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Research Article

PHYTOCHEMICAL SCREENINGAND EVALUTION OF ANTIOXIDANT POTENTIAL OF *CRYPTOCARYA STOCKSII* PLANT EXTRACTS

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ABSTRACT

Cryptocarya stocksii is an important medicinal plant which belongs to the family Lauraceae. This plant is exclusively found in the Western Ghats regions of Karnataka State, India.Although this endangered plant has been found to possess pharmacological potential, there are no systematic reports on the phytochemical content and radical scavenging activity.The phytochemical analysis of *C. stocksii* extracts revealed the presence of significant levels of alkaloids, flavonoids and moderate amounts of steroids, and phenols. Further, the chloroform and ethanolic extract possesses significant free radical scavenging property and a clear correlation exists between the antioxidant activity,phenolic content and flavonoid content.

Keywords: *Cryptocarya stocksii*, DPPH, antioxidant, Phosphomolybdenum and phenolic compounds.

INTRODUCTION

Aerobic organisms produce a number of reactive free radicals (molecules or atomshaving unpaired electrons) continuously in cells during respiration, metabolism and phagocytosis. Out of these, the most important source of free radicals being produced by respiratorychain. Free radicals are produced by oxidation reaction, involves transfer of electrons from substance to an oxidizing agent, that results in initiation of chain reactions,damaging different cellular components, which includes nucleic acids and enhance a number of degenerative diseases such as atherosclerosis, cancer, prematureaging, cardiovascular disease,neurodegenerative disease,including Alzheimer's disease,Parkinson's as well as inflammation caused by cutaneous aging[1-3].

The antioxidant protectionis the key cell machinerycarried by neutralising the damaging effects of free radicals, through endogenous enzymatic defence system such as super oxide dismutase(SOD),glutathione peroxidase(GPX) and catalase(CAT) are present in human system.Free radicals may be either oxygen derivedreactive oxygen species(ROS)or nitrogen derived reactive nitrogen species(RNS)[4].

Since time immemorial, plants are considered as potent biochemical factories and have been the major components of phytomedicines.Various phytochemicals and their effects on health, especially supressing active oxygen species by natural antioxidantsfrom teas, spices and herbs have been intensively studied[4]. Flavonoids and phenoliccompounds are found to be effective plant raw materials, particularly in herbs and fruits,seeds[1]. Much attention has been focussed in screening the medicinal plants with potential antioxidant properties,due to their increasing concern for safe and non-toxic alternative antioxidants[2]. In this regard,efforts

are being made, by systematic screening of the plants to screennovel phytochemicals present in different parts of the plant.

In India, the Western Ghatsare the rich repositories of medicinal plants.Several medicinal plants that are indigenous to this region have been in use by local traditional healer to cure various diseases. Among these, *C. stocksii* is an endemic plant that is exclusively present in WesternGhats, belongs to the family lauraceae.Nearly 250-350 species are located in tropical and subtropical regions of the world.*C. stocksii* is a tall plant of 10-15 feet height that is located in Bababudangiri hills region of Western Ghats, Karnataka, India.Till today, there is no data regarding the isolation and characterization of active components present in this plant.In the present study, an attempt has been made to systematicallystudy the extracts of *C.stocksii* plant for its application in the pharmacy.Thus the objective of this study is to investigate the antioxidant capacity and estimation of various phytochemicals of the plant.

MATERIALS AND METHODS

Plant material

Bark of *C. stocksii* plant was collected from the Western Ghats of Chikmagalore district, Karnataka, India. The plant material was identifiedand Voucherspecimen was deposited in department for future reference.

Preparation of the plant extracts

The bark of the plant was washed,shade dried,powdered mechanically, and subjected to Soxhlet extraction using petroleum ether(60-80ºC), chloroform, and ethanol, successively for 48 hrs till the solvent in the thimble becomes colourless.The solvent was recovered using rotatory vacuum evaporator under reduced pressure and the extract was stored at 4ºC till use.The crude extract thus obtained was used for further investigations.

Phytochemical screening

The phytochemical analysis of all the three extracts was carried out to detect the presence of secondary metabolites such as flavonoids, tannins and steroids, using standard phytochemical screening procedure[6].

