

Antioxidant Potential of Hydroethanolic Extract *Ctenolepis garcinii*

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ABSTRACT: The present study was to evaluate the antioxidant activity of hydroethanolic extract of dried leaves of five medicinally important herbs *Cleome aspera*, *Ctenolepis garcinii*, *Aslepias curassavica* L. *Urenalobata* and *Sidacordifolia*. Antioxidant activity was studied by superoxide radical scavenging assay, hydrogen peroxide non radical scavenging assay, total antioxidant capacity and non enzymatic Hb-glycosylation assay with varying plant concentrations (200, 400, 600, 800 and 1000 µg/ml). The free radical scavenging potential of the plant extracts also proved that *Ctenolepis garcinii* have the highest activity and the results suggest that *Ctenolepis garcinii* has promising antioxidant activity and may serve as a source of natural antioxidants.

I. INTRODUCTION

The second leading disease that cause human death is cancer continuously spreading in the 21st century with increasing incidence (Jemal *et al.*, 2011). The human population by the year of 2020 predicted to be 15 (Bray and Moller, 2006). An International Agency of Research on Cancer called GLOBOCAN identified that India's cancer incidence would be nearly double by next 20 years to around 1.7 million 2035 (Ferlay *et al.*, 2008) and this may be due to very low prevention and diagnosis awareness and treatment facilities available. Different types of cancer affecting the Indian people includes breast, rectum, stomach, prostate, liver, skin, lungs, cervix, mouth, esophagus, bladder, blood etc. The reason for high incidence rate may be genetic, poor immune condition, mutations, poor food habits, social cause, industrialization, population etc (Imran *et al.*, 2011).

Liver being the largest organ perform superior role in metabolism by producing various metabolites like fibrinogen, blood clotting factors, prothrombin, heparin and secretes bile (Vijayalakshmi, 2015). One of the major reasons for morbidity and mortality in human is liver disease mainly due to hepatotoxicity (Nadeem *et al.*, 1997). A leading form of liver disease includes liver cancer which takes 5th place next to lung, prostate, colon and rectum and pancreas related cancer and the primary liver cancer is hepatocellular carcinoma (Rocken and Carl-McGrath, 2001). The aggressive nature and poor survival rate, liver cancer remains a significant health related issue (Altekruse *et al.*, 2009).

The primary liver cancer diseases are mostly hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) (Melissa & Ahmedin, 2011). In which hepatocellular carcinoma prevailing 9.1% in cancer mortality globally (Wong *et al.*, 2017) with the inducible risk factors for HCC includes exposure to aflatoxin in diet, chronic hepatitis B virus (HBV) & hepatitis C virus (HCV) infection, diseases like fatty liver, cirrhosis induced by alcohol, iron overload and diabetes, obesity and smoking (Blonskiet *et al.*, 2010, Parkin & Bray 2006, Perzet *et al.*, 2006). In Indian subcontinent the incidence of HCC for male is between 0.7 and 7.5 and for female is between 0.2 and 2.2/100,000 population per year (Acharya, 2014). Globally HCC accounts for 7.5% in men and 3.5% in women (Parkin *et al.*, 2001).

The herbal medicines holds a good source of bioactive compounds which maintains human health and disease management (Lord *et al.*, 2011). Plant extracted products have received considerable interest now-a-days because of its extensive pharmacological activity which includes cytotoxic and cancer curing effects. India holds itself with diverse plant source with potent compounds with anticancer activity. Till date, many numbers of natural products are screened for anticancer potential using various experimental models. This results in identification of more than 30 effective anticancer agents. (Ramnath *et al.*, 2002).

The present study aims to free radical scavenging activity of *Ctenolepis garcinii*. The plant has good medicinal values. *Ctenolepis garcinii* Burm.f belongs to the family Cucurbitaceae, a climber. The plant was widely distributed in southern parts of India and in some parts of Ceylon. Various parts of *Ctenolepis garcinii* have been reported to have antibacterial activity and antifungal activity, anticancer activity and anti inflammatory activity (Vijayakrishnan, 2013). The plant *Ctenolepis garcinii* Burm. F, which is a climber grouped under family cucurbitaceae. It is distributed in south India, Srilanka etc., and the roots are given for ailments like fever, anorexia, indigestion and rheumatism. For boils and swellings leaf paste is used, also fruits are taken for gonorrhoea and the fruit pulp is applied on foot sole to reduce body temperature (Vijayakrishnan, 2013).

