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Biological Assay of *In Vitro* Antioxidant and Antibacterial Activity of the Whole Plant Material *Cleome gynandra* Linn

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ABSTRACT

The current scientific knowledge stated that the excessive production of free radicals in the living organisms, and the imbalance between the development and neutralization of pro-oxidants may lead to processes such as aging and several diseases. The free radical theory of aging has received wide-spread attention which proposes that deleterious actions of free radicals are responsible for the functional deterioration associated with aging. The protein oxidative damage is indicates by carbonyl content and activities of antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase. The present research article describes about the *in vitro* biological antioxidant and antibacterial activities of whole plant - *Cleome gynandra Linn*. The crude plant extracts showed significant antibacterial activity against the selected bacterial strains.

Keywords: *Cleome Gynandra Linn,* SOD, CAT, GSH antioxidant activity, Antibacterial activity.

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INTRODUCTION

In the Past decades, research scientists focused on increasing human infections caused by pathogenic bacteria, fungi and virus. Microorganisms have shown adverse effects on the quality and safety of human beings life. A variety of synthetic chemicals are widely used against these microorganisms, unfortunately they develop resistance to many antibiotics due to the indiscriminate uses of commercial antibiotics [1, 2]. In addition, these antibiotics sometimes cause allergic reaction and immunity suppression. Plant-mediated phytocompounds play an important role in the chronic disease caused by these microorganisms.

Cleome Gynandra Linn belongs to the family *Capparidaceae*. The Common names of the plant material are spider flower and cat whiskers. It is a herb indigenous to the tropical and pan tropical regions. The herb is edible and grows up to about 60cm high [3-5]. *Cleome Gynandra* leaves has contains high percentage of vitamin C and taken as a pot herb in soups, fresh (or) dried [6, 7]. Leaves and seeds of *Cleome gynandra* is used for earache, epileptic fits, stomach-ache, constipation and inflammation in most of the countries [8-11], fresh leaves of *Cleome gynandra* is used in ayurveda and siddha medicine for a variety of disease conditions [12]. The plant material is used as an anthelmintic drug in ayurveda for ear diseases, pruritus and several other diseases like gastrointestinal disorders and gastro-intestinal infections, etc [13]. The phytochemical studies revealed that it has contains several constituents like Carotenoids, Cardiac glycosides, Cyanogenic glycosides, Flavanoids, Saponins, Triterpenes, sugars, Tannins, etc [14].

Various human diseases has causes oxidative damage that results from imbalance between the development and neutralization of pro-oxidants [15]. Oxidative damage are caused by free radicals, such as superoxide anions, hydrogen peroxide, hydroxyl, nitric oxide and peroxynitrite. The role of free radicals is becoming increasingly recognized in the pathogenesis of the many human diseases including cancer, aging and atherosclerosis [16]. They are some synthetic antioxidant compounds such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) these compounds show some side effects [17]. However there is so far no report about the antioxidant activities of the plant material using biological assay method. In other hand, the traditional use of the plant revealed that, the plant material possessing a significant antibacterial activity against bacterial strains. Therefore, the aim of the present study is to assess the antibacterial and antioxidant activities of the plant (*Cleome Gynandra Linn*).

MATERIALS AND METHODS

Plant Authentication

The whole plant material *Cleome gynandra* L. were collected in the month of December 2010 from its natural habitat from nearby Thiruvallam village, Vellore district of Tamilnadu. The plant was authenticated from Herbal Garden, VIT University Vellore district, Tamil nadu. The whole plant materials were cleaned and dried under the shade to avoid degradation of volatile oil. The whole plant materials were dried in hot air woven at 55°C for 3 days and at 40°C for the next 4 days.

Plant Extraction for Antibacterial Activities

The dried plant materials were crudely powdered and extracted with solvents like n-hexane, petroleum ether, ethyl acetate, ethanol, chloroform and water using Soxhlet apparatus [18] at 50°C. The solvent was completely removed and the dried crude extracts were used for investigation. Further the crude plant extracts were subjected to phytochemical study as well as antioxidant screening.

Plant Extraction for Antioxidant Activities

Weigh 100g of the plant material, and then extracted with 70% Methanol at 80^{o}C up to 3hrs. The extracts are filtered and evaporated to dryness. The extracts powder were dissolved in Dimethyl sulfoxide (DMSO) and then diluted with phosphate buffered saline (PBS, p^{H} 7.4) to give the last concentrations in range 0.8 to 100μg/ml.