Total phenolic content

The total phenolic content of the extracts was determined by Folin- Ciocalteu reagent(FCR) method.In brief, a diluted extract(0.5ml) was mixed with 0.5ml of FC reagent (1:1diluted) and 1ml of sodium carbonate (20%). The reaction mixture was allowed to stand for 30 minutes and optical density was read at 765nm.A standard curve was plotted using different concentration of Gallic acid(0-200µg/ml) and the phenolic content was expressed as mg of gallic acid equivalents (GAE/100mg) of dried extract[7].

Total flavonoid determination

Aluminium chloride method was used forflavonoid determination[8]. Briefly, 0.5 ml of each plant extracts were separately mixedwith 4.5 ml of distilled water. To this, 0.3ml of 1M Na_2NO_3 was added, followed by 0.3 ml of 10% aluminium chloride, and kept at room temperature for 6 min. Further, 2.0 ml of1M NaoH and 3.4ml of distilled waterwere added and kept at room temperature for 30minutes. The absorbance of the reaction mixture was then measured at 510 nm. The total flavonoid contents wascalculated as catechol equivalents from a calibration curve using different concentrations of Catechol (10-100µg/ml) and flavonoid content of *c.stocksii* bark was expressed as catechol equivalents/100mgof extract[8].

Free radical scavenging activity (DPPH method)

The antioxidant activity of the plant extracts and standard were assessed on the basis of the radicalscavenging effect of the stable DPPH free radical[10].About 10-100 µl of each extract or standard was added to 3 ml of DPPHin methanol (0.33%) in a test tube. After incubation at 37ºC for 30 minutes the absorbance of each solution was determined at 517 nm.The corresponding blank reading were also measured and the DPPH scavenging activity was calculated by using the followingformula,

% DPPH radicalscavenging activity = [Abs (control) – Abs (test sample)/Abs of control]×100.

Where, Abs (control): Absorbance of DPPH radical +methanol, Abs (standard): Absorbance of DPPH radical + extract/standard.

FRAP method

The reducing powers of the extracts were determined by ferric reducing assay[12]. Different concentrations of the extracts of *C.stocksii* (20–100 µg/ml) in 1.0 ml of methanol were mixed with phosphate buffer (0.5ml, 2 M, pH 6.6) and potassium ferricyanide (0.5 ml, 1%). The mixture was incubated at 50ºC for 20 min. A portion of trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water

(2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%). The absorbance was measured at 700 nm and compared with standards. Increased absorbance of the reaction mixture indicated increased reducing power of the extracts.

Evaluation of total antioxidant capacity (TAC)

The total antioxidant capacity of the extracts was evaluated by thePhosphomolybdenummethod. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acidicp[H]. A 0.5 ml extract was combined with 5 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of reaction mixture was measured at 695nm against blank after cooling to room temperature. Methanol (0.5 ml) in the place of extract was used as the blank. The antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic (20, 40, 60, 80, and 100 µg/ml) with methanol[9].

RESULTS

Phytochemical analysis

The preliminary phytochemical screening of the extracts revealed the presence of alkaloids, flavonoids, tannins, andsteroids in the bark extracts of *C.stocksii*. Ethanol extract shows the significant amount of alkaloids,flavonoids, and steroids.Chloroform extract exhibited moderate amount of alkaloids,flavonoids and steroids. Alkaloids and terpenes are absent in petroleum ether extract.The summary of these results aredepicted in Table1.

Total phenolic and flavonoid content

Polyphenolic phytochemicalsare widely found in food products derived from plant sources, andthey have been shown to possess significant antioxidant activities. The ethanolic extract of *C.Stocksii* shows high phenolic and flavonoid content followed by Chloroform and Petroleum ether extracts.The results are shown in the Table 1and 2.

DPPH radical scavenging activity

The stable DPPH method was widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity[5].Nitrogen centeredcolour of DPPH changes from violet to yellow upon reduction by either the process of H^+or electron donation.The more rapid absorbance decreaseindicates the more potent antioxidant activity in terms of hydrogen donating ability[16].The bark extracts showed dose dependent scavenging of free radicals.Among all the extracts,ethanol extract showed 81% and chloroform extracts showed 67.7% of scavenging activity followed by pet ether extracts which is 35%, compared with standard ascorbic acid of 96.2% (Figure1).