II. MATERIALS AND METHODS

Chemicals

Aflatoxin-B1 used in the present study was obtained from Sigma-Aldrich. All the other chemicals, acids, bases, solvents and salts used were of analytical grade. HepG2 cell line was obtained from National Centre for Cell Science, Pune and Gene expression kit was obtained from Thermo Scientific.

Collection of plant material and extract preparation

Fresh leaves of selected medicinal plants namely *Cleome aspera*, *Ctenolepis garcinii*, *Aslepias curassavica* L. *Urenalobata* and *Sidacordifolia* were collected randomly in and around Kolli hills (11.2485° N, 78.3387°) near Namakkal, Tamil Nadu, India. The plants were then authenticated by the Botanical Survey of India, Coimbatore, (BSI/SRC/5/23/2013-14/Tech. 1080) and also by ABS botanical garden, Salem, India (AUT/PSG/047). The fresh leaves were washed under running tap water, air dried under shed for totally 3 days and the dried leaves were ground to a coarse powder and stored in polythene containers at room temperature for further study. 100g of each plant's dried leaves were extracted using 200ml of 50% hydroethanol in a soxhlet apparatus. The extracts were dried under reduced pressure by the use of rota evaporator at 40°C. The yield of the selected plants extract namely *Cleome aspera*, *Ctenolepis garcinii*, *Aslepias curassavica* L. *Urenalobata* and *Sidacordifolia* was found to be 4.5%, 8.16%, 5.01%, 5.39% and 4.5% respectively. The extracts were stored in an air-tight desiccator for future use (Amarasingham et al., 1964).

Superoxide radical scavenging activity (Winterbourne et al., 1975)

The photoreduction of riboflavin produces superoxide radical ($O_2^{\cdot-}$) and was studied by nitrobluetetrazolium dye (NBT) reduction method. The assay mixture contained sample (200-1000 µg/ml) with 0.1 ml of nitrobluetetrazolium (1.5 mM), 0.2 ml of EDTA (0.1M), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M). The control tubes were also maintained where in DMSO was added instead of sample. The reaction mixture was illuminated for 30 mins and the absorbance at 560 nm was read against the control samples. Quercetin was used as the standard. All the tests were done in triplicate and the results were averaged. The percentage inhibition was calculated by considering the results of control and test samples.

Hydroxyl radical scavenging activity (Elizabeth and Rao, 1990)

The reaction mixture contained 0.1 ml of 2.8mM deoxyribose, 0.1 ml of 0.1mM EDTA, 0.1 ml of 1mM H_2O_2 , 0.1 ml of 0.1mM Ascorbate, 0.1 ml of 20mM KH_2PO_4 -KOH buffer, pH 7.4 and various concentrations of plant extract (200-1000 µg/ml) having final volume of 1 ml. The prepared reaction mixture was incubated for 1 hour at about 37°C. The percentage inhibition was calculated by deoxyribose degradation which was measured as TBARS. Mannitol was used as standard.

FRAP assay (Benzie and Strain, 1996 modified)

The stock solutions include 300 mM acetate buffer, pH 3.6, 10 mM TPTZ (2, 4, 6-tripyridyl-S-triazine) solution mixed in 40 mM HCl with 20 mM $FeCl_3 \cdot 6H_2O$. The working solution was prepared fresh by mixing 25 ml acetate buffer, 2.5 ml TPTZ and 2.5 ml ferric chloride solution. The temperature of the solution was maintained at 37°C. 0.15 ml of hydroethanolic leaves extract at various concentration ranging from 200-1000 µg were allowed to react with 2.85 ml of FRAP solution for 30 mins in the dark atmosphere. The coloured product of ferrous tripyridyltrozine complex was read at 593nm. Results are expressed in µM (Fe (II) /g dry mass and compared with that of ascorbic acid.

