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EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY OF A FEW WILD CUCURBITACEAE PLANTS AGAINST CANCER

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Abstract: The methanolic extract of whole plant of *Diplocyclos palmatus*, roots of *Corallocarpus epigaeus* and fruits of *Lagenaria siceraria* were evaluated for their antioxidant efficacy by DPPH radical scavenging assay. Reactive Oxygen Species (ROS) and many of the free radicals are natural by-products of metabolism of drugs, environmental chemicals and other Xenobiotics as well as endogenous chemicals, especially stress hormones like adrenalin and noradrenalin. ROS has a high reactive potential and is responsible for many of the human diseases like diabetes, cancer, viral infections, cardiovascular diseases and inflammations and is known to cause oxidative damage to DNA, proteins and lipids in humans. Antioxidants nowadays are increasingly sought as components of the diet owing to their benefits upon human health through protection of cells against oxidative stress which might otherwise lead to cell damage and death. The antioxidants present in the medicinal plants quench the free radicals by donating a hydrogen atom. The antioxidant activity of the medicinal plants namely, *Diplocyclos palmatus, Corallocarpus epigaeus* and *Lagenaria siceraria* were estimated by using 2, 2–Diphenly 1-Picryl Hydrazyl (DPPH) assay. The present study aims at evaluating therapeutic potential of these medicinal plants due to their high free radical scavenging activity.

Keywords: *Diplocyclos palmatus, Corallocarpus epigaeus, Lagenaria siceraria,* cancer, antioxidants, DPPH

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INTRODUCTION

Cellular damage, triggered by imbalance between reactive oxygen species (ROS) generation and scavenging system has been implicated in the pathophysiology of a diverse range of human diseases including cancer, diabetes, cardio diseases, ageing process, immune-depression and other degenerative conditions [1]. Free radicals (super oxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and proxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [2, 3]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurogenerative diseases and others [4-9]. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [10-12]. Hence propensity to scavenge free radicals can be used effectively in mitigating the oxidative stress process. The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders. Recently, there has been growing interest in natural antioxidants of plant origin because they have greater application in the food industry for increasing the stability and shelf life of food products. Moreover, they also find use as nutraceuticals and phytoceuticals as they have significant impact on the status of human health and disease prevention [13]. Hence the present study was undertaken to establish the antioxidant capacity of a few selected wild cucurbitaceae plants by using the DPPH radical scavenging assay.

MATERIALS AND METHODS

Selection, identification and processing of plant material [14]

For the present study, the following three plants viz., *Corallocarpus epigaeus, Mukia, Diplocyclos palmatus* and *Lagenaria siceraria* belonging to the family Cucurbataceae were selected. These plants were collected from Mysore (Karnataka) and Chittor (Andra Pradesh) districts in the month of April and September where they were growing profusely. The plants have been identified and authenticated by experts from National Ayurveda Dietetics Research Institute, Bangalore.

The collected plant materials were washed with running tap water and shade dried (± 28° C). The dried samples were powered using a stainless steel mixer grinder. After

pulverization, the powder was sieved with a commercial sieve of mesh size approximately 0.3mm to make the particle size uniform and labeled and stored in airtight bottles for further use.

Extraction of plant material [15]

The plant materials were extracted with methanol using soxhlet extraction apparatus continuously for 16 hours. For extraction, the dried plant material was used. Initially 50g of plant material was packed in filter paper and loaded into the thimble of soxhlet apparatus. 300ml of solvent was poured into the flask (distilling pot) and the whole apparatus was set. The soxhlet extraction was performed for 12- 16 hours until the collected solvent in siphon tube appears to be clear. Later the extracted solvent was evaporated under reduced pressure using a rotary vacuum evaporator to get solid/ semi solid extract. The extracts were dried, weighed and physical characters were noted. The extracts were suitably labeled and stored in clean and dry specimen bottles.

DPPH Assay [16]

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with hydrogen donor changes to yellow color. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in methanol and the decrease in absorbance was measured at 490nm.

Reagents

2, 2–Diphenly 1-Picryl Hydrazyl solution (DPPH, 100μM): 22mg of DPPH was accurately weighed and dissolved in 100ml of methanol. From this stock solution, 18ml was taken and diluted to 100ml using methanol to obtain 100μM DPPH solution.

Preparation of test solutions: 21mg each of the extract was dissolved in distilled DMSO separately to obtain solutions of 21mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations.

