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EVALUATION OF ANTIOXIDANT ACTIVITY OF ETHANOL AND CHLOROFORM EXTRACTS OF WILD AND MICROPROPAGATED *CLEOME VISCOSA* LINN.

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ABSTRACT: Herbal medicines are now creature in modern dosage forms using modern manufacture in and processing techniques in pharmaceutical industry. Antioxidants assuage oxidative stress in cells and thereby help in the prevention and treatment of many diseases of humans. The aim of this study was to assess the antioxidant activity of ethanol and chloroform extracts of wild and micro-propagated *Cleome viscosa*. Antioxidant activity was performed by DPPH ((2, 2 – diphenyl 1, picryl hydrazyl) radical scavenging method for ethanol and chloroform extracts of wild and micro-propagated *C. viscosa*. The ethanol and chloroform extracts results were exhibited by strong antioxidant DPPH radical scavenging activity with percentage of antioxidant potential concentration wild and micro-propagated were found to be 77.74%, 76.45% and 63.54%, 55.16% respectively. The percentage was compare to positive standard compounds of ascorbic acid. The results were concluded that ethanol extracts possess effective antioxidant activity than that chloroform extracts.

INTRODUCTION: Phytomedicine also known as herbal medicine has become a mainstream phenomenon worldwide. Recently, it has been reported that more than 80% of the world population is dependent on herbal medicine [1]. Plant besides therapeutic agents is also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design [2].

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Since, people are aware of organic pesticides and are intended towards using plant extracts to offer alternatives to synthetic insecticides [3]. Moreover, the excessive use of medicinal plants for drug formulation increases the need of more biomass of plants which can be met with the biotechnological tools like micro-propagation [4].

Cleome viscosa Linn. commonly known as "wild or dog mustard," is an annual, sticky herb belonging to family Cleomaceae found as a common weed all over the plains of India and throughout the tropics of the world. The whole plant and its parts (leaves, seeds, and roots) are widely used in traditional and folkloric systems of medicine. In Asia and Africa the leaves and seeds used to treat infections, fever, rheumatism and headache. The whole herb is used in treatment of inflammation of the ear pain and applied on wounds and ulcers.

A decoction is used as an expectorant and digestive stimulant and the vapour from a steaming decoction of the whole plant is inhaled to treat headache [5]. Traditionally, this plant is used in various disorders such as diarrhoea, fever, inflammation, liver diseases, bronchitis, skin disease and malarial fever [6]. Antioxidants act as a defense mechanism that protects oxidative damage, and include compounds to repair damage molecules. It can prevent the oxidation caused by free radicals and sufficient intake of antioxidants is supposed to protect against diseases [7]. Antioxidants may be classified according to their mode of action as being free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen closed system [8].

Free radicals are natural by-products of human metabolism. These are charged molecules which attack cells, breaking cellular membranes, reacting with the nucleic acids, proteins, and enzymes present in the cells. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and eventually result in cell dysfunction. They are continuously produced by our body's use of oxygen, such as in respiration and some cell mediated immune functions. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation, air-pollution, pesticides [9]. Hence, in the present study *C. viscosa* (*in vitro* and wild plant) extracts was employed to study its antioxidant property.

MATERIALS AND METHODS:

Collection of Plant Material: The healthy plants of *Cleome viscosa* L. were collected during the month of March from the natural habitats of Kanchipuram district, Tamil Nadu, India. The plant specimen was identified and authenticated by Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. Specimens Reference number - BSI/SRC/5/23/2016/Tech/1195.

Sterilization and Inoculation of Explants: The nodal segment of the plant was chosen as explants for the present investigation. Actively growing shoots were selected as the source for explants. The explants were sterilization followed by Muthusamy Govarthanan *et al*., 2015 [10]. The nodal segments were cut into 5 mm in size and carefully transferred to the sterile MS basal medium supplemented with 3% sucrose, 0.8% agar and different concentration of PGRs such as 6-Benzylaminopurine (BAP), Kinetin (KIN), Naphthalene-3-acetic acid (NAA). The inoculated cultures were maintained in growth chamber. Data was recorded after 28 days [11].

Preparation of Plant and Callus Extracts: The wild and micro-propagated plants were washed thoroughly in sterile distilled water. The plants were shade dried and ground to fine powder using mortar and pestle. One gram (dry weight) of powdered extract was soaked in 10 ml of ethanol for 3 hours and sonicated in an Ultrasonic Sonicator at 20 pulses for 20 min. The extract was centrifuged at 10,000 rpm for 10 min and the supernatant was freeze-dried and stored at 4 °C until further use [12].

Antioxidant Activity by *in vitro* Techniques: The free radical scavenging ability of the crude extracts of *Cleome viscosa* was evaluated by DPPH free radicals scavenging assay.

