In vitro antioxidant studies of *Sitopaladi Churna*, a polyherbal Ayurvedic formulation

Inder Kumar Makhija, H. N. Aswatha Ram*, C. S. Shreedhara, Vijay Kumar S, Raviraj Devkar

Department of Pharmacognosy, Manipal college of Pharmaceutical Sciences, Manipal University, Manipal - 576 104, Karnataka, India

ABSTRACT

Introduction: "*Sitopaladi churna*" is a reputed Ayurvedic polyherbal medicine prescribed for pleurodynia, intercostal neuralgia, cough associated with bronchitis, supportive agent for allergy, viral respiratory infection, and in pharyngeal and chest congestion. Antioxidants play an important role in protecting cellular damage by reactive oxygen species. Plants containing phenolic compounds have been reported to possess strong antioxidant properties. **Methods:** Antioxidant potential of the *Sitopaladi churna* was studied using different *in vitro* free radical models like DPPH, ABTS, nitric oxide and superoxide scavenging radical models. **Results:** The extracts showed good dose dependent free radical scavenging property in all the models. IC_{50} values of methanolic and aqueous extracts of *Sitopaladi churna* were found to be 65.6 and 44.88 µg/ml for DPPH, 74.5 and 66.46 µg/ml for ABTS, 191.77 and 170.15 µg/ml for nitric oxide, 94.79 and 62.03 µg/ml for superoxide radical, respectively. Total phenolic contents of aqueous and methanolic extract were found to be 256.25 and 298.2 µg equivalent of gallic acid. **Conclusions:** The antioxidant potential may be directly linked to the phenolic compounds present in the ingredients of *Sitopaladi churna*. The data obtained in this study suggest a possible use of *Sitopaladi churna* as a natural antioxidant agent.

Keywords: Free radicals, antioxidant, *Sitopaladi churna*, phenolic compounds

***Correspondence:** Dr. Aswatha Ram H. N., Associate Professor, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal – 576 104, Karnataka, India.

E-mail: aswatharam@gmail.com

Mobile: +91 98454 29662

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INTRODUCTION

Oxygen is essential element for the survival of all living creatures on this earth. During the process of oxygen utilization in a normal physiological and catabolic process, approximately 5% of oxygen gets univalently reduced to oxygen derived free radicals (ROS) like superoxide anions (O_2^-), hydroxyl (.OH) nitric oxide (NO), which damage cellular components causing tissue injury through covalent binding.[1,2] Free radicals have been implicated in causation of diseases such as diabetes, inflammation, cancer, neurodegenerative disorders, atherosclerosis, liver cirrhosis, nephrotoxicity etc.[3] It has been suggested that fruits, vegetables, plants are the main source of antioxidant in the diet. Natural antioxidants may have free-radical scavengers, reducing agents, complexers of pro-oxidant metals, quenchers of singlet oxygen etc. Recently interest has been increased considerably in finding natural occurring antioxidants for use in foods or medicinal products to replace synthetic antioxidants, which are being restricted due to their adverse reaction such as carcinogenicity. Antioxidant constituents from natural resources possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance.[4-6]

"Sitopaladi churna" (SPC) is a polyherbal Ayurvedic medicine prescribed for pleurodynia, intercostal neuralgia, cold, cough associated with bronchitis, pneumonia, tuberculosis, burning sensation in extremities, supportive agent for allergy, viral respiratory infection, digestive impairment and in pharyngeal and chest congestion. The ingredients present in *Sitopaladi churna* have been reported to possess antioxidant activity.[7-9] But there is no scientific proof to support antioxidant potential of *Sitopaladi churna*. The present study therefore carried out to investigate the antioxidant potential of aqueous and methanolic extract of *Sitopaladi churna*.

MATERIAL AND METHODS

Chemicals

All chemicals and solvents were of analytical grade and were procured from Ranbaxy Fine Chemicals, Mumbai, India. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS), gallic acid were procured from Sigma Chemicals, USA. The other chemicals used were ascorbic acid, sodium nitroprusside, ammonium persulfate, sulphanilamide, potassium superoxide, dimethyl sulfoxide (DMSO), O-phosphoric acid, naphthyl ethylene diamine dihydrochloride, phosphate buffer, nitroblue tetrazolium, Folin-Ciocalteau reagent, sodium carbonate and solvent used were of analytical grade.