Preparation of standard solutions: 10mg each of ascorbic acid or rutin were weighed separately and dissolved in 0.95ml of Dimethyl sulfoxide (DMSO) to get 10.5mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

Procedure: The assay was carried out in a 96 well microtitre plate. To 200μl of DPPH solution, 10μl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 1000,

500, 250, 125, 62.5, 31.25 and 15.625, 7.812μg/ml. The plates were incubated at 37^{o}C for 30 min and the absorbance of each solution was measured at 490nm, using a microplate reader.

RESULTS AND DISCUSSION

The methanolic extract of whole plant of *Diplocyclos palmatus* exhibited potent antioxidant activity against DPPH radical followed by the roots of *Corallocarpus epigaeus* and fruits of *Lagenaria siceraria* when compared to the standard antioxidant rutin.

Scavenging activity of free radicals of 2, 2-Diphenyl 1-Picryl Hydrazyl assay has been widely used to evaluate the antioxidant activity of many of the natural products from plants [13]. The whole plant of *Diplocyclos palmatus* exhibited strong DPPH radical scavenging activity with the determined IC_{50} value of 108. 33 ± 2.89, followed by the roots of *Corallocarpus epigaeus* (728.33 ± 2.89) and fruits of *Lagenaria siceraria* (846.67 ± 2.89) when compared to the standard antioxidant rutin.

Diplocyclos palmatus is commonly known as Lollipop climber and marble vine, Shivalingi and Bankakra in Hindi and Linga konde balli in Kannada. In the traditional system of medicine the different parts of the plant (leaves, stem, flower, seeds and even whole plant) are used in the treatment of various ailments such as jaundice, inflammation and fever [17]. In Ayurveda the fruits are used in the area of reproductive medicine (female infertility, aphrodisiac, tonic, leucorrhoea) [18].

The chloroform and methanolic extracts of aerial parts of *Diplocyclos palmatus* contained alkaloids, flavonoids, tannins, saponins, diterpenes, triterpenoids, glycosides and steroids. Phenols were present in methanolic extract only. Petroleum ether extract contained tannins, triterpenoids, glycosides and steroids. Anthraquinones were totally absent in all the three extracts [14].

The flavonoids are known to inhibit tumor growth and also serve to protect against gastrointestinal infections and are of pharmacognostic importance thus giving evidence of the use of these plants in ethnomedicine. Some of these bioactive compounds which are synthesized as secondary metabolites as the plant grows also serve to protect the plant against microbial attacks and predation by animals. The increasing reliance on the use of medicinal plants by a sizeable proportion of the people in the so-called industrial world has been traced to the extraction and development of several drugs and chemotherapeutic agents from these plants as well as from traditionally used herbal remedies [19].

The results of the present study support the folkloric use of these three plants viz., *Diplocyclos palmatus, Corallocarpus epigaeus* and *Lagenaria siceraria* in the treatment and

management of cancer. Flavonoids have been reported to expert multiple biological effects such as anti-inflammatory, anti-allergic, anti-viral and anti-cancer activities [13].

Thus, the findings of the present study reinforce the fact that among the three plants tested for the antioxidant properties, *Diplocyclos palmatus* possess potent antioxidant and anti-cancer properties. However, to know the exact mode of action underlying the properties, further investigations are to be carried out to isolate & characterize the specific bio-active principles that can be effectively used in the treatment and management of cancer.

Table 1: Antioxidant activity of *Diplocyclos palmatus, Corallocarpus epigaeus* and *Lagenaria siceraria* plant extracts

Sl. No.	Samples	IC_{50} values ± SE µg/ml by DPPH method
1	DpW	108.33 ± 2.89
2	CeR	728.33 ± 2.89
3	LsF	846.67 ± 2.89
4	Rutin*	3.91 ± 0.10

DpW- *Diplocyclos palmatus* Whole plant; CeR- *Corallocarpus epigaeus* Roots; LsF- *Lagenaria siceraria* Fruits; IC_{50}-Half Maximal Inhibitory Concentration; SE- Standard Error; µg/ml-Microgram per Milliliter; DPPH- 2, 2–Diphenly 1-Picryl Hydrazyl and *- Standard

Figure 1: Plot of IC_{50} values for the DPPH radical scavenging activity of whole plant of *Diplocyclos palmatus* (Data from Table 1)

