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# *In vitro* antioxidant activities of *Cleome monophylla* L. A sporadic medicinal herb

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**Abstract**
*Cleome monophylla* is a common weed in cultivated land in hills, Ethanolic extract of *Cleome monophylla* was subjected to *in vitro* antioxidant assays such as 2, 2-diphenyl-1-picryl hydrazyl radical (DPPH), Super oxide radicals (SOS) and Nitric oxide radicals (NOS). Scavenging activities of the DPPH radical by the ethanolic extract of *Cleome monophylla* was found to be moderate as compared to the standard ascorbic acid. The $IC_{50}$ value of plant extract was 81.42 µg/ml. The superoxide radical scavenging ability of ethanol extract of *Cleome monophylla* (49.88 µg/ml as $IC_{50}$) was competent and slightly lower than standard ascorbic acid (24.19 µg/ml). The ethanol extract of *Cleome monophylla* showed a high forceful free radical scavenging activity in the nitric oxide assay at 40 µg/ml concentration.

**Keywords:** *Cleome monophylla,* DPPH, superoxide, nitric oxide scavenging activities, ascorbic acid

## Introduction

Medicinal plants are nature's gift to man. These medicinal plants have the ability to cure all diseases that affect humans. Humans have been using herbs to protect themselves from disease since time immemorial. Herbs are one of the foremost traditional Indian medicines in Siddha and Ayurveda. The side effects of using herbs are minimal and also inexpensive, knowing the benefits of the herbs, our Indian people use it in their daily diet. In the recent studies, the medicinal values of plants have been explored due to their effective antioxidant properties. Antioxidants are an inhibitor of the process of oxidation even at relatively small concentration and thus have diverse physiological role in the body and the antioxidant constituents of the plant material act as radical scavenger and facilitate in converting the radicals to less reactive species (Kumar, 2014) [1]. The formation of free radical is usually controlled naturally by antioxidants (Nagarajappa *et al*., 2015) [2]. The plant origin antioxidants with free radical scavenging properties may have great therapeutic importance in free radical mediated diseases as reported by Nagulendran *et al*. (2007) [3]. Human body is equipped with certain antioxidants such as superoxide dismutase and catalase which can counteract the deleterious actions of the reactive oxygen species and protect tissues against cellular and molecular damage (Singh *et al*., 2003) [4]. Usually the therapeutic effects of several plants and vegetables, used in traditional medicine are recognized to their antioxidant compounds. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids. Plant based antioxidants are now preferred to the synthetic ones because of safety concerns (Akinmoladun *et al*., 2007) [5]. Hence, in the present study, *Cleome monophylla* which is used by the traditional healers for many diseases is investigated for antioxidant activities.

A medicinal herb *Cleome monophylla* L. belongs to the family Capparidaceae was selected to analyse *in vitro* antioxidant activities. *Cleome monophylla* is a common weed in cultivated land in hills, It is an only single leaved species in *Cleome* (compound leaf) genus. It is an annual herb, aerial, erect, branched and has an unpleasant smell with acrid taste. It is commonly known as "Spindle pod" and Tamil vernacular names are "Naai kadugu", "Kaatu kadugu" and "Elluku sakkalathi" and it is widely distributed in India, Sri Lanka, tropical and subtropical regions of Africa and Nigeria. *Cleome monophylla* L. is used to treat different ailments such as the whole plant paste is externally applied for swellings, crushed leaves are applied for head ache, the leaves and seeds alone or mixture are applied to ulcers, boils and to prevent the formation of pus (Kannan *et al*., 2016) [6], bile enlargement (Bandhana and Sudhanshu, 2000) [7]. The leaf extract possess anti HIV - 1 reverse transcriptase activity (Hurinathan, 2013) [8] and an essential oil extracted from the seeds possess repellent activity (Ndungu *et al*., 1995) [9].

