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Antioxidant Activity of Ethanolic Extract of *Stereospermum Colais* and *Pedilanthus Tithymaloides*

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ABSTRACT
The present study is aimed to investigate antioxidant activity of ethanolic extract of Stereospermum colais (S. colais) and Pedilanthus tithymaloides (P. tithymaloides) leaves. The anti-oxidants and free radical scavenging activity of ethanolic extract of S. colais and P. tithymaloides leaves were assayed by using in-vitro models like 1,1-diphenyl, 2-picryl hydrazyl (DPPH) and nitric oxide scavenging activity. Ascorbic acid was used as a standard. Further plants extracts were screened for the presence of phytoconstituents. Both plants exhibited potent and concentration dependent free radical scavenging activity in both test models. In DPPH method, IC_{50} values for the S. colais, P. tithymaloides and ascorbic acid were found 41.58, 209.56 and 14.33 µg/ml respectively, whereas, in nitric oxide scavenging method the IC_{50} value of S. colais, P. tithymaloides and ascorbic acid were found 48.48, 67.82 and 14.45 µg/ml respectively. The phytochemical analysis of plants extract was showed presence of flavonoids, tannins and other antioxidant components. The ethanolic extract of S. colais and P. tithymaloides leaves have appreciable antioxidant activity and can be used as a prospective source of natural antioxidant.
Keywords: *Antioxidant, DPPH, Nitric Oxide, In-vitro, Ascorbic Acid.*

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INTRODUCTION

In several biomedical fields, hepatotoxicity is a topic of great significance and interest [1]. The nature of the hepatotoxic substances, the characteristics of the injury and the mechanism of the hepatotoxic effects are complex. Numerous chemicals and drugs such as high doses of paracetamol [2], antitubercular medications [3], toxic compounds carbon tetrachloride [4], thioacetamide [5], dimethyl nitrosamine D-galactosamine [6] and excessive alcohol consumption, can have a toxic impact on the liver [7]. Several medicinal plants have been reported to possess hepatoprotection activity and scientifically investigated to identify the phytochemical constituents responsible for this activity and ascertain their effectiveness. The *Stereospermum colais* leaves are used to treat odantalgia, otalgia, malarial fever, rheumatalgia and wounds. Leaves decoction is used to treat chronic dyspepsia and as an antipyretic effect [8]. The root is one of the important ingredients in Dasamula in ayurvedic formulation [9]. *Pedilanthus tithymaloides* (*Euphorbiaceae)* used as antiviral, antibacterial, anti-hemorrhagic, antitumor, abortive, anticancer, anti-inflammatory agent, larvicidal. The leaves used for treating asthma, mouth ulcers, venereal troubles, ringworms and insect stings [10,11].

MATERIAL AND METHODS

Plant material

The fresh leaves of *Stereospermum colais* were collected from Mandya, Mysore, Karnataka and *Pedilanthus tithymaloides* leaves were collected locally and authenticated by Dr. Y. S. Sarangdevot, Professor, B.N. College of Pharmacy, Udaipur, Rajasthan. Dried leaves of plants were pulverized and extracted (500 g) with ethanol separately at room temperature for 7 days. The ethanolic extracts were filtered through a cotton plug and finally with a Whatman No. 1 filter paper. Then the extracts were concentrated by rotary evaporator (RE-2 Aditya Scientific, India) and allowed to air dry for complete evaporation of ethanol. The dried extracts were kept in desiccators for further uses [12,13].

Chemicals:
DPPH, ascorbic acid, methanol, Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, sodium nitroprusside, sulphanilic acid reagent, naphthylethylene diamine dihydrochloride and glacial acetic acid.

Phytochemical Screening
The tests were carried out on aqueous and alcoholic solution of powdered drug kept for 24 h and then filtered using Whatman filter paper. The filtrates were screened for presence of phytoconstituents [14,15].

Determination of 1,1, dipheny-2-picrylhydrazyl (DPPH) radical scavenging activities
The free radical scavenging capacity of the ethanolic extracts of *S. colais* and *P. tithymaloides* was determined using DPPH assay. The DPPH solution (0.1mM) was prepared in methanol. The different concentrations for *S. colais*, ascorbic acid (20-100 µg/ml) and *P. tithymaloides* (50-250 µg/ml) were prepared. To 0.5 ml of each dilution of the extracts was added freshly prepared DPPH solution (0.1mM). The mixture was shaken well and incubated at dark place for 20 min at room temperature. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (M1700, Shimadzu Japan). Control was prepared with equal volume of DPPH in methanol without extract and ascorbic acid. The ascorbic acid was used as a reference standard [16,17]. The percentage inhibition was calculated by the formula as follows [18]

$$\% \text{ scavenging activity} = [(A_{control} - A_{test})/A_{control}] \times 100$$

Where; $A_{control}$ is the absorbance of control and
A_{test} is the absorbance of test or standard
IC_{50} values for each extract were calculated from curve fitting method.