Non-enzymatic haemoglobin glycosylation assay (Pal and Dutta, 2006)

The degree of non-enzymatic hemoglobin glycosylation helps to investigate the antioxidant activity of herbal extract which was measured colorimetrically at 520nm. The reagents 2% glucose, 0.06% hemoglobin and 0.02% Gentamycin solutions were prepared in 0.01M phosphate buffer with pH 7.4. 1.0 ml each of above solutions are mixed well. After which 1.0 ml of different concentrations of the sample ranging from 200 µg/ml to 1000 µg/ml was added mixed well and incubated in dark at room temperature for 72 hours. At 520nm the degree of glycosylation of hemoglobin was measured colorimetrically.

$$\text{Inhibition \%} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

Statistical method

The results of all experiments performed were expressed as Mean \pm SD of three determinants. The statistical analyses were performed using the statistical package SPSS (Statistical Package for 146 Social Science, SPSS Inc., Chicago, IL).

III. RESULTS AND DISCUSSION

Superoxide radical scavenging activity

The superoxide radical is omnipresent in aerobic cells. Reduction of molecular oxygen into water produces superoxide anion radical ($\text{O}_2^{\cdot-}$). This radical is formed by electron leakage from electron transport chain (Ismail share *et al.*, 2014). So it is highly reactive molecule produced by oxidative enzymes through non-enzymatic reaction like autooxidation with catecholamines (Hammana and Paritar, 1998). Superoxide anion acts as a precursor for singlet oxygen and hydroxyl radicals. The superoxide radical reduces nitrobluetetrazolium (NBT) to a blue coloured formoxon. Increase in antioxidant concentration decreases the absorbance value which indicates the uptake of superoxide anion in the reaction mixture (Khanamet *et al.*, 2004). The percentage scavenging ability of superoxide inflated with the increasing concentration of extract. The figure 1 portrays the superoxide radical scavenging activity of selected plants and the standard antioxidant quercetin.

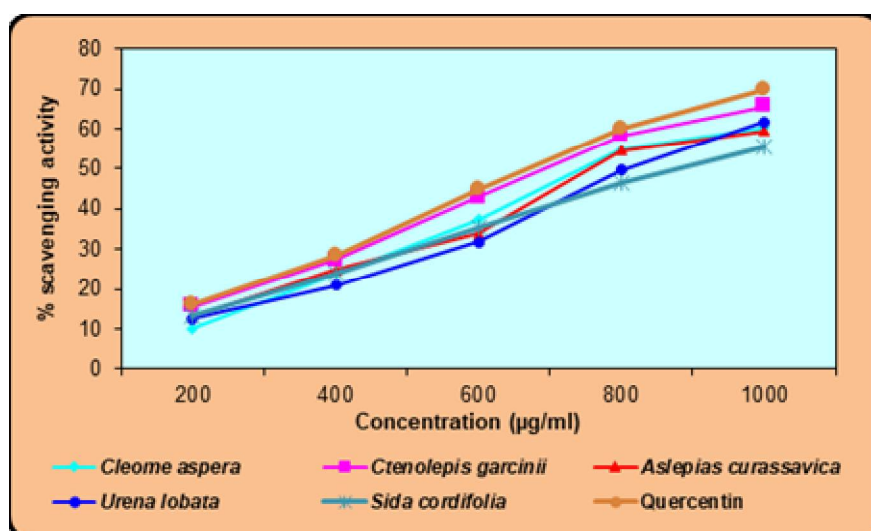


Figure 1 : Super oxide radical scavenging activity of different plant extracts

The maximum scavenging activity was observed at 1000 µg/ml was detected to be 60.43%, 65.63%, 59.5%, 61.8%, 55.6%, and 69.73% for *Cleome aspera*, *Ctenolepis garcinii*, *Aslepias curassavica* L., *Urena lobata*, *Sida cordifolia* and Quercetin respectively. All extracts exhibited good superoxide anion scavenging activity due to the presence of phytochemicals flavonoids, polyphenols, terpenoids etc. *Ctenolepis garcinii* has shown the highest ability among the other plants and the IC_{50} values for *Cleome aspera*, *Ctenolepis garcinii*, *Aslepias*

curassavica L. *Urenalobata*, *Sidacordifolia* and Quercetin was calculated as 830 µg/ml, 670 µg/ml, 780 µg/ml, 920 µg/ml and 760 µg/ml and 660 µg/ml.