Biochemical Assays

Measurement of Glutathione (GSH)

Total reduced glutathione was estimated using the standard protocol mentioned by Kakkar *et al* 1984 [19]. A 0.1ml of homogenous plasma was precipitated with 5% solution of TCA. The resulting solution was mixed well for their complete precipitation of proteins and then centrifuged. To an aliquot of clear supernatant, 2.0ml of 0.6mM DTNB reagent and 0.5ml phosphate buffer (p^{H} 8.0) were added to get a final volume of 4.0ml. The absorbance was read at 412nm against a blank containing 5% TCA instead of sample. A series of standard treated in a similar way was obtained to determine the glutathione content. The amount of glutathione expressed in terms of mg/100g (or) g/100g control polluted soil, and vemicompost mixed soil in milligram (or) gram.

Measurement of Superoxide Dismutase (SOD)

SOD was assayed using the standard method revealed by Misra and Fridovich *et al* 1972 [20]. Based on the inhibition of Epinephrine-Adrenochrome transition by the enzyme. A 0.5ml of homogenous plasma was taken in a reaction vessel and then 0.5ml of distilled water was added to dilute the sample. To this 0.25ml of ice cold ethanol and 0.15ml of chloroform were added to precipitate the reaction mixture. The reaction mixture was shaken well for about 5minutes at 4^{o}C and then centrifuged. The enzyme activity in the supernatant solutions was determined using spectrophotometer. The Adreno-Chrome produced in the reaction mixture, contains 0.2ml of EDTA (0.6mM), 0.4ml of Na_2CO_3 (0.25M) and 0.2ml of Epinephrine (3.0mM), final volume of was adjusted to 2.5ml and then the absorbance readings were measured at 420nm in a UV-Visible recording Spectrophotometer. The Transition of Epinephrine to Adreno-Chrome was determined by the addition of the required quantity of enzyme to assess the enzyme activity expressed in terms of units/minute/mg protein.

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Measurement of Catalase

CAT was assayed using standard protocol given by Beers and Siezer 1952 [21]. The breakdown of H_2O_2 on addition of the enzyme is followed by absorbing the decrease in light absorption of peroxide solution in the UV region was determined. A 3ml of reaction mixture containing 1.9ml of phosphate buffer, [(0.05M) of p^H 7.0], 1.0ml of substrate H_2O_2 and 0.1ml of diluted enzyme was used in this assay. The activity was measured as change in optical activity/density at 240nm at 30sec interval for about 3minutes. The CAT activity was expressed in terms of μmole of H_2O_2 consumed/minute/mg protein.

Bacterial Strains

The microorganisms were used for antibacterial activity study; *Staphylococcus aureus* (ATCC 700699), *Escherichia coli* (ATCC10412), *Pseudomonas aeruginosa (*ATCC 27853), *Klebsiella* (ATCC 2719), *Salmonella typhi* (ATCC 700931), *Bacillus subtilis* (ATCC 11778), *Salmonella paratyphi* (ATCC13426).

Standardization of Microorganisms

Exactly 0.2ml of overnight cultures of each organism was dispensed into 20ml of sterile nutrient broth and incubated for about 3-5hrs to standardize the culture to 10^6 cfu/ml. A loop full of the standard cultures was used for antibacterial assay. The Mueller Hinton Agar solid media was used for culturing of bacteria. The Agar Diffusion assay was carried out to check the antibacterial activity as described by Perez *et al* in 1990 [22]. The plates were incubated at 37^{o}C for about 24hrs during which the antibacterial activity was evidenced by the presence of a zone of inhibition surrounding the well. The antibacterial activity of the plant extracts were compared along with the standard drug.

RESULTS AND DISCUSSION

The crude plant extracts obtained using soxhlet extraction was screened for their antibacterial activity. Dimethyl sulfoxide was employed as the solvent to dissolve the test compound and negative control. Penicillin (5μg/disc) was used as positive reference standards to determine the sensitivity of each microbial species tested. The results of the antibacterial activity clearly showed significant activities against all the selected bacterial strains. Both the positive and negative bacterial strains showed a very good activity and the results are illustrated in the table 1. The antibacterial activity of the crude plant extracts is based on the presence of secondary metabolites concentration in the plant content. In the previous research paper, it was reported that the presence of secondary metabolites like alkaloids, tannins, saponins, steroids, terpenoids, flavanoids, phenols, tannins, phytosterols, fixed oil, fats and cardiac glycosides, etc.