Determination of nitric oxide radical scavenging activity
The nitric oxide radical inhibition is estimated by use of Griess Illosvoy reaction. The Griess Illosvoy reagent was modified by using naphthylethylene diamine dihydrochloride (0.1%w/v) instead of 1-napthylamine (5%). The different concentrations for *S. colais*, ascorbic acid (20-100 µg/ml) and *P. tithymaloides* (50-250 µg/ml) were prepared. The reaction mixture 6 ml, containing 4 ml of sodium nitroprusside (10 mM), 1 ml of phosphate buffer saline (PBS, pH 7.4) and 1ml of extracts and ascorbic acid of different concentration was incubated at 25°C for 150 minutes. After incubation, 0.5 ml of the reaction mixture was mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acid) and was allowed to stand for 5 minutes for completion of diazotisation. Then, 1 ml of naphthylethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 min in diffused light. A pink colored chromophore was formed. The absorbance was measured at 540 nm against blank solution. The entire procedure was repeated without extract or ascorbic acid which give the control reading. The ascorbic acid was used as the reference compound. The percentage inhibition was calculated by the formula as follows [19,20].

$$\% \text{ scavenging activity} = [(A_{control} - A_{test})/A_{control}] \times 100$$

Where; $A_{control}$ is the absorbance of control and
A_{test} is the absorbance of test or standard
IC_{50} values for each extract were calculated from curve fitting method.

RESULTS AND DISCUSSION

Phytochemical analysis
The *S. colais* extract revealed the presence of carbohydrates, alkaloids, phenolic compounds and tannins, flavonoids. *P. tithymaloides* leaves extract give the positive results for carbohydrates, alkaloids, glycosides, phenolic compounds, tannins, flavonoids and saponin.

DPPH radical scavenging activities
In the present investigation antioxidant activity of ethanolic extract of *S. colais* and *P. tithymaloides* leaves were studied. The *S. colais* and *P. tithymaloides* demonstrated remarkable *in-vitro* DPPH radical scavenging activities in a dose-dependent manner. DPPH radical scavenging activity exhibited by ascorbic acid, *S. colais* and *P. tithymaloides* were 88.24 ± 0.19, 73.15 ± 0.23 and 52.92 ± 0.11 % respectively as given in Table 1. The concentration of extracts of the *S. colais* and *P. tithymaloides* required to scavenge 50% of the DPPH radicals (IC_{50}) were also determined. The IC_{50} values for the *S. colais* and *P. tithymaloides* were found 41.58 µg/ml and 209.56 µg/ml respectively. On the other hand, the IC_{50} value of the ascorbic acid (standard) was found 14.33 µg/ml.

Table 1. DPPH scavenging activities of ascorbic acid, *S. colais* and *P. tithymaloides*

***S. colais* (µg/ml)**		***P. tithymaloides* (µg/ml)**		**Ascorbic acid (µg/ml)**	
Conc. (µg/ml)	**% DPPH scavenging**	**Conc. (µg/ml)**	**% DPPH scavenging**	**Conc. (µg/ml)**	**% DPPH scavenging**
20	41.13 ± 0.11	50	30.63 ± 0.19	20	52.27 ± 0.22
40	47.34 ± 0.14	100	36.35 ± 0.11	40	61.20 ± 0.12
60	58.47 ± 0.41	150	43.22 ± 0.23	60	72.54 ± 0.13
80	69.68 ± 0.10	200	51.35 ± 0.13	80	84.64 ± 0.11
100	73.15 ± 0.23	250	52.92 ± 0.11	100	88.24 ± 0.19
IC_{50} 41.58		IC_{50} 209.56		IC_{50} 14.33	

Data are Measured ± SEM of three measurements

Nitric oxide scavenging activities

This method is based on the inhibition of nitric oxide radical generation from sodium nitroprusside in buffer saline. The amount of NO generated is measured by Griess reagent. Both the plants extracts and standard ascorbic acid were demonstrated dose dependent increase in the nitric oxide anion scavenging property. Ascorbic acid was exhibited 82.34 ± 0.10 % scavenging activity. Whereas, *S. colais* and *P. tithymaloides* were shown 66.31 ± 0.18 and 72.86 ± 0.21 % respectively of nitric oxide scavenging activity. The IC_{50} value for *S. colais, P. tithymaloides* and standard ascorbic acid were found 48.48 µg/ml, 67.82 µg/ml and 14.45 µg/ml respectively as given in Table 2.

