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RESEARCH ARTICLE

# PHYTOCHEMICAL AND*IN-VITRO* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *LACTUCA SCARIOLA ANDCELOSIA ARGENTEA LEAVES*

***G H Urmila[1], B.Ganga Rao [2], T.Satyanarayana[2]**

[1]Nargund college of Pharmacy, Banashankari 3rd stage Bangalore, Karnataka, India 560 085.

[2]A.U College of Pharmaceutical Sciences, Andhra University, Visakhapatnam, A.P, India-530003

**Corresponding Author's Email: urmilagk@yahoo.co.in, contact: 9482020128*

**ABSTRACT**

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Highly reactive free radicals and oxygen species can initiate degenerative diseases. Antioxidant compounds like phenolic acids, polyphenols and flavonoids are commonly found in plants have been reported to have multiple biological effects, including antioxidant activity.Methanolic leaf extracts of*Lactucascariola Linn* and *Celosia argentea* Linnwere assessed for the In vitro Antioxidant activity using DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical , Nitric oxide(NO) and hydrogen peroxide ($H_2O_2$)scavenging models using Ascorbic acid as positive control. Analysis of free radical scavenging activities of the extracts revealed a concentration dependent free radical scavenging activity resulting from reduction in DPPH, Nitric oxide and Hydroxyl radical. The methanolic extract of *Lactucascariola Linn* showed significant DPPH, Nitric oxide and hydrogen peroxide scavenging activity compared to *Celosia argentea*Linn extract. IC50 values by DPPH , Nitric oxide and hydrogen peroxide model for *Lactucascariola*and *Celosia argentea*were found to be 192.5±9.014,*394.2*±6.009, 434.2±18.78,233.3±20.73 , 521.4±4.061and 494.2±5.465respectively.

**Keywords**:*Lactucascariola , Celosia argentea,* Antioxidant activity,methanolic extracts.

## INTRODUCTION

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress.[1]Theantioxidant which when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of substrate. The term 'oxidizable substrate'includes almost everything found in the living cells including proteins,lipids, DNA and carbohydrates.

Biological antioxidants have been defined as compounds that protect biological systems against the potentially harmful effects of processes or reaction that can cause excessive oxidation. Our body is rich in endogenous antioxidants, the substances that have the ability to stop free radicals formation or to limit the damage they cause. [2]

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables.

Highly reactive free radicals and oxygen species can initiate degenerative diseases. Free radicals arising from metabolism or environmental sources interact continuously in biological systems and their uncontrolled generation correlates directly with molecular level of many diseases. Free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are particularly important in antioxidative-defence in protecting cells from the injury of free-radical. Plants are the good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals and may have therapeutic potential for free radical associated disorders. Free radicals are the major cause of various chronic and degenerative diseases, such as coronary heart disease, inflammation, stroke, diabetes mellitus and cancer.[3]

The present studywas aimed to carry out the Antioxidant activity by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical , Nitric oxide and hydrogen peroxide scavenging modelsusing the methanolic leaf extracts of*Lactucascariola*Linn *and Celosia argentea*Linn.

*Lactucascariola*is a common weed in cultivated fields belonging to family Asteraceae.Leaves are used as vegetable,eaten raw or cooked.[4] *Celosia argentea* belonging to family Amaranthaceae grows as a weed during rainy season throughout India and other tropical regions of the world such as Sri Lanka, South Asia, Africa and America.[5]

## MATERIALS AND METHOD

### Plant Material

*Lactucascariola leaves* were collected from Ravoor village, Gulbarga district, Karnataka and leaves of *Celosia argentea* were collected from local market Bangalore.The samples were were authenticated by Dr.Santhanu,botanist,Natural RemediesPvt limited, Bangalore.

### Preparation of extract

Collected leaves were shade dried, pulverized to a coarse powder and was extracted in Soxhlet extractor consecutively using solvents of non polar to Polar grade(Petroleum ether, Methanol and Aqueous), obtained crude extracts were evaporated to dryness in rotary evaporator.

### Preliminary phytochemical screening[6,7,8,9]

 

The methanolicleaf extract of *LactucaSerriola*(LSME) *and Celosia argentea*(CAME)were subjected to different chemical tests for the detection of phytochemical constituents such as carbohydrates, glycosides, alkaloids, amino acids, phenolics, flavonoids, triterpenoids, steroids, etc.