Plant Material

Sitopaladi churna consist of five ingredients viz., *Saccharum officinarum* (Sugar candy), *Bambusa arundinacea* (Siliceous concretion), *Piper longum* (dried fruit), *Elettaria cardamomum* (dried seed), and *Cinnamonum zeylanicumm* (Stem bark). All these ingredients were procured from the local market of Udupi, Karnataka, India, and were authenticated by botanist Dr. K. Gopal Krishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, Karnataka. A voucher specimen of the same was deposited in the museum of Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, for future reference.

Preparation of Sitopaladi Churna

The churna was prepared as per the procedure given in Ayurvedic Formulary of India.[10] All the ingredients were powdered separately, passed through 80 # sieve and then mixed together in specified proportions to get uniformity blended churna.

Preparation of Sitopaladi Churna Extract

The dried *Sitopaladi churna* (100 g) was extracted exhaustively with methanol (350 ml) at 60 °C using soxhlet apparatus for 48 h. The methanolic extract was concentrated under reduced pressure at 40 °C using a rotary evaporator and lyophilized at –40 °C to obtain a reddish-brown syrupy residue (yield 28.34% *w/w*). Aqueous extract was prepared by maceration of powdered drug in water: chloroform (98:2) for 7 days with intermittent shaking. The resulting extract was concentrated, lyophilized, to obtain a brownish residue (yield 35.98 % *w/w*). Both residues were stored in a dessicator until use.

Preliminary Phytochemical Screening

Aqueous and methanol extract of SPC samples were tested for screening of alkaloids, glycosides, flavonoids, tannins, sterols, terpenes, fixed oil, resin, protein and gums.[11]

DPPH Radical Scavenging Assay

DPPH scavenging activity was measured by spectrophotometric method. To an ethanolic solution of DPPH (200 μM), 0.05 ml of aqueous and methanolic extract dissolved in ethanol was added at different concentrations (10-100 μg/ml). An equal amount of ethanol was added to the control. After 20 min the decrease in absorbance of test mixtures (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition was calculated using the following equation: % inhibition = (control–test)/control × 100.[12]

ABTS Radical Cation Depolarization Assay

ABTS radical cations ($ABTS^{+}$) were produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium persulfate. The mixture was allowed to stand in the dark at room temperature for 12-16 hr before use. Different concentrations (10-140 μg/ml) of the aqueous and methanolic extract (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up to 1 ml with ethanol. Absorbance was read at 745 nm and the % inhibition was calculated by using the above formula.[13-14]

Nitric Oxide Radical Scavenging Assay

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction as described previously. Sodium nitroprusside (5 mM) in standard phosphate buffer solution was incubated with different concentrations (25-400 μg/ml) of aqueous and methanolic extract dissolved in phosphate buffer (0.025 M; pH: 7.4) and the tubes were incubated at 25 °C for 5 h. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 h, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess', reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride). The absorbance of the chromphore formed during

diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm.[15-17]

Superoxide Radical Scavenging Assay

The potassium superoxide scavenging activity was performed by using alkaline DMSO. Potassium superoxide and dry DMSO were allowed to stand in contact for 24 h and the solution was filtered immediately before use. The filtrate (200 µl) was added to 2.8 ml of an aqueous solution containing nitroblue tetrazolium (NBT) (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 mM). Extracts or standard at various concentrations (20-140 µg/ml) were added and the absorbance was recorded at 560 nm using pure DMSO as a control.[18-19]

Total Phenolic Content

Total phenolic content of the two extracts of SPC was determined using Folin-Ciocalteau reagent method. 0.5 ml of each extract at concentrations of (125, 250, 500, 1000 µg), 2.5 ml of Folin-Ciocalteau reagent (1/10 dilution) and 2 ml of 7.5% sodium carbonate (w/v) were added and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance of the blue color that developed was measured at 765 nm with sodium carbonate solution (2 ml of 7.5% sodium carbonate and 2.55 ml of distilled water) as control. The concentration of total phenols was expressed as gallic acid equivalents in µg/ml of dry extract.[20]

Statistical Analysis

Data were expressed as mean ± standard of triplicate determinations. Linear regression analysis was used to calculate the IC_{50} values.

RESULTS

Preliminary Phytochemical Screening

Preliminary phytochemical testing revealed the presence of constituents like alkaloid, carbohydrates, flavonoid, tannins, saponins and fats in both SPC extracts.