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S.No	Microorganisms	n-hexane	Pet-Ether	$CHCl_3$	Ethyl acetate	C_2H_5OH	Penicillin
1	*E.coli*	-ve	-ve	+ve (0.7)	+ve (0.7)	+ve (0.8)	+ve (1.0)
2	*Klebsiella*	-ve	+ve(0.6)	-ve	+ve(0.2)	+ve (0.3)	+ve (0.3)
3	*P.vulgris*	+ve (0.6)	-ve	+ve (0.6)	-ve	-ve	-ve
4	*S.typhi*	+ve (0.4)	+ve (0.6)	-ve	+ve (0.8)	+ve (0.4)	+ve (0.4)
5	*S.aureous*	-ve	-ve	+ve (0.8)	+ve (1.8)	+ve (0.9)	+ve (0.9)
6	*S.para*	-ve	-ve	-ve	+ve (1.4)	+ve (0.7)	+ve (0.7)
7	*B.subtilis*	+ve (0.4)	-ve	-ve	-ve	-ve	+ve (0.7)

Table 1: Antibacterial activity of the whole plant crude extracts - Cleome gynandra Linn.

Biological assay - Antioxidant Activity for the plant material

The antioxidant activity of the plant *cleome gynandra* was investigated with three different methods; Reduced Glutathione, Superoxide Dismutase and Catalase test. The biological assay antioxidant activity results and figure are illustrated in the table -2 and Figure-1.

Factors	*Control*	*Polluted Soil*	*Vermicompost mixed Soil*
AOA %	97	61	128
SOD	624±0.22	810±0.22	526±0.22
GSH	16.2±0.12	19.4±0.12	13.8±0.12
Catalase	7.2±1.12	12.6±1.12	5.6±1.12

Table -2: Antioxidant properties of the whole plant material (Cleome Gynandra)

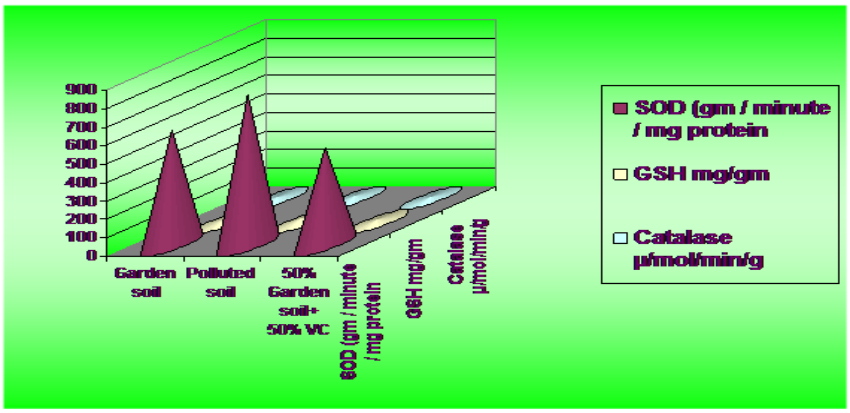

Figure -1: Antioxidant properties of the whole plant material (Cleome gynandra Linn)

The antioxidant activity was found to be the maximum (128%) in the whole plant material exposed to vermicompost. In the case of heavy metal contaminated soil exposed to plant material, the antioxidant activity was reduced into 61%, Whereas in the case of garden soil (Control) is 97%. This shows that the free radical Scavenging activity is reduced much in the plant exposed to heavy metal contaminated soils. The enzyme superoxide dismutase, catalase and glutathione were reduced significantly in the plant sample exposed to heavy metal contaminated soils, whereas the same has been increased significantly in the vermicompost amended soils showing that organic matter in the soil increases the antioxidant properties of the plants. In nature, the plant material possesses a very good antioxidant potential.

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CONCLUSION

The following conclusion was drawn based on the results; crude plant extracts showed significant antibacterial and antioxidant activity. The crude plant material is subjected to antioxidant activity test and it gives positive results to the whole plant materials. Antibacterial activity of the various crude extracts showed positive results against microorganisms used, due to the presence of bioactive compounds like Carotenoids, Cardiac glycosides, Cyanogenic Glycosides, Flavanoids, Saponins, Triterpenes, sugars, Tannins, etc. Based on the antioxidant activity results, the plant material can be used as a drug for radical scavenging diseases and bacterial infections in future.

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