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Phytochemical Estimation and Antioxidant Potential of *Cinchona officinalis* L. Stem Bark Extracts

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Abstract

Stem bark of Cinchona sp. (Rubiaceae) is one of the well known drugs for its therapeutic values in traditional as well as modern medicine. Even though a lot of work has been carried out on quinoline alkaloids of Cinchona, its phenolic constituents received very little attention. The aim of the present study was to evaluate *in vitro* antioxidant activities, qualitative and quantitative phytochemical analysis of *Cinchona officinalis* L (*C. officinalis*) stem bark collected from Bhopal region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolic content were determined by the well-known test protocol available in the literature. The *in vitro* antioxidant activity of aqueous extract of the stem bark was assessed against DPPH radical scavenging assay methods using standard protocols. Phytochemical analysis revealed the presence of, carbohydrates, flavonoids, diterpines, saponins, protein and phenols. The total phenolic content of aqueous stem bark extract of *C. officinalis* was found to be 0.548mg/100mg respectively. The activities of aqueous extracts against DPPH assay method were concentration dependent. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *Cinchona officinalis* L, Rubiaceae, Antioxidant activity, DPPH assay method.

INTRODUCTION

Indian medicinal plants are considered a vast source of several pharmacologically active principles and compounds, which are commonly used in home remedies against multiple ailments[1]. Reactive oxygen species (ROS) are highly reactive molecules which may be both important mediators of some physiological functions and also potential prooxidants. Imbalance between ROS generation and antioxidant capacity induces a condition known as oxidative stress which may play a major role in the initiation and progression of numerous pathologies including cardiovascular dysfunction associated with vascular disease, hyperlipidemia, diabetes mellitus, hypertension and ischemia/reperfusion injury. The potential damage caused by an excess of ROS is controlled by a series of antioxidant defence mechanisms and among them, a key protective role is played by the antioxidant enzymes gluthatione (GSH) peroxidase, superoxide dismutase (SOD) and GSH reductase[2]. Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage[3]. These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation[4, 5].

Cinchona is a large medicinal plant. Cinchona plant lives in the tropical rain forest region. Cinchona is believed to originate from the Andes Mountains in South America. It is believed that the name of cinchona was taken from the name of a Royal princess in Peru in 1638 the princess affected Malaria. After cinchona treatment the princess was recovered from malaria[6]. In India cinchona is widely distributed throughout the Western Ghats (Nilgiris hills in Nilgiri district) and Annamali hills (in Coimbatore district) in tamilnadu) and it is also grown in west Bengal. Cinchona is commonly known as Jesuits bark, Peruvian bark, koina plant etc. Among all other countries Indonesia is largest producer of cinchona[7]. Cinchona is an evergreen plant, growing 5m to 15m in height. The plant is simple and oppositely arranged (simple panicle) leaves. Cinchona flowers are small and are rose or creamy white in colour. Flowers are in terminal clusters, the fruit capsule contain numerous seeds[8]. From ancient times this medicinal plant used for curing many diseases. During Second World War cinchona was the only effective drug against malarial infection[9]. But today the growth of these plantations decreased. Cinchona Bark has been used as traditional medicine for thousands of years. Cinchona plant is mainly cultivated for its Bark and it is considered as the most useful bark medicine. Cinchona Bark rich in alkaloids, Phytochemicals and other acids etc. Different types of alkaloids present in cinchona bark[10]. Alkaloids present in the cinchona are collectively referred to as quinoline. *Cinchona officinalis*, Peruvian bark, is a family member of Rubiaceae, originally from South America. The bark is mainly used for medicinal purposes and possesses a strong antimalarial effect. It consists of 16% quinine, 15% alkaloids, 0.25% to 3.0% cinchonidine quinidine, cinchonine, and cinchonine in

combination with other vigorous compounds like tannin. In addition, minerals, essential oils, acids, flavonoids, and phytosterols have also been identified. Its medicinal effects include antimicrobial factors, antiarrhythmic, anti-obesity, antioxidant, anti-inflammation, and anticancer properties. Quinine works on cancer cells by inducing apoptosis and preventing cell proliferation depending on dose and duration[11]. The present study was focused to evaluate the phytochemical analysis and antioxidant activity of stem bark *C. officinalis*.