## Materials and Methods

### DPPH* radical scavenging activity (Blois, 1958) [10]

The plant extract at different concentrations (40 - 1000 µg) was taken and the volume was adjusted to 100 µl with various solvents. 5 ml of 0.1 mM solution of DPPH was added and allowed to stand for 20 minutes at 27°C. The absorbance of the sample was measured at 517 nm. Ascorbic acid was used as a positive control. Radical scavenging activity was calculated using the formula:

DPPH* radical scavenging activity % = (control OD - sample OD / control OD) × 100

### Superoxide radical scavenging activity (Beauchamp and Fridovich, 1971) [11]

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich (1971) [11]. The assay was based on the capacity of the extract to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin- light - NBT system. Each 3 ml reaction mixture contained 50 µM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 µM EDTA, 0.1 mg NBT and various concentrations (40 - 200 µg) of sample extracts. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination, the absorbance was measured at 590 nm. The entire reaction

assembly was enclosed in a box lined with aluminium foil. The percentage inhibition of superoxide anion generation was calculated as follows:

SO radical scavenging activity % = (control OD - sample OD / control OD) × 100.

Ascorbic acid was used as a positive control.

**Nitric oxide radical scavenging activity (Sreejayan and Rao, 1997) [12]**

The nitric oxide scavenging activity of extract was measured according to the method of Sreejayan and Rao (1997) [12]. 3 ml of 10 mM sodium nitro prusside in 0.2 M phosphate buffered saline (pH 7.4) was mixed with different concentrations (40 - 200 µg/ml) of extract and incubated at room temperature for 150 mins. After incubation time, 0.5 ml of Griess reagent (1 % sulphanilamide, 0.1 % naphthylethylene diamine dihydrochloride in 2 % $H_3PO_4$) was added. The absorbance of the chromophore formed was read at 546 nm. Nitric oxide radical scavenging activity was calculated using the formula:

NO radical scavenging activity % = (control OD- sample OD / control OD) × 100.

Ascorbic acid was used as a standard.

**Results**

In the current study, the different *in vitro* antioxidant assays such as 2, 2-diphenyl - 1 - picryl hydrazyl radical (DPPH), Super oxide radicals (SOS) and Nitric oxide radical (NOS) were investigated using ethanolic extract of *Cleome monophylla.*

**DPPH radical scavenging activity**

The results on DDPH radical scavenging activity of ethanol extracts of *Cleome monophylla* at six different concentrations and standard ascorbic acid are depicted in the Table - 1 and represented in the Fig - 1. When the concentration (10, 20, 40, 60, 80, 100 µg/ml) of the samples increased, the DPPH radical inhibition percentage also increased. However, the ethanol extract of *C.monophylla* showed moderate DPPH scavenging activity, when compared to the standard. The ethanol extract showed the $IC_{50}$ values as 81.42 µg/ml. The inhibitory percentage varies from 30 % to 72 % for ascorbic acid. The ethanolic extract of *Cleome monophylla* inhibits the DPPH radicals from 16 % to 59 % at various concentrations. The ethanol extract of *Cleome monophylla* recorded percent inhibition as 16.20, 18.57, 33.43, 41.89, 48.75 and 59.24 at 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml concentrations respectively. The ethanolic extract exhibited a remarkable effect at different concentrations. Lower concentration showed a less radical scavenging activity. Higher concentrations (80 % and 100 %) of ethanolic extract showed a noticeable DPPH radical scavenging activity. The $IC_{50}$ value of the ethanolic extract ($IC_{50} =$ 81.42 µg/ml) of test plant is drastically higher than the standard, ascorbic acid ($IC_{50} =$ 42.67 µg/ml).

**Table 1:** DPPH radical scavenging activity of ethanol extract of *Cleome monophylla* L. and standard (ascorbic acid)

| S. No. | Concentration (µg/ml) | Standard (ascorbic acid) | Ethanol Extract |
|---|---|---|---|
| | | Percent inhibition | Percent inhibition |
| 1. | 10 | 30.23 ± 0.41 | 16.20 ± 0.25 |
| 2. | 20 | 43.50 ± 0.23 | 18.57 ± 0.41 |
| 3. | 40 | 54.78 ± 0.12 | 33.43 ± 0.38 |
| 4. | 60 | 59.16 ± 0.57 | 41.89 ± 0.36 |
| 5. | 80 | 65.37 ± 0.39 | 48.75 ± 0.78 |
| 6. | 100 | 72.52 ± 0.22 | 59.24 ±1.6 |
| | $Ic_{50}$ values | $Ic_{50} =$ 42.67 µg/ml | $Ic_{50} =$ 81.42 µg/ml |

Values are means of three independent analyses of the extract ± standard error (n = 3).