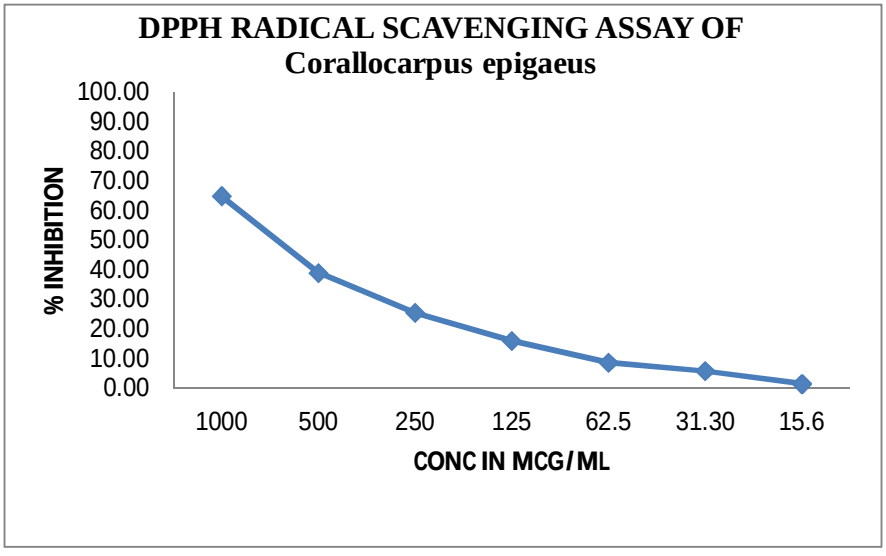


Figure 2: Plot of IC_{50} values for the DPPH radical scavenging activity of roots of *Corallocarpus epigaeus* (Data from Table 1)

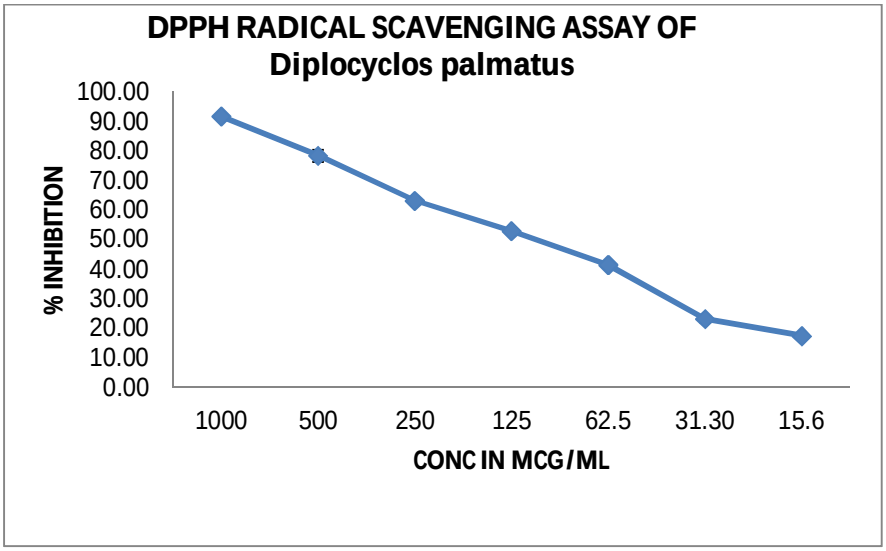
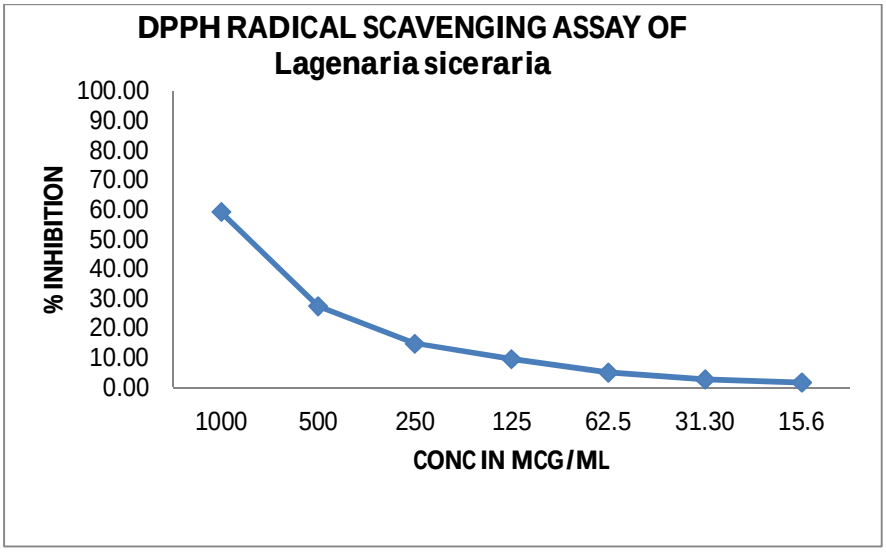


Figure 3: Plot of IC_{50} values for the DPPH radical scavenging activity of fruits of *Lagenaria siceraria* (Data from Table 1)



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