DPPH Radical Scavenging Assay: The effect of ethanol and chloroform extracts of *Cleome viscosa* on DPPH (2, 2 - diphenyl 1, picryl hydrazyl) radical was estimated according to the procedure described by Von Gadow *et al*., 1997 [13]. 2 ml of 6 $\times 10^{-5}$ M methonolic solution of DPPH were added to 50 µl of an ethonolic solution (1 mg/ml) of the sample. Absorbance measurements commenced immediately. The decrease of absorbance at 515 nm was continuously recorded in a spectro-photometer for 16 min at room temperature. Methanolic solutions of pure compound (ascorbic acid) were tested at 1 mg/ml concentration. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration as follows: The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994) [14].

$$IP = [(AC (0) - AA (t) / AC (0))] \times 100$$

Where AC (0) is the absorbance of the control at t = 0 min; and AA (t) is the absorbance of the antioxidants at t = 16 min.

RESULTS AND DISCUSSION: *C. viscosa* was efficiently regenerated from nodal explants from field grown young plants on MS medium on supplemented with different concentration of cytokinins and auxins BAP, KIN and NAA. The callus was observed in 15 days old culture on media tested and it was found the number of shoots developed on nodal explants (**Fig. 1A** to **Fig. 1C**).

A **B** **C**

FIG. 1: *IN VITRO* SHOOT PROLIFERATION OF *C. VISCOSA* ON MS MEDIUM WITH DIFFERENT CONCENTRATION OF CYTOKININS AND AUXINS

Scavenging activity for free radicals of 2, 2-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. The antioxidant potential of the ethanol and chloroform extracts of wild and micro-propagated *C. viscosa* was shown 77.74%, 76.45% and 63.54%, 55.16% respectively. The positive standard of ascorbic acid compound showed 94.83% of antioxidant potential (**Table 1** and **Fig. 2**).

TABLE 1: DPPH RADICAL SCAVENGING ACTIVITY OF ETHANOL AND CHLOROFORM EXTRACTS OF WILD AND MICROPROPAGATED *C. VISCOSA*

S. No	Name of the Plants	O.D. at 515 nm		Antioxidant Potential concentration (%)
		Initial O.D. (0 min)	Final O.D. (16 min)	
1	*C. viscosa* ethanol (Wild)	0.620	0.138	77.74
2	*C. viscosa* ethanol (callus)	0.620	0.146	76.45
3	*C. viscosa* chloroform (wild)	0.620	0.226	63.54
4	*C. viscosa* chloroform (callus)	0.620	0.278	55.16
5	Ascorbic acid	0.620	0.009	94.83

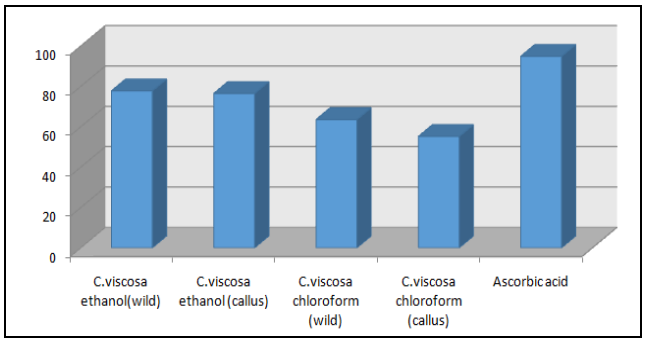

FIG. 2: EFFECT OF WILD AND CALLUS EXTRACTS IN DPPH RADICAL SCAVENGING ASSAY WITH COMPARED STANDARD COMPOUND ASCORBIC ACID

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation.

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [15]. Previous authors analyzed the efficacy of free radical scavenging by using DPPH radical scavenging assay with ethanol crude extracts of *C. viscosa* showed value of IC_{50} of leaf, stem, pod and root were 8.32, 12.26, 21.62 and 35.99 mg/ml, respectively [16]. The present study was ethanol and chloroform extracts of the wild and callus exhibited significant antioxidant activities determined by DPPH assay. *Cleome viscosa* showed higher activities in antioxidant assay.

Ethanol and chloroform extracts wild and callus is results was observed in 77.74%, 76.45% and 63.54%, 55.16% respectively at the concentration of 1 mg/ml. The DPPH radical scavenging activity methanolic extracts of *C. viscosa* has the lowest free radical scavenging potential. The maximum highest antioxidant potential percentage value was reported to be 50% [17]. In the present study, wild and callus ethanol extracts of *C. viscosa* was effective DPPH radical scavenging activity compared with chloroform extracts.

CONCLUSION: In conclusion the current study describes the *in vitro* antioxidant activity of ethanol and chloroform extracts of wild and micro-propagated *Cleome viscosa*. The extracts showed efficient free radical scavenging activity compared with standard compound ascorbic acid. Further the phytochemical screening of *C. viscosa* showed the presence of various chemical constituents of important pharmacological actions and needed to find the compounds which are responsible for treating radical-related pathological damage.

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CONFLICTS OF INTEREST: Nil

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