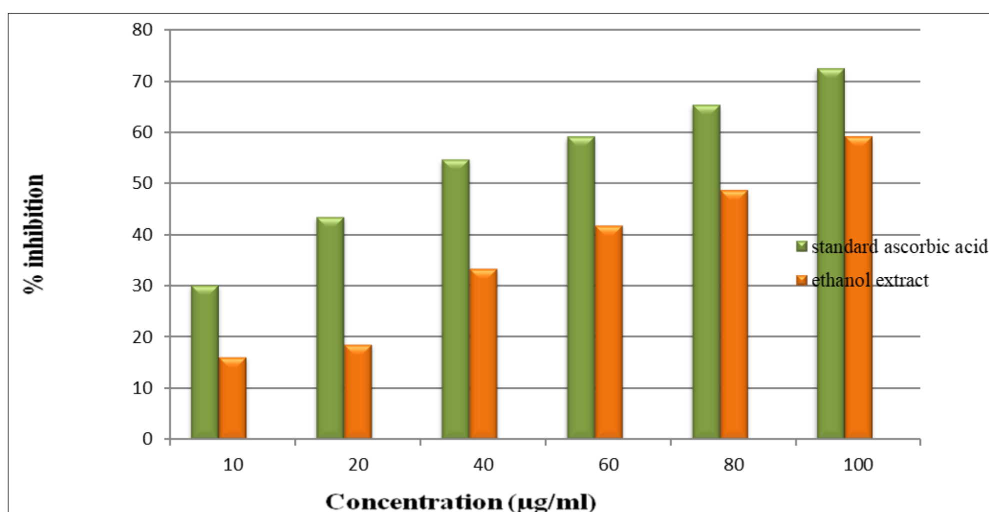


**Fig 1:** DPPH radical scavenging activity of ethanol extract of *Cleome monophylla* L. and standard (ascorbic acid)

**Super oxide radical scavenging activity**

The results on superoxide radical scavenging activity are presented in Table - 2 and graphically shown in Fig - 2.

The percent inhibition of ethanol extract of *Cleome monophylla* registered as 19.74 %, 31.89 %, 43.41 %, 59.66 %, 67.17 % and 72.72 % respectively at 10 µg/ml, 20

µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml concentration. The ethanolic extract of *Cleome monophylla* exhibited a remarkable antioxidant effect at various concentrations. Of the six tested concentrations, higher concentrations (80 µg/ml, 100 µg/ml) have proved a strong super oxide radical scavenging activity. Lower concentrations of ethanol extracts (10 µg/ml, 20 µg/ml and 40 µg/ml) showed a moderate inhibition rate. When the concentration of extracts increased, free radical scavenging capacity also increased.

The $IC_{50}$ values of standard and ethanol extracts are respectively 24.93 µg/ml and 49.88 µg/ml. The ethanol extract $IC_{50}$ value is more than the $IC_{50}$ value of standard ascorbic acid.

**Table 2**: Super oxide radical scavenging activity of ethanol extract of *Cleome monophylla* L. and standard (ascorbic acid)

| S. No. | Concentration (µg/ml) | Standard (ascorbic acid) | Ethanol Extract |
|---|---|---|---|
| | | Percent inhibition | Percent inhibition |
| 1. | 10 | 30.34 ± 0.13 | 19.74 ± 0.17 |
| 2. | 20 | 43.71 ± 0.15 | 31.89 ± 0.22 |
| 3. | 40 | 59.62 ± 0.34 | 43.41 ± 0.26 |
| 4. | 60 | 62.38 ± 0.25 | 59.66 ± 0.19 |
| 5. | 80 | 79.10 ± 0.22 | 67.17 ± 2.1 |
| 6. | 100 | 92.57 ± 0.31 | 72.72 ± 2.3 |
| | $Ic_{50}$ values | $Ic_{50}$ = 24.93 µg/ml | $Ic_{50}$ =49.88 µg/ml |

Values are means of three independent analyses of the extract ± standard error (n = 3).

