicrobial and antitumor activities, it is a diffusible free radical. Nitrate and peroxy nitrite anions, which function as free radicals, are produced when oxygen combines with excess nitric oxide. The nitric oxide in this study was produced by the reaction of sodium nitro prusside with oxygen to form nitrite, which then reacts with griss reagent at physiological PH. The nitrite was then incubated with varying concentrations of plant extracts to form a chromophore, which was measured at 546 nm.

Table 5 & Figure 4. shows the measure of nitric oxide scavenging activity of aqueous extracts of *C. officinalis*, *Centella asiatica* L. and *Euphorbia hirta* Linn. The aqueous extracts of selected plant sources produced moderate to good nitric oxide scavenging activity and the activity was found to be less significant with respect to the inhibition observed at concentration of 100,200 and 300 µg/ ml concentration, while at higher concentration it was found to be highly significant(400,500 µg/ ml). The aqueous extracts of all the selected plants at higher concentration(500 µg) exhibited highest activity and the values are 79.40% (*C. officinalis*) followed by 77.25% (*Centella asiatica* L),78.8% (*Euphorbia hirta* Linn.). Among the three selected plants, *C. officinalis* had significant nitric oxide radical scavenging activity.

From the above results, it was noticed that the aqueous extracts of *C. officinalis*, *Centella asiatica* L. and *Euphorbia hirta* Linn. showed high flavonoid content which has contributed directly to the antioxidant activity by neutralising the free radicals.

Summary

From the results of the present study was concluded that the aqueous extract of flowers of *Calendula officinalis* Linn., leaves of *Centella asiatica* L. and leaves of *Euphorbia hirta* Linn. exhibited optimum levels of antioxidant activities in all the models tested. The antioxidants efficacy of different solvent extract from these plants will be evaluated in the future. And also further in depth studies coupled with *in vivo* model resulted in the development of safe, efficacious and cost effective natural antioxidant.

Scientific Ethics Declaration

The authors say that they are responsible for the scientific, moral, and legal aspects of this chapter, which is published in Current Studies in Health and Life Sciences 2023.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this chapter.

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