Antioxidant potential

In vitro antioxidant studies of aqueous and methanolic extract showed scavenging activity of DPPH, ABTS, nitric oxide generation, superoxide dismutase in a concentration dependent manner up to the given concentration in all the models. The percentage scavenging for different dosage of the two SPC extracts along with ascorbic acid equivalence are depicted in Figure 1-4.

The maximum inhibitory concentrations (IC_{50}) in different models are presented in Table 1. IC_{50} values

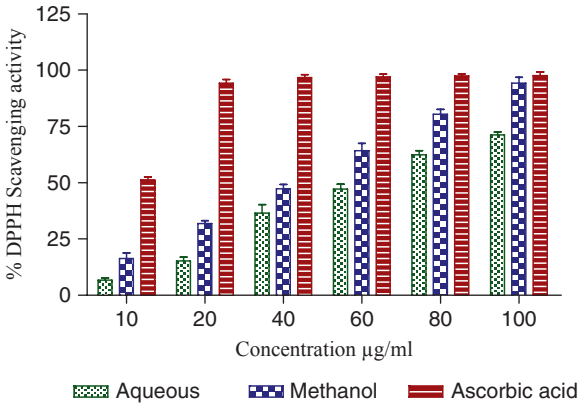

Figure 1. Percentage DPPH radical scavenging activity of aqueous and methanolic extract of *Sitopaladi churna*. Data expressed are as mean ± SD (n = 3)

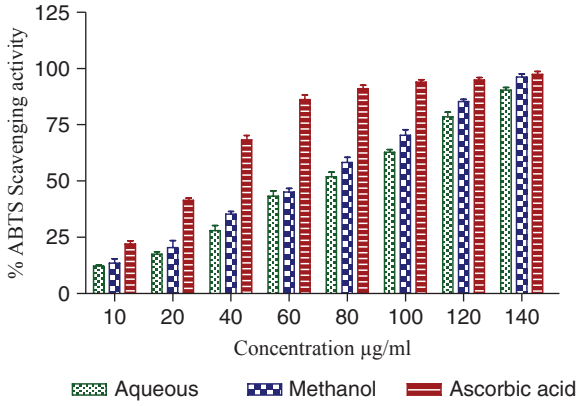

Figure 2. Percentage ABTS radical scavenging activity of aqueous and methanolic extract of *Sitopaladi churna*. Data expressed are as mean ± SD (n = 3)

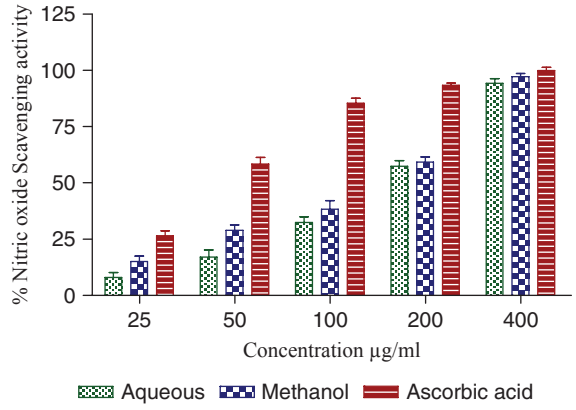

Figure 3. Percentage nitric oxide radical scavenging activity of aqueous and methanolic extract of *Sitopaladi churna*. Data expressed are as mean ± SD (n = 3)

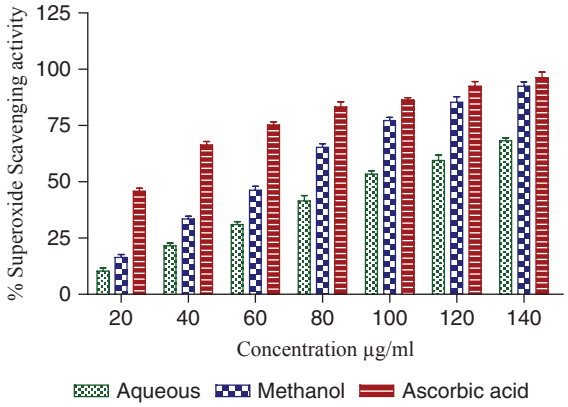

Figure 4. Percentage superoxide radical scavenging activity of aqueous and methanolic extract of *Sitopaladi churna*. Data expressed are as mean ± SD (n = 3)