Our results are in good agreement with the investigation of Ramesh Balu and Jayabharathi, (2017), for the search of natural antioxidant found in hydroethanolic leaf extract of *Acacia catechu* (L.f) Willd. The *Annonamuricata* leaf extract was found to be with higher superoxide radical scavenging activity comparing to standard Quercetin (Nalini and Brindha, 2018). Thus from the above study, *Ctenolepis garcinii* is found to have potent superoxide radical scavenging activity by neutralizing the highly reactive peroxynitrite anion.

Hydrogen peroxide scavenging assay

Hydrogen peroxide (H_2O_2) is a weak oxidizing agent and produce hydroxyl radical which can cause sugar fragmentation, loss of nitrogenous base, DNA breakage, lipid peroxidation and cause tremendous biological damage (Manianet *al.*, 2008). The herbal extracts are capable of scavenging H_2O_2 in a dose dependent manner. Different concentrations (200-1000 µg/ml) of plant extracts were screened for its H_2O_2 scavenging potential with standard ascorbate. The table 1 exhibited that increased amount of scavenging activity was noticed in 50% hydroethanolic extract of *Ctenolepis garcinii* at 1000 µg/ml and the IC_{50} value was 610 µg/ml and it was compared with ascorbate which showed a IC_{50} value of 580 µg/ml. The other plant extract showed less percentage of hydrogen peroxide scavenging ability.

Table 1: Hydrogen peroxide radical scavenging activity of different plant extracts

Conc. of plant extract (µg/ml)	Percentage scavenging activity of Hydrogen peroxide radical					
	Ascorbate	Cleome aspera	Ctenolepis garcinii	Asclepias currasavica	Urenalobata	Sidacordifolia
200	30.53±0.31	20.78±0.22	29.27±0.42	21.91±0.32	19.8±0.44	25.43±0.35
400	42.27±0.3	33.36±0.3	40.89±0.7	29.76±0.3	31.84±0.41	38.6±0.3
600	52.67±0.72	39.78±0.32	49.65±0.69	35.15±0.42	36.36±0.35	41.67±0.38
800	60.16±0.58	49.76±0.7	58.45±0.44	47.93±0.7	50.08±0.37	53.39±0.35
1000	73.23±0.4	63.21±0.75	69.38±0.68	64.79±0.7	61.9±0.49	65.87±0.46
IC_{50} (µg/ml)	580	760	610	780	800	725

Values are expressed as mean ± SD of three replicates.

Hydrogen peroxide (H_2O_2) can be formed from two ways. One, upon two-electron reduction of molecular oxygen and second one-electron reduction of superoxide anion ($O_2^{\cdot-}$). As H_2O_2 has no unpaired electron it is not a radical but can act as oxidant and possess two important biological properties. One, it can easily diffuse across biological membrane and second it can react with transition metals like copper and iron by homolytic cleavage to produce most highly reactive hydroxyl radical (OH^{\cdot}) (Bahorunet *al.*, 2006). The reduction of Fe^{3+} to Fe^{2+} by superoxide anion enhances H_2O_2 to readily decompose to OH^{\cdot} and OH^- . This decomposition requires Fe^{2+} ion and this reaction is called Fenton reaction. Hence the combined presence of H_2O_2 , superoxide and iron ion leads to the synthesis of very reactive hydroxyl radical, hence this non radical scavenging activity is required.