## ANTIOXIDANT ACTIVITY

### Determination of Free radical scavenging activity by DPPH method[10]

2ml of 0.33% methanolic solution of DPPH was added to different concentration of methanolic*LactucaSerriola and Celosia argentea*(100-500µg/ml) extract .After 30 minutes; absorbance was measured at 517nm using UV-Visible spectrophotometer. All the tests were performed in triplicate and averaged. Ascorbic acid was used as standard. Percentage scavenging of the DPPH free radical was calculated using the following equation.

DPPH radical scavenging activity (%) = $(A_{control}-A_{Test})/A_{control}X$ 100.

Where $A_{control}$is the absorbance of the control reaction and $A_{test}$ is the absorbance in the presence of the extracts or standard.

### Determination of Nitric oxide(NO) scavenging activity[11]

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent (1% sulphanilamide,2% orthophosphoric acid,0.1% naphthyl ethylene diaminedihydrochloride).

1mlsodium nitroprusside (5mM, in phosphate buffer saline pH 7.4) was mixed with 1ml of different concentration of *LactucaSerriola and Celosia argentea*(100-500µg/ml)methanolic extract and incubated for 3 hours at 25˚C. To 1ml of incubated solution, 1 ml of Griess reagent was added. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylenediaminedihydrochloride was measured at 546 nm. All the tests were performed in triplicate and averaged. Ascorbic acid was used as standard.

Percentage of Nitric oxide scavenged was calculated using the following equation

Nitric oxide scavenged (%) = $(A_{control}-A_{Test})/A_{control}$ X 100.

Where $A_{control}$ is the absorbance of the control reaction and $A_{test}$ is the absorbance in the presence of the extracts or standard.

### Determination of Hydrogen peroxide($H_2O_2$)scavenging activity[12,13]

Different concentration of *Lactucascariola*and *Celosia argentea*methanolicextract (100-500µg/ml) were added to 0.6ml hydrogen peroxide solution (40 mM in phosphatebufferpH 7.4). The absorbance of hydrogen peroxide at 230 nm was determined after ten minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard.All the tests were performed in triplicate and averaged .Percentage ofhydrogen peroxide scavenging by the extracts and standard compounds were calculated using the following equation:

% scavenged $[H_2O_2] = [(Ao - A1)/Ao] \times 100$

whereAo was the absorbance of the controland A1 was the absorbance in the presence of extracts or standard.

## RESULT AND DISCUSSION

### Phytochemical screening

Preliminary phytochemical screening of the *Lactucascariola*and *Celosia argentea*methanolicleaves extract revealed the presence of different phytochemical constituents which are represented in **Table 1**.

**Table 1**: Phytochemical constituents of methanolic extracts of *Lactucascariola*and *Celosia argentea* leaves

| TestS | *Lactucascariola*methanolic extract (LSME) | *Celosia argentea*methanolic extract (CAME) |
|---|---|---|
| Alkaloids | - | - |
| Glycosides | + | + |
| Saponins | + | + |
| Phytosterols | + | - |
| Phenolic compounds | + | + |
| Flavanoids | + | + |
| Tannins | + | + |
| Carbohydrates | + | + |
| Tri terpenoids | + | - |

*(+): Indicates the presence of chemical constituents. (-): Indicates the absence of chemical constituents.*

### Free radical scavenging activity by DPPH method[14]

DPPH is a stable free radical and can be reduced in the presence of an antioxidantmolecule. The ability of a molecule to donate a hydrogen atom to a radical determines its antioxidant potentials. DPPH accepts a hydrogen atom from an antioxidant and becomes a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm.

*Lactucascariola and Celosia argentea*methanolic extracttestedfor antioxidant activity using DPPH free radicalshowed antioxidant activity.**(Table 2, Figure I ).**

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**Table 2**:DPPH radical scavenging activity ±SD of three replicates

| Concentration | Ascorbic acid(%inhibition) | LSME(%inhibition) | CAME(%inhibition) |
|---|---|---|---|
| 100µg/ml | 36.14±1.019 | 40.98±0.6583 | 41.20±1.150 |
| 200µg/ml | 73.95±0.5006 | 50.99±1.508 | 49.58±1.327 |
| 300µg/ml | 81.72±0.5816 | 68.53±1.357 | 50.66±1.231 |
| 400µg/ml | 90.83±0.3014 | 76.32±2.003 | 55.63±0.8541 |
| 500µg/ml | 102.5±0.5533 | 84.50±1.336 | 59.73±0.6384 |
| $IC_{50}$ | 138.3±2.205 | 192.5±9.014 | 233.3±20.73 |

$IC_{50}$value showed for *Lactucascariola*and *Celosia argentea*leaf extractswere192.5±9.014 and 233.3±20.73as compared withstandard Ascorbic acid 138.3±2.205µg/ml.

