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Research Article

FREE RADICAL SCAVENGING AND ANTIOXIDANT POTENTIAL OF *FICUS LACOR* BUCH. HUM.

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ABSTRACT

Objectives: The objective of the present study was to evaluate the antioxidant potential of ethanol extract from aerial roots of *Ficus lacor.*
Methods: Ethanol extract of aerial roots of *Ficus lacor* was used to study their total phenolic and flavonoids contents, in vitro antioxidant including radical scavenging of 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide (NO).
Results: The ethanol extract showed significant results Total phenolic contents were estimated to be equivalents to 46.65 ± 1.75 mg of gallic acid equivalent and total flavonoids contents were 38.42 ± 2.46 mg of catechin/g equivalent. The percentage of DPPH and NO scavenging activity increased with increasing various concentration of extract.
Conclusions: The results concluded that the extract have potential source of antioxidants of natural origin that could have great importance as therapeutic agents for biological system liable to free radical mediated reactions.

Keywords: Antioxidant, *Ficus lacor,* DPPH, NO, phenolic content, flavonoids content

INTRODUCTION

Plants are used as medicine around the world and plant based medicine has been the stronghold of traditional societies in dealing with health problems [1]. The World Health Organization has estimated that 80% of the population rely upon traditional medicine for their primary health care needs [2, 3]. The overall, medicinal plants are the backbone of the traditional medicine. Plants are a natural source of biologically active compounds known as phytoconstituents [1, 4]. The phytoconstituents have been found to act as antioxidants by scavenging free radicals, and many have therapeutic potential for free radical associated diseases. Reactive oxygen species (ROS) like hydrogen peroxide, superoxide anions, hydroxyl radicals, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various types of diseases [5]. In healthy individuals, the production of free radicals is balanced by the antioxidative defence system of body. The oxidation of lipids, DNA, proteins, carbohydrates, and other biological molecules by toxic ROS may cause DNA mutation and serve to damage target cells or tissues, and this often results in cell senescence and death [6]. Biological antioxidants are natural molecules, which can prevent the uncontrolled formation of free radicals and activated oxygen species [7]. Biological antioxidants include anti-oxidative enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, and small non-enzymatic antioxidant molecules, such as glutathione and vitamins C and E [8]. In recent years there has been an increasing interest in finding natural antioxidants since they can protect the human body from oxidative damage [9]. In the prevention of overproduction of free radicals in biological systems, the reinforcement of endogenous antioxidants via intake of dietary antioxidants (mainly from plant sources) may be of particular importance in decreasing the cumulative effects of the oxidatively damaged molecules [10].

The traditionally used medicinal plants look forward to such standardization and the medicinal properties of plants have also been investigated in the light of recent scientific developments throughout the world, due to their effective pharmacological activities, low toxicity and economic viability [11]. Some of these plants have shown potent antioxidant activity [12]. However, majority of plants have not yet been evaluated for such activity. So, in order to contribute further the knowledge of Indian traditional medicinal plants, our present study is focussed on anti-oxidant activity of ethanol extract isolated form aerial roots of *Ficus lacor.* It is also konown as *Ficus infectoria* Roxb. It is locally known as pilkhan. It is widely distributed in tropical and subtropical regions of the world. It also grows in various humid regions in India . The bark of the plant in traditional system of India is used for treatment of ulcers, for expelling round worms, and for treatment of leucorrhoea. The leaves are also used for treatment of various skin problems [13]. This is first ever antioxidant and free radical scavenging activity on aerial roots of *Ficus lacor.*

Materials and Methods

Plant Collection

The plant of *Ficus lacor* aerial roots were collected during the month of the July 2009 from Panchkula Sector-17 (Haryana), North India. The plant material was taxonomically identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum division, with ref.no. NISCAIR/RHMD/Consult/2010-11/1638/236. The voucher specimen has been deposited in the herbarium section of the Phytochemistry and Pharmacognosy Division, Chitkara College of pharmacy, Chitkara university, Panjab for further reference.

Preparation of Extracts

The dark brown coarse powder 500g was extracted with ethanol in sohxlet apparatus 72 h. The dark brown mass of extract (20.48g) was obtained by concentrating ethanol extract in rotary vacuum evaporator.

Preliminary Phytochemical Studies

The Ethanol extract screened for preliminary phytochemical studies [2]. The presence of flavonoids, saponins, phenolic compounds, and sterol in extract were observed.

METHODS FOR SCREENING OF ANTIOXIDANT ACTIVITY:

Total phenolic content

Total phenolic content in the extract was determined using the Folin-Ciocalteu's reagent (FCR) according to Molan et al. [14]. Each sample (0.5 ml) was mixed with 2.5 ml of FCR (diluted 1:10, v/v), and 2 ml of Na2CO3 (7.5%, w/v) was added. The absorbance was then measured at 765 nm after incubation at 30ºC for 90 minutes. Results were expressed as gallic acid equivalents (mg of gallic acid/g of dried extract).

Total flavonoids content

The total flavonoid content of ethanol extract was determined using a colorimetric method [15]. Briefly, each sample (0.5 ml) was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a NaNO2 solution (15%, w/v). After 6 minutes, 0.15 ml of an AlCl3 solution (10%, w/v) was added and allowed to stand for 6 minutes, then 2 ml of NaOH solution (4%, w/v) was added to the mixture. Instantaneously, water was added to bring the final volume to 5 ml, and then the mixture was carefully mixed and allowed to stand for another 15 minutes. Absorbance of the mixture was determined at 510 nm versus prepared water blank. Results were expressed as catechin equivalent (mg of catechin/g of dried extract).

