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Phytochemical screening and in vitro antioxidant activity of extracts of jasminum sessiliflorum

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ABSTRACT

Objectives

The aims of this research were to carry out the preliminary phytochemical screening and antioxidant activity of different extracts of J. sessiliflorum. The different anti-oxidant methods carried out were DPPH scavenging method, NBT