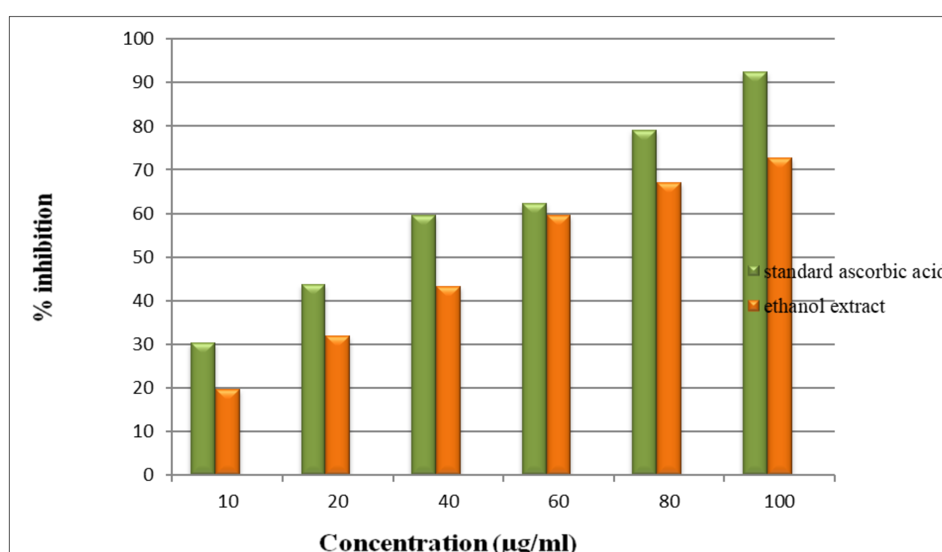


**Fig 2:** Super oxide radical scavenging activity of ethanol extract of *Cleome monophylla* L. and standard (ascorbic acid)

**Nitric oxide radical scavenging activity**

The results on nitric oxide radical scavenging activity of standard ascorbic acid and ethanol extract of *Cleome monophylla* are given in the Table - 3 and shown in Fig - 3. The *Cleome monophylla* ethanol extract exhibited a significant Nitric oxide radical scavenging activity. The extract has a high inhibitory activity against nitric oxide radicals. The inhibition percentage has increased, when the concentration of the extract increased. The $IC_{50}$ values of the ethanol extract was recorded as 49.94 µg/ml and standard ascorbic acid was recorded as 42.44 µg/ml. $IC_{50}$ value of plant sample was slightly higher than the $IC_{50}$ value of the standard ascorbic acid.

This result showed that the plant extract was comparatively potential as the standard sample in scavenging the nitric oxide radicals.

**Table 3:** Nitric oxide radical scavenging activity of ethanol extract of *Cleome monophylla* L. and standard (ascorbic acid)

| S. No. | Concentrations (µg/ml) | Standard (ascorbic acid) | Ethanol Extract |
|---|---|---|---|
| | | Percent inhibition | Percent inhibition |
| 1. | 10 | 26.81 ± 0.15 | 20.56 ± 0.71 |
| 2. | 20 | 33.28 ± 0.23 | 25.81 ± 0.82 |
| 3. | 40 | 47.40 ± 0.21 | 39.62 ± 1.1 |
| 4. | 60 | 58.23 ± 0.78 | 54.70 ± 1.7 |
| 5. | 80 | 65.78 ± 0.31 | 59.81 ± 1.9 |
| 6. | 100 | 77.14 ± 0.19 | 61.44 ± 0.91 |
| $Ic_{50}$ values | | $Ic_{50}$ = 42.44 µg/ml | $Ic_{50}$ = 49.94 µg/ml |

Values are means of three independent analyses of the extract ± standard error (n= 3)