Free radical scavenging activity

Scavenging activity of diphenyl- 2-picrylhydrazyl (DPPH) radicals of ethanol extract or catechin was measured according to the method reported by Molan et al., [14], with minor modifications. Assays were performed in 3 ml reaction mixtures containing 2.0 ml of 0.1 mM DPPH-ethanol solution, 0.9 ml of 50 mM Tris-HCl buffer (pH 7.4), and 0.1 ml of deionized H2O (as control) or test plant extracts. After 30 minutes of incubation at room temperature, absorbances of the reaction mixtures at 517 nm were taken. The inhibitory effect of DPPH was calculated according to the following formula:

Percentage inhibition=[(Absorbance control –Absorbance sample)/Absorbance control] × 100

NO scavenging activity

The scavenging effect of ethanol extract on NO was measured according to Marcocci et al., [16]. Briefly, sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.4) was mixed with different concentrations of the test sample (100-1000 mg/ml) and incubated at 25 ºC for 150 minutes. After incubation, nitrite produced from sodium nitroprusside was measured by Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% 1-naphthylethylenediamine dihydrochloride in water). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with 1-naphthylethyelenediamine dihydrochloride was immediately read at 570 nm. Catechin was used as a positive control. The percentage of NO scavenging was calculated using the following formula:

Percentage inhibition = [(Absorbance control –Absorbance sample)/Absorbance control] × 100

Results and Discussion

Phenolic and Flavanoid contents

Phenolics are the majority wide spread secondary metabolite in natural drugs. The antioxidant activity of phenols are mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [17]. 1995). Total phenolic were estimated to be equivalents to 46.65 ± 1.75 mg of gallic acid. Flavonoids are a class of plant phenolics with prevailing antioxidant properties [18]. Total flavonoid contents were 38.42 ± 2.46 mg of catechin/g of dried aerial roots extract, respectively.

Free radical scavenging activity

The DPPH antioxidant assay is based on the ability of DPPH a stable free radical, to decolorize in the existence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. The percentage of free radical scavenging activity of alcoholic extract and ascorbic acid determined at different concentrations (10, 20, 40, 80 and 160) and at higher concentration it was found to be 49.18% and 62.24% for ascorbic acid respectively as shown in table 1. The absorbance was decreased as concentration of extract increasing as shown in table 1.

Table 1: Free radical scavenging activity of ethanol extract

Sr. No	Concentration (µg/ml)	Absorbance (517nm)		DPPH % inhibition	
		Ethanol Extract	Ascorbic acid	Ethanol Extract	Ascorbic acid
1.	10	0.540	0.503	11.48	17.38
2.	20	0.512	0.465	16.07	23.79
3.	40	0.480	0.405	21.32	33.60
4.	80	0.410	0.314	32.35	44.10
5.	160	0.308	0.225	49.18	62.24

*** Values are expressed as mean ± SEM of 3 observations**.

NO scavenging activity

The NO scavenging activity was observed by various concentrations (6.91%, 15.13%, 23.19%, 30.31 % and 52.26% at extract concentrations of 100, 200, 400, 800 and 1000 µg/ml, respectively). This is shown in figure 1. Incubation of a sodium nitroprusside solution in phosphate buffered saline at 25 ºC for 150 minutes resulted in linear time-dependent nitrite production, which was reduced by ethanol extract in a concentration-dependent manner. The absorbance was decreased as concentration of extract increasing as shown in figure 2.

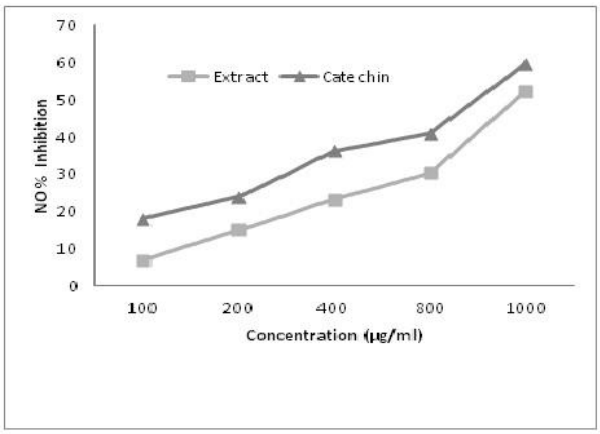

Figure 1: NO % scavenging activity of Ethanol extract of Ficus lacor aerial roots in different concentrations (µg/ml) comparison with standard (Catechin), NO = Nitric oxide

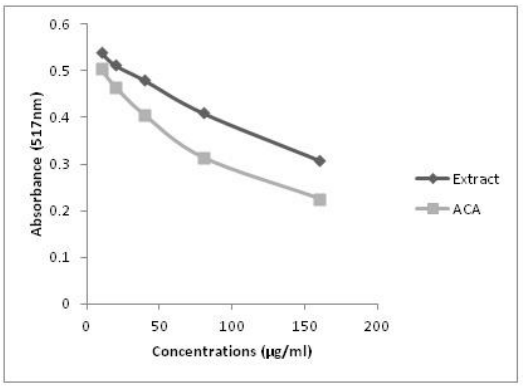

Figure 2: Absorbance of NO scavenging activity of Ethanol extract of Ficus lacor aerial roots at different concentrations (µg/ml) comparison with standard (Catechin), NO = Nitric oxide.

CONCLUSION

The alcoholic extract of aerial roots of *Ficus lacor* had shown very significant total phenolic content, total flavanoids content and free radical scavenging activity by DPPH and NO methods. The results concluded that the extract have potential source of antioxidants of natural origin that could have great importance as therapeutic agents for biological system liable to free radical mediated reactions. The above results proved the effectiveness of ethanol extract for its antioxidant activity. Further investigation on the isolation and identification of antioxidant component in this plant may show the way to chemical entities with potential for clinical use.

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