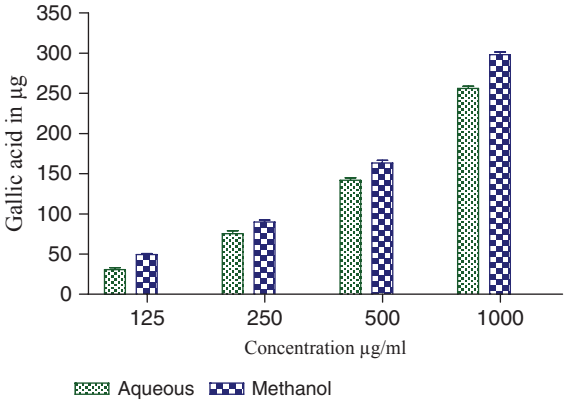

Figure 5. Total phenolic content in varying concentrations of *Sitopaladi churna* extracts. Data is given in mean ± SD (n = 3). Gallic acid of the extracts is given in µg/ml.

Table 1. IC_{50} value of aqueous and methanolic extract of *Sitopaladi churna* in different *in vitro* models

In vitro models	IC_{50} values (µg/ml)	
	Aqueous extract	Methanolic extract
DPPH	65.6	44.88
ABTS	74.5	66.46
Nitric oxide	191.77	170.15
Superoxide	94.79	62.03

of aqueous and methanolic extracts were found to be 65.6 and 44.88 µg/ml, 74.5 and 66.46 µg/ml, 191.77 and 170.15 µg/ml, 94.79 and 62.03 µg/ml for DPPH, ABTS, nitric oxide and superoxide model, respectively. The order of effectiveness of extracts in different free radical activity was DPPH>ABTS>superoxide>nitric oxide, respectively. On comparative basis, strongest activity was found in quenching DPPH and less activity in nitric oxide assay.

Total phenolic content

Phenolic compounds have been reported to be responsible for the antioxidant activity. Quantitative estimation shows that both extracts have considerably high phenolic compounds, which increase with extract concentration. Total phenolic contents of aqueous and methanolic extract (1000 µg/ml) were found to be 256.25 and 298.2 µg/g gallic acid equivalent. The total phenolic content in the different dosage of SPC extracts are depicted in Figure 5. Based on the above observations, it can be concluded that extracts of *Sitopaladi churna* may find use as supplementary antioxidant for protection arising from the oxidative stress.

DISCUSSION

Oxidative stress has been involved in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing.[3] Antioxidants may offer action against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease.[21]

DPPH is a relatively stable nitrogen centered free radical. The assay is based on the measurement of the scavenging ability of extracts towards the stable radical DPPH. The results claimed that *Sitopaladi churna* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in extract antioxidant principles.[12] DPPH radicals react with suitable reducing agents, unpaired electron of DPPH gets paired off and the solution loses colour stoichiometrically depending on the number of electrons taken up.[22] ABTS cation decolorization assay is based on the inhibition of the absorbance of the radical cation $ABTS^+$, which has a characteristic long wavelength absorption spectrum.[12] The results obtained suggest the activity of the extract either by inhibiting or scavenging activities of antioxidants towards $ABTS^+$ radicals.[23] Nitric oxide is a very unstable radical, react with oxygen to produce stable product nitrate and nitrite through intermediates NO_2, N_20_4 and N_30_4. It is estimated using Griess reagent. In the present investigation the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate saline buffer at 25 °C was reduced by the extracts of *Sitopaladi churna*. It may be due to the extract antioxidant constituents which compete with oxygen to react with nitric oxide and inhibits the generation of nitrite.[24]

Superoxide dismutase catalyses the dismutation of highly reactive superoxide anion to oxygen and hydrogen peroxide. Superoxide anion is the first reduction product of oxygen which is measured in terms of inhibition of generation of $0^{2\bullet}$.[22] In the present study, extracts showed dose dependent inhibition of generation of superoxide in the *in vitro* reaction mixture.

CONCLUSION

The present study shows the significant antioxidant potential of aqueous and methanolic extract of *Sitopaladi churna* in all *in vitro* study. Further, both extract was found to possess significant amount of total phenolic content. Different ingredients of *Sitopaladi churna* have been claimed in different studies to possess biological properties related to antioxidant mechanisms. Hence, the significant antioxidant activity of aqueous and methanolic extract in the present study may be attributed to these aforementioned potent antioxidant ingredients of *Sitopaladi churna*.

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