MATERIAL AND METHOD

Plant material

Stem bark *C. officinalis* free of diseases were collected from local market in separate sterile bags from Bhopal, Madhya Pradesh. Plant material (Stem bark part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Defatting of plant material

150 gram shade dried powder of stem bark of *C. officinalis* was extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction with aqueous solvents by maceration method

Plant material was extracted with aqueous solvent. Powdered plant materials were extracted by maceration method. The resultant content was filtered with whatman filter paper no.1 and kept for evaporation of solvent to get the dry concentrated extract. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis[12].

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures[13, 14]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Total phenol determination

The total phenolic content was determined using the method of Joshi *et al.,* 2019[15]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Antioxidant activity

DPPH radical scavenging assay

DPPH scavenging activity was measured by modified method of Dutta *et al.,* 2020[16]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher is the antioxidant activity.

RESULTS AND DISCUSSION

The stem bark of *C. officinalis* was collected from the local market of Bhopal, MP, India. Air-dried and extracted by maceration extraction process. The crude extracts so obtained after each of the maceration extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the stem bark of the plants was founds to be 7.357% w/w. The results of qualitative phytochemical analysis of the crude powder stem bark of *C. officinalis* are shown in Table 2. Aqueous extracts of stem bark sample of *C. officinalis* showed the presence of carbohydrates, flavonoids, diterpines, saponins, protein and phenols. The total phenolic content of the extracts was expressed as percentage of gallic acid equivalent per 100 mg dry weight of sample. The total phenolic estimation of aqueous extracts of stem bark of *C. officinalis* showed the content values of 0.548 Table 2 & Figure1. DPPH radical scavenging assay measured hydrogen donating nature of extracts[17]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *C. officinalis* aqueous extract was found to be 71.72µg/ml as compared to that of ascorbic acid (16.59µg/ml). A dose dependent activity with respect to concentration was observed Table 3.

Table 1 Phytochemical evaluation of *C. officinalis* stem bark extracts

S. No.	Constituents	Aqueous extract
1.	Alkaloids Hager's Test:	 -ve
2.	Glycosides Legal's Test:	 - ve
3.	Flavonoids Alkaline Reagent Test: Lead acetate Test:	 + ve + ve
4.	Diterpenes Copper acetate Test:	 + ve
5.	Phenol Ferric Chloride Test:	 + ve
6.	Proteins Xanthoproteic Test:	 + ve
7.	Carbohydrate Fehling's Test:	 + ve
8.	Saponins Froth Test:	 + ve

Table 2 Results of phenolic content of stem bark extracts of *C. officinalis*

S. No	Extracts	Total phenolic content (mg/ 100 mg of dried extract)
3	Aqueous	0.548

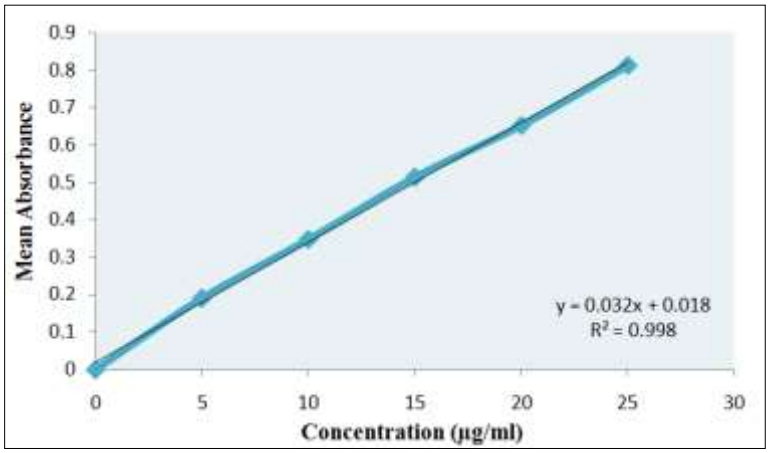

Figure 1: Graph of estimation of total phenolic content

Table 3 % Inhibition of ascorbic acid and aqueous extract of *C. officinalis* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Aqueous extract
1	10	46.54	23.23
2	20	50.32	37.45
3	40	67.21	44.67
4	60	71.23	48.62
5	80	79.41	55.36
6	100	86.13	61.54
	IC_{50}	16.59	71.72

CONCLUSION

It can be concluded that from present investigation the phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The total phenolic content in aqueous stem bark extract is further proved by in vitro antioxidant studies. The extract, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated in this study clearly indicates the potential application value of the plant. Further studies, on the use of above plant for their antioxidant role in various systems may provide potential natural antioxidants.

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