Reducing power assay

In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+} to $Fe^{2+}$by donating an electron[2]. The presence of reductants in *C. stocksii* barkcauses thereduction of the ferricyanide(Fe^{3+}) complex to the ferrous(Fe^{2+}) form. Therefore, the Fe^{2+}can be monitored by measuring the formation ofPerl's Prussian blue at 700nm. The increase absorbance of the reaction indicated the increased reduceing power. From figure 2it is clear that theabsorbance of ethanol extract of *C.Stocksii* increases with increase in concentration of reaction mixtureas compared tochloroform and pet ether extracts.

Total antioxidant by phospomolybdenum method

The results of total antioxidant capacity are expressed as equivalents of ascorbic acid. The Phosphomolybdenum method is animportant antioxidant assay based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of greenphosphate/Mo (V) complex with a maximal absorption at 695nm.The antioxidant capacity of bark is expressed as the number of ascorbic acid equivalents.The ethanolic extract showed high antioxidant capacity of 0.90mg/100mg of extract followed by chloroform extract of 0.43mg/100mg of extract and pet ether extract of 0.29mg/100mg of extract.This is due to presence of high content phenols in the ethanol extract, as polyphenols plays an important role as antioxidants in living systems due to the presence of hydroxyl groups in *ortho*- and *Para*positions (Table4).

DISCUSSION

The relation between disease and free radicals has been proved by many studies. Radiation, smoking, alcohol consumption stress and high cholesterol consumption can increase the process of cell oxidation[19].This study is aimed to establish a platform for *invitro* evaluation of antioxidant capacity of *C.Stocksii* barkplant extracts.

A number studies, so far, have clearly indicated a close correlation between the antioxidant capacity andthe amount of

polyphenols,flavonoidsandflavonols present in the plant. The total polyphenols play a vital role in antioxidization as well as biological functions such as anti-inflammatory,anticarcinogenic and anti-atherosclerosis,activities.These biological properties may be due to their antioxidant activities. The free radicals causes decrease in membrane fluidity, and damage to membrane protein leading to death. Phenolic compounds are known to be a powerful chain breaking antioxidants and are important constituents of plants[14]. The phenolic compounds also have inhibitory effects on mutagenesisand carcinogenesis in humans.

As presented here, the DPPH activity of the *C. stocksii* plant extracts shows dose response curve, compared with standard ascorbic acid, which may be,due to their electron donating ability. Extracts have proton donating ability and could serve as free radical scavengers, acting possibly on primary antioxidants[13].

The existence of the reductones are the key reducing power, which exhibit their antioxidant activities through the action of breaking the free radical chain by donating hydrogen atom. Increase in absorbance at 700nm indicates an increase in reductive ability of the extracts[10].Phosphomolybdenum model evaluates the both water soluble and fat soluble antioxidant capacity of the extracts. From the result, it is clear that the extract retainsreductive potential and could serve as electron donors terminating the radical chain reactions. Chloroform and ethanolic extracts showed much quantity of antioxidant activity.

CONCLUSION

On the basis of the results obtained in the present study, it is concluded that chloroform and ethanolic extracts of *C. stocksii* stem bark, which contains large amount of phenolic and flavonoids, high antioxidants and free radical scavenging activity. These result encourage the researcher to do further invitro and invivo studies, which will explore the role of bioactive constituents responsible for these activities.Hence further study is need to be carried out to isolate and identify the antioxidant compound present in the plant extract,which play an important role in curingmany health disorder related to oxidative stress and cancer.

Table 1: Phytochemical analysis of *cryptocarya stocksii* extracts

Tests	Petroleum ether	Chloroform	Ethanol
Alkaloids:			
i. Mayer's test	–	++	+++
ii.Dragendorff'stest	–	++	+++
Flavonoids:			
i.Ferric chloridetest	+	++	+++
ii. Lead acetate test	+	++	+++
(i. NaOH solution test)			
Steroids:			
i. Salkowski'stest	++	+	+++
ii. Libermann-Burchard'stest	++	+	+++
iii.Sulphur test	++	+	+++
Terpenoids:			
i.Salkowski'stest	+	+	+++
ii. Libermann-Burchard'stest	+	+	+
Tannins &phenolics			
i. Ferric chloride test	–	-	++
ii. Gelatin test	–	-	++
Saponins:			
i. Foam test	–	–	–
Proteins & amino acids			
i. Biuret test	–	–	–
ii.Xanthoproteic test	–	–	–
Quinones	+	+	+
Coumarines	+	+	-

Table 2: Total Phenolic Contents of the extract expressed in mg of Gallic acid equivalents/100mg of the dried extracts