ThagrikiDluyaet *al.*, (2017) reported the antioxidant activity of five medicinal plant extracts namely *Ficusgnaphalocarpa*, *Vitexdonniana*, *Sennaoccidentalis*, *Pakiabiglobosa* and *Euphorbia convolvuloide* in which *Euphorbia convolvuloide* revealed maximum radical scavenging ability compared with other extracts. This finding is in accordance with our finding that *Ctenolepis garcinii* exhibited maximum percentage inhibition compared to other four plants and this may be due to presence of phenolic compounds such as tannins, flavonoids phenolics and diterpenes. AdilFarookWaliet *al.*,(2016) studied the hydrogen peroxide radical scavenging assay in propolis, bee glue a resinous material

FRAP Assay

In numerous diseases from the past 50 decades oxidative stress is studied to be key phenomenon, which was under great consideration for doctors and biologists (Coulidiati, 2011). Jaromirgumulecet *al.*, (2012) implied that the reason for stress is due to either overproduction of reactive oxygenated species or is due to antioxidant system failure. Hence the antioxidant system of various plant extracts plays an important role and one such important method is Ferric ion reducing antioxidant power (FRAP). FRAP rely on the antioxidants present in plant extracts ability to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri (2-pyridyl)-S-triazine (TPTZ) and the reducing ability was calculated as mmoles of $FeSO_4/g$ of extract with ascorbate as standard.

From the figure 2 we infer that FRAP values for 50% hydroethanolic extract of different plants viz., *Cleome aspera*, *Ctenolepis gacini*, *Aslepias currasvica*, *Urenalobata*, *Sid codifolia* were found to be 0.68mM, 0.85mM, 0.73mM, 0.69mM, 0.81mM respectively. For the ascorbate the FRAP value is 0.98mM equivalents of $FeSO_4/g$ at 1000 $\mu g/ml$

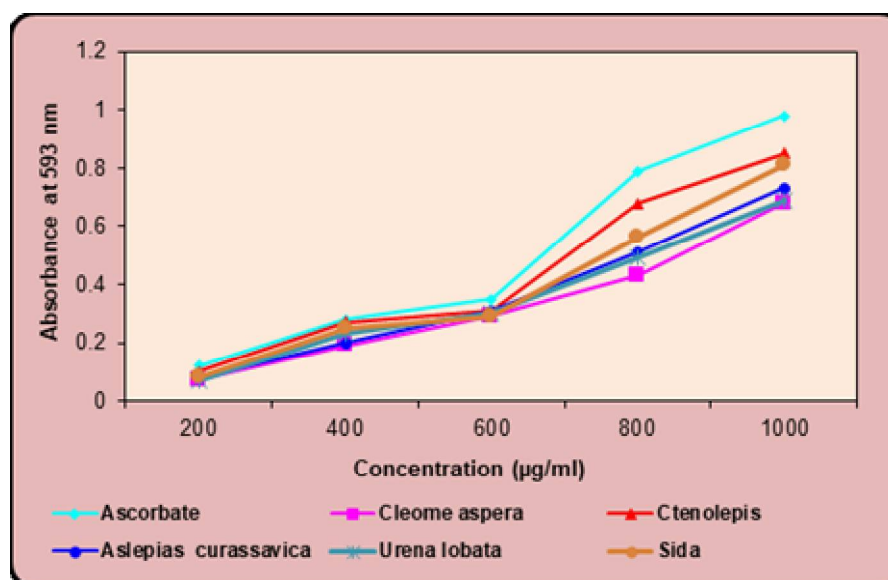


Figure 2: Ferric reducing ability of different plant extracts

Compared to other plants, the *Ctenolepis garcinii* extract showed highest FRAP value due to the presence of antioxidants. The potentiality is attributed by mechanisms like prevention of chain formation, binding of catalyst like transition metal ion, peroxide decomposition, hydrogen prevention, radical scavenging (Diplock, 1997). Many scientists like Meir *et al.*, (1995), Benzie and Strain (1996) expressed that this method is a potent one to determine the total antioxidant power. The ferric reducing potential of bioactive compound extract in *Murray koenigii L* was studied by Kali Ghasemzadehet *al.*, (2014).

Non-enzymatic Hemoglobin glycosylation assay

Among various methods available to evaluate antioxidant capacity non-enzymatic hemoglobin glycosylation assay gives good support for the assay of total antioxidant capacity of herbal extracts. Hemoglobin glycosylation is an oxidation reaction and hence an antioxidant is expected to inhibit the reaction. The degree of hemoglobin glycosylation *in vitro* is measured colorimetrically with the presence of different concentration of plant extracts and is presented in figure 3.