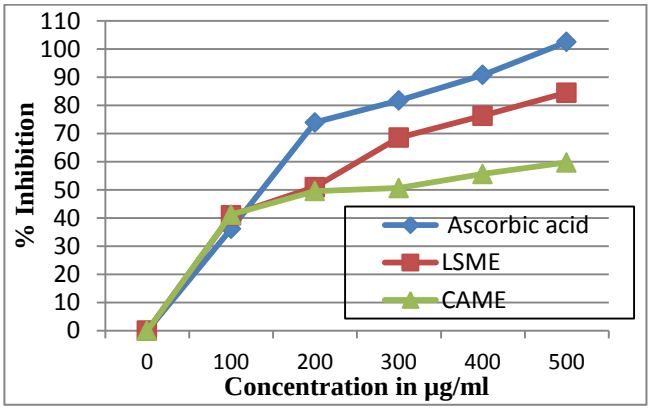


**Figure 1**: Graphical representation of % Inhibition against concentration(µg/ml) on DPPH model

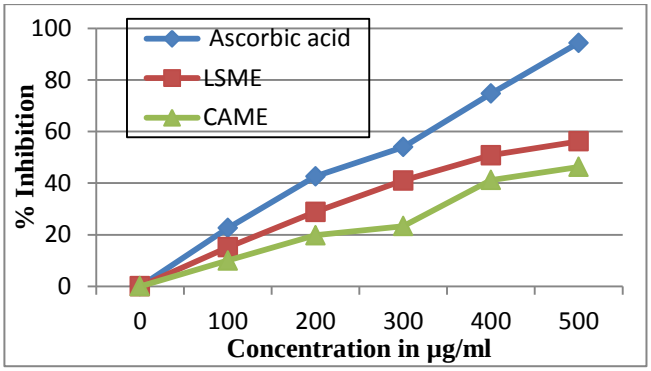


**Figure II**: Graphical representation of % Inhibition against concentration (µg/ml)on NO model

**Nitric oxide scavenging activity**

Nitric oxide (NO) has also been involved in a variety of biological functions, includingneurotransmission, vascular homeostasis, antimicrobial, and antitumor activities.

SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduced production of NO.[11]

The LSME at varied concentration showed remarkable inhibitory effect of NO scavenging activity compared to CAME. In the NO scavenging activity Ascorbic acid, LSME and CAME plant extracts increased gradually with increase in concentration which showed scavenging activity to be dose dependent. $IC_{50}$valuefor*Lactucascariola*and *Celosia argentea*leaf extracts was found to be394.2±6.009 and 521.4±4.061respectively and forAscorbic acid the $IC_{50}$value was found 270.3±1.740µg/ml.**(Table 3, Figure II).**

**Hydrogen Peroxide scavenging activity**

The measurement of $H_20_2$ scavenging activity is one of the useful methods of determiningthe ability of antioxidants to decrease the level of pro-oxidants such as $H_2O_2$.It can crossmembranes and may slowly oxidize a number of compounds. Hydrogen peroxide itself is not very reactive, but sometimes it can be toxic to cells because of rise in the hydroxyl radicals in the cells.[15]$IC_{50}$value for Ascorbic acid in the $H_20_2$ model was found to be 183.3±7.949µg/ml and for LSME and CAME itwas found to be434.2±18.78 and 494.2±5.465µg/ml respectively.