Table 2. Nitric oxide scavenging activity of ascorbic acid, *S. colais* and *P. tithymaloides*

***S. colais* (µg/ml)**		***P. tithymaloides* (µg/ml)**		**Ascorbic acid (µg/ml)**	
Conc. (µg/ml)	**% NO scavenging**	**Conc. (µg/ml)**	**% NO scavenging**	**Conc. (µg/ml)**	**% NO scavenging**
20	40.17 ± 0.10	50	44.56 ± 0.20	20	50.93± 0.10
40	45.26 ± 0.18	100	57.36 ± 0.18	40	59.64 ± 0.31
60	54.56 ± 0.25	150	62.28 ± 0.23	60	71.58 ± 0.25
80	64.20 ±0.18	200	69.12 ± 0.12	80	78.36 ± 0.20
100	66.31 ± 0.18	250	72.86 ± 0.21	100	82.34 ± 0.10
IC_{50} 48.48		IC_{50} 67.82		IC_{50} 14.45	

Data are Measured ± SEM of three measurements

Pro-oxidants and oxidants are collectively referred to as reactive oxygen species (ROS). The most significant free radicals created during metabolic processes are oxygen-derived radicals, ROS [21]. Radicals are species that can contain at least one unpaired electron in the shells around the atomic nucleus and are capable of independently existence [22]. The examples of radicals include oxygen radical, superoxide, hydroxyl, peroxyl radical, alkoxy radical, nitric oxide and nitrogen dioxide. Enzymatic and nonenzymatic reactions produce ROS radical. The ROS generated from enzymatic reactions able to involved in respiratory chain, phagocytosis, prostaglandin synthesis and cytochrome P450 system. Superoxide radical is generated by NADPH oxidase, xanthine oxidase and peroxidases. The nonenzymatic reactions can also be responsible for free radical production *i.e.,* when oxygen reacts with organic compounds or when cells are exposed to ionizing radiations [23].

The body has various mechanisms to counteract oxidative stress by producing antioxidants either naturally generated endogenous or exogenous sources. The roles of these antioxidants are to neutralize the excess of free radicals to protect the cells against their toxic effects and help to prevent diseases [24]. Various synthetic antioxidants used against oxidative stress, but have adverse effects like hepatotoxicity and cancer. Now day, there is need to replace synthetic antioxidants with plants origin antioxidants because they are considerably safer, low cost and affordable.

The objective of the present study was to assay for the *in-vitro* antioxidant activities of ethanolic extract of *S. colais* and *P. tithymaloides*. The antioxidant activity of the extracts was evaluated by the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. In this method, molecules are considered to be antioxidants if they can scavenge and reduce DPPH free radicals *in-vitro*. The DPPH radical scavenging activity is observed by a characteristic change in colour from blue to yellow, which is analysed at 517 nm [25]. In the present study, we found that DPPH scavenging capacity of the ethanolic extracts of *S. colais* and *P. tithymaloides* exhibited a concentration-dependent relationship as previously demonstrated by Loganayaki et al. (2020) in extracts of *Helicteres isora* and *Ceiba pentandra* [26].

Moreover, the nitric oxide is a potent mediator for various physical activities such as relaxation of smooth muscle, inhibition of platelet aggregation and regulation of cell mediated toxicity. However, the toxicity and damage due to NO• is multiplied as they react to produce reactive peroxynitrite which leads to serious toxic reactions with biomolecules [20]. Plant extracts may possess the ability to inhibit NO creation, hence minimizing the harmful effects of excessive NO production in the human body. Further, the scavenging activity may also help in stopping the sequence of initiated by excess generation of NO to more reactive peroxynitrate. Suppression of NO• generation may be partially attributed to direct NO• scavenging by *S. colais* and *P. tithymaloides* extracts which reduced the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro*. The scavenging of nitric oxide by the *S. colais* and *P. tithymaloides* extracts were also increased in a dose dependent manner.

Current research has shown that medicinal plants contain active principles like flavonoids, phenols and tannins, which are responsible for antioxidant activity. Various phytochemicals of antioxidant value found in medicinal plants are responsible for this bioactivity. Qualitative phytochemical screening of *S. colais* and *P. tithymaloides* extracts showed the presence of flavonoids, phenols, and tannins among other antioxidant phytochemicals, which may have contributed to the antioxidant potency of *S. colais* and *P. tithymaloides* extracts.

CONCLUSIONS

On the basis of obtained results, it was concluded that the ethanolic extract of *S. colais* and *P. tithymaloides* leaves have appreciable antioxidant activity by scavenging DPPH and NO methods. Furthermore, the extracts were found to contain flavonoids, tannins and other phytoconstituents which play an important role in controlling oxidation generated by free radicals. The results of present study suggest that the ethanolic extract of *S. colais* and *P. tithymaloides* can be used as a prospective source of natural antioxidant. However, the phytoconstituents responsible for the antioxidant activity of the extracts is still elusive. Further studies are needed to isolate and identify the antioxidant compounds present in the plants extracts.

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