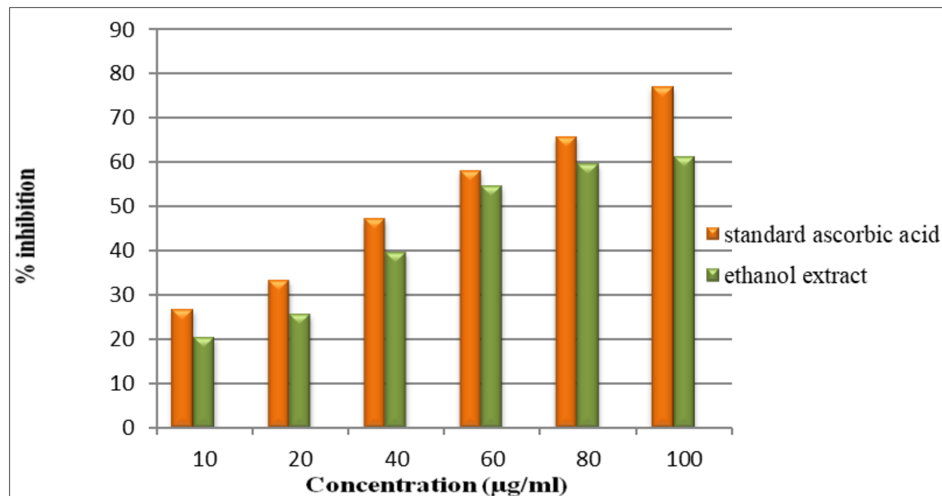


**Fig 3:** Nitric oxide radical scavenging activity of ethanol extract of *Cleome monophylla* L. and standard (ascorbic acid)

## Discussion

Antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2011) [13]. Duan *et al.* (2006) [14] reported that the reactive oxygen species and reactive nitrogen species in the living organisms are known to cause damage to proteins, enzymes, lipids and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including cancer, diabetes, liver injury, Alzheimer, Parkinson and coronary heart pathologies. The reactive oxygen and nitrogen species include diverse entities namely super oxide radicals, hydroxyl radicals, peroxyl radicals, peroxynitrite and nitric oxide radicals as well as non free radicals species as hydrogen peroxide, nitrous acid and hypochlorous acid (Mavi *et al*., 2004) [15].

Medicinal plant parts are commonly rich in chemical compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity (Packer *et al*., 1999) [16]. Phenolic compounds constitute one of the major groups of phytochemicals acting as free radical scavengers and antioxidants (Stevanovic *et al*., 2009) [17]. Phenolic compounds are class antioxidants that act as free radical terminators (Shahidi and Wanasundara, 1992) [18].

Previously, many reporters have studied different methods in antioxidant analysis that further support for current results. In the present exploration, ethanolic extract of *Cleome monophylla* was analysed for different *in vitro* antioxidant assays *viz*., DPPH, super oxide and nitric oxide. These were used to reduce the development of free radicals. Similarly, various reporters have studied antioxidant activities in other *Cleome* species Meda *et al.* (2013) [19] in *Cleome gynandra*; Sangeetha *et al*. (2014) [20] in *Cleome viscosa*; Shanmuganathan and Karthikeyan (2014) [21] in *Cleome gynandra*; Sumitha and Gurulakshmi (2015) [22] in *Cleome chelidonii*; Suresh *et al*. (2018) [23] in *Cleome viscosa*; Al-Humaidi *et al*. (2018) [24] in *Cleome amblyocarpa* and *Cleome ramosissima* and Nasseri *et al.* (2019) [25] in *Cleome heratensis*.

## DPPH radical scavenging activity

The DPPH assay method is based on the reduction of DPPH, a stable free radical (Warrier *et al*., 1994) [26]. A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2, 2-Diphenyl-1- picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate antioxidant activity (Kirtikar and Basu, 2006) [27]. In the present investigation, scavenging activities of the DPPH radical by the ethanolic extract of *Cleome monophylla* was found to be moderate as compared to the standard ascorbic acid. The $IC_{50}$ value of plant extract was 81.42 µg/ml. Similar observations were made by Samrath and Krishna (2007) [28] in *Aleurites trispesma, Elaeocaspus multiformis, Livestona chinensis* and *Nagia nagi*, Shanmuganathan and Karthikeyan (2014) [29] in *Cleome gynandra* and Sharma *et al*. (2015) [30] in *Bauhinia variegata.* Generally, the DPPH radical scavenging activity is mainly related to the nature of phenolic compounds and their electron transfer or hydrogen donating capability (Williams *et al*., 1995) [31].

In the present study, the $IC_{50}$ value of the study plant extract displayed a DPPH radical scavenging activity. This radical scavenging activity may be due to the phytocompounds present in the *Cleome monophylla*. This is in line with research of Al - Humaidi *et al*. (2018) [24] who reported that total phenolic and flavonoid compounds in the aerial parts of *Cleome amblyocarpa* and *Cleome ramosissima* possess good antioxidant activities. The results from the phytochemical screening are in consonance with previous work; for instance, the antioxidant activity of *Cleome viscosa* showed abundance of chemical compounds such as alkaloids, phenols, flavonoids, anthocyanins and glycosides which are responsible for the antioxidant property of this species (Gayathri *et al.*, 2013) [32]. In general, it is well known that phenolic compounds are widely distributed in plant kingdom and they have shown to possess strong antioxidant properties (Badami *et al*., 2003) [33].