**Table 3**: Nitric oxide scavenging activity ±SD of three replicates

| Concentration | Ascorbic acid(%inhibition) | LSME(%inhibition) | CAME(%inhibition) |
|---|---|---|---|
| 100µg/ml | 22.56±1.425 | 15.11±0.9700 | 9.977±0.9617 |
| 200µg/ml | 42.64±1.152 | 28.83±1.621 | 19.82±0.9180 |
| 300µg/ml | 53.95±0.5487 | 41.03±2.312 | 23.36±1.645 |
| 400µg/ml | 74.70±1.067 | 50.81±0.9333 | 41.20±1.860 |
| 500µg/ml | 94.33±1.224 | 56.23±1.135 | 46.28±1.454 |
| $IC_{50}$ | 270.3±1.740 | 394.2±6.009 | 521.4±4.061 |

**Table 4**: Hydrogen Peroxide scavenging activity ±SD of three replicates

| Concentration | Ascorbic acid(%inhibition) | LSME(%inhibition) | CAME(%inhibition) |
|---|---|---|---|
| 100µg/ml | 31.62±1.436 | 20.43±0.6409 | 9.480±0.3880 |
| 200µg/ml | 54.50±2.154 | 30.81±0.7337 | 19.23±0.1867 |
| 300µg/ml | 79.15±0.5487 | 39.88±0.2132 | 29.21±1.118 |
| 400µg/ml | 93.31±1.656 | 47.83±1.353 | 38.32±0.54 |
| 500µg/ml | 97.82±1.299 | 53.43±0.6929 | 50.83±0.7435 |
| $IC_{50}$ | 183.3±7.949 | 434.2±18.78 | 494.2±5.465 |

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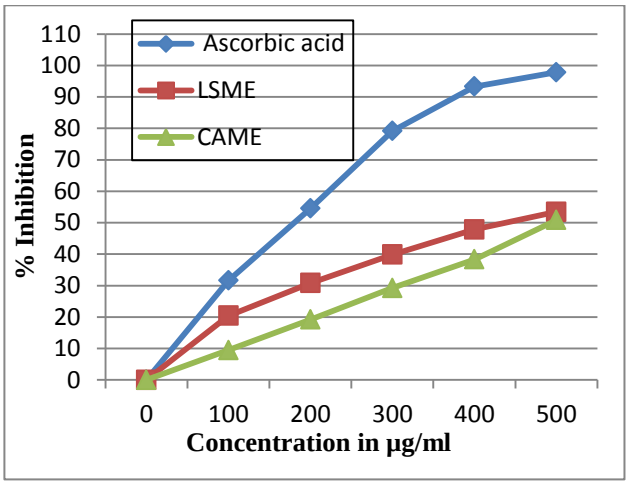


**Figure III**: Graphical representation of % Inhibition against concentration(µg/ml) on $H_2O_2$ model

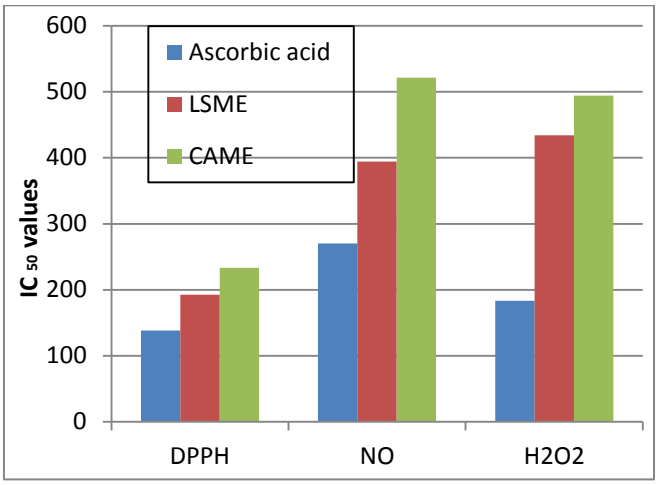


**Figure IV**: Graphical representation of 50% Inhibition concentration($IC_{50}$) on DPPH, NO and $H_2O_2$ model

## CONCLUSION

In vitro antioxidant activity was carried out with methanolic leaf extracts of*Lactucascariola*Linn*and Celosia argentea*Linnby DPPH, NO and $H_2O_2$ radical scavenging activities models. The result of the present study revealed that both extracts exhibited antioxidant activitybut *Lactucascariola*Linndisplayed better activity compared to *Celosia Argentea*.Thephytochemical constituents present in the plant extract may be responsible for showing the anti-oxidant properties. *Lactucascariola* Linnand*Celosia argentea*Linn can beproposed as promising natural sources of antioxidants suitable forapplication in nutritional/pharmaceutical fields, in the prevention of free radical- mediated diseases.

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