Extracts	mg of Gallic acid equivalents/100mg of dried extracts
Pet ether	0.85
Chloroform	2.0
Ethanol	20

Table 3: Total Flavonoid content of the extracts expressed in mg of Catechol Equivalents/100mg of dried Weight of the plant material

Extracts	mg of Catechol equivalents/100mg of dried extracts
Pet ether	0.15
Chloroform	0.475
Ethanol	10.93

Table 4: Total Antioxidant by Phosphomolybdenum Method

Extracts	mg of ascorbic acid equivalents/100mg of dried extracts
Pet ether	0.29
Chloroform	0.430
Ethanol	0.90

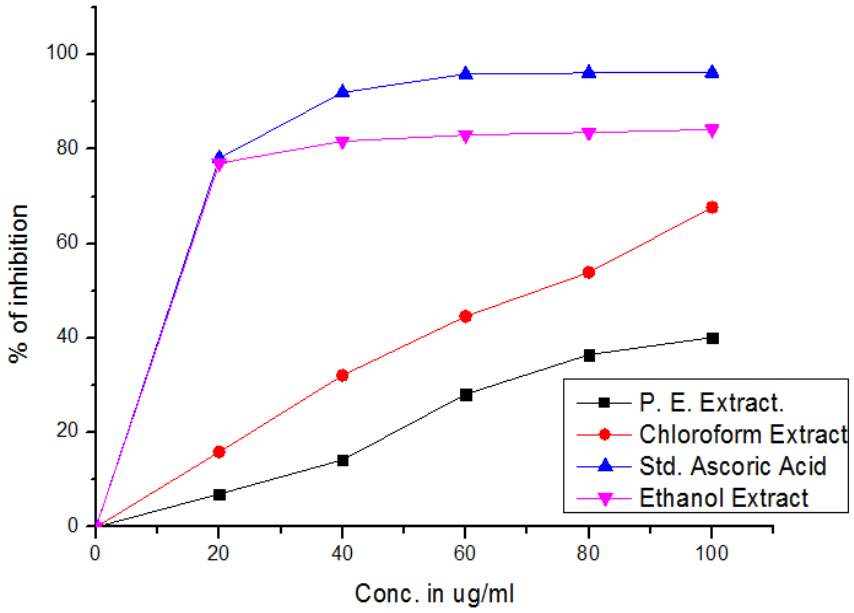

Fig. 1: DPPH Activity of *Cryptocarya Stocksii* Plant Extracts

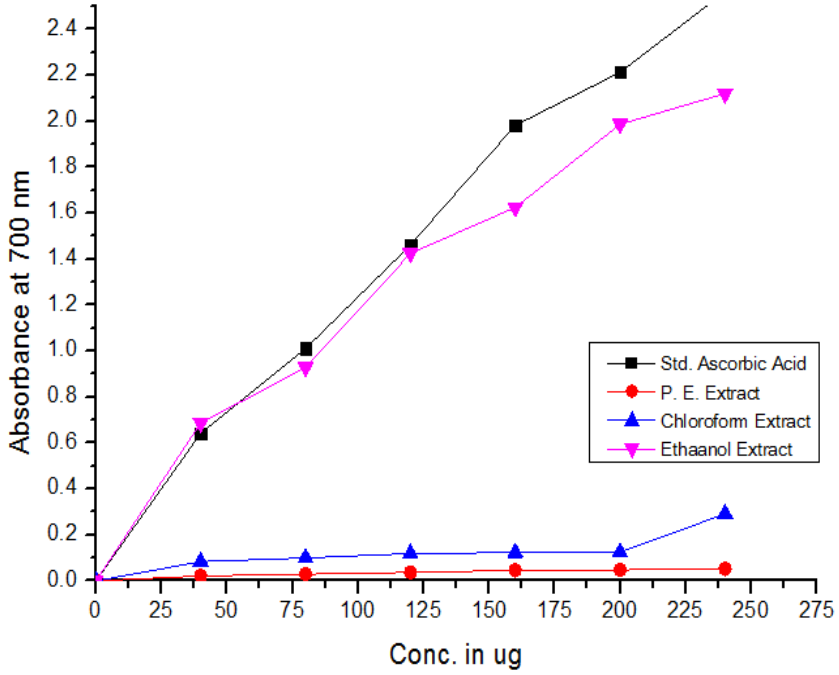

Fig. 2: Antioxidant by Ferric Reducing Power Assay

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