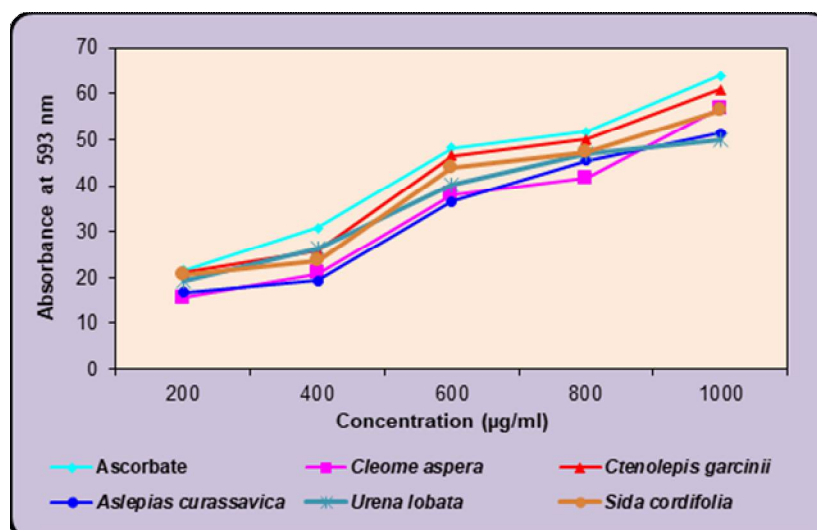


Figure 3: Hb-glycosylation assay of different of different plant extracts

The results obtained indicate that 50% hydroethanolic extract of different plant extracts revealed increased affinity for glycosylated haemoglobin from 200-1000µg/ml concentration and the percentage inhibition was found to 56.9%, 60.8% , 51.4%, 50.1%, 56.4% for *Cleome aspera*, *Ctenolepis garcinii*, *Aslepias curassavica*, *Urenalobata* and *Sidacordifolia* respectively . For the standard ascorbate the value obtained was 63.9% inhibition. From the figure it was inferred that the hemoglobin glycosylation capacity for *Ctenolepis garcinii* was found to be high compared with other plant extracts. The non-enzymatic *in vitro* analysis of antioxidant potential of *Ctenolepis garcinii* showed good result in inhibiting erythrocyte hemolysis in dose dependant manner. As the concentration level of plant extract increased the level of glucose-hemoglobin complex formation decreased indicating promising result of antioxidant capacity of *Ctenolepis garcinii*.

Ashok kumaret al., (2010) identified methanolic extract of *Amaranthusspiousus* exhibited increased the percentage inhibition of hemoglobin glycosylation. Karnawatet al., (2013) screened *Ocimum sanctum*, *Allium sativum* and *Tinosporacordiolia* for its hemoglobin glycosylation property. Adding to this, in *Premnacorymbosa* glycosylated haemoglobin inhibition and preventing further formation of reactive oxygen species was studied by Radhikaet al., (2013). Our study results are found to be in concordance with the other researchers who have investigated the antioxidant potential in medicinal plants.

In the present work, it was found that *Ctenolepis garcinii* exhibited higher antioxidant activity. So these findings are in agreement with previous reports that there is a linear relation between antioxidant activity and total phenolic contents.

IV. CONCLUSION

Results acquired in this examination demonstrate that *Ctenolepis garcinii* leaf extract, wealthy in phenolics displayed most elevated cancer prevention agent and decreasing exercises. Add up to phenolic content had positive connection with cell reinforcement limit. The finding of this examination recommends that this plant leaves could be a

potential wellspring of normal cell reinforcement that could have extraordinary significance as restorative specialists in avoiding or moderating the advance of maturing and age related oxidative pressure related degenerative infections. Assist examination on the disconnection and portrayal of the cell reinforcement constituents is anyway required. Along these lines the plant *Ctenolepis garcinii* could be utilized as a hotspot for medications and furthermore to enhance the wellbeing status of the purchasers because of the nearness of different exacerbates that are fundamental for good wellbeing.

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