In this way, the phenolic compounds known as antioxidants could be served as free radical scavengers and protect oxidative damages as reported by Zhu *et al.* (2017) [34] in *Agrimonia pilosa.*

## Superoxide radical scavenging activity

Super oxide anions are indirectly initiating the lipid oxidation. In cellular oxidation reactions, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of cell

damaging free radical and oxidising agents (Aruoma, 1996) [35]. Inhibitory effect of *Cleome monophylla* extract on superoxide radicals depends on the concentration of extracts. Higher inhibition of super oxide radicals are observed at higher concentrations of ethanol extract of *Cleome monophylla.*

In the present study, the superoxide radical scavenging ability of ethanol extract of *Cleome monophylla* (49.88 µg/ml as $IC_{50}$) was competent and slightly lower than standard ascorbic acid (24.19 µg/ml). With increasing concentration of extracts, free radical scavenging capacity was also increased. The ethanolic extract of *C.monophylla* was capable to scavenge superoxide anions. The following other earlier studies agreed to this findings. Sathisha *et al.* (2011) [36] reported that *Curcuma* extract was markedly a more potent scavenger of superoxide anion. Usha and Suriyavathana (2012) [37] reported that ethanolic extract of *Desmodium gangeticum* aggressively scavenged the superoxide radicals.

**Nitric oxide radical scavenging activity**

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and involved in the regulation of various physiological processes (Ranka and Karthik, 2017) [38]. Nitric oxide or reactive nitrogen species, formed during their reaction with oxygen or with super oxides. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Inhibition of solutions of sodium nitroprusside in phosphate buffer saline resulted in linear time dependent nitrite production which is reduced by the tested ethanolic extracts of *Cleome monophylla*. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite. The ethanol extract of *Cleome monophylla* showed a high forceful free radical scavenging activity in the nitric oxide assay at 40 µg/ml concentration. Hence, this extract exhibited a great antioxidant effect. The $IC_{50}$ value of ethanol extract and ascorbic acid showed as 49.94 µg/ml and 42.44 µg/ml respectively. Nitric oxide scavenging activity has been found to increase in dose dependent manner. Suresh and Suriyavathana (2012) [39] reported a minimum inhibition (28 %) at 100 µg / ml and the maximum inhibition of 64 % at 500 µg / ml in *Anisomeles malabarica*. Banerjee *et al*. (2011) [40] reported that the maximum inhibition (88.66 %) of NO was observed at the highest concentration of ethanolic extract of *Ixora coccinea*. Nitric oxide scavenging effect was found to be maximum in methanolic extracts of *Phyllanthus fraternus, Triumfetta rhomboidea* and *Casuarina litorea* (Parul *et al*., 2013) [41]. This is in line with the present investigation.

From the result of nitric oxide, it may be postulated that the ethanol extract was found to have 6 % of less inhibition at 60 µg/ml concentration, when compared with standard. Hasler and Blumberg (1999) [42] reported that phytocompounds are biologically active, naturally occurring chemical compounds found in plants which provide health benefits for humans. In the present study, *Cleome monophylla* showed positive response to carbohydrate, protein and aminoacid, flavonoids, glycosides, phenols, tannins, quinones, anthraquinones, terpenoids and coumarins in hexane, chloroform, ethanol and aqueous extracts. The therapeutic effect of *Cleome monophylla* might be distributed to the phytochemicals present in it. Many of these compounds have been recognised to possess antioxidant properties from the above results. It can be speculated that these chemical constituents might be responsible for the observed nitric oxide scavenging activity. It is well known that nitric oxide plays an important role in various inflammatory processes such as carcinomas, juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Hazra *et al*., 2008) [43].

**Conclusion**

In recent years, there has been an exponential increase in search of antioxidant properties in medicinal plants. Therefore, it is time to explore and to identify our traditional therapeutic knowledge and plant sources and to interpret it according to the recent advancements to fight against oxidative stress. The results of this study indicate that *Cleome monophylla* plant possesses antioxidant properties and could serve as free radical inhibitor or scavenger or act possibly as primary antioxidant. The results of antioxidant activity in this research are in justification with the medicinal importance of *Cleome monophylla* as naturally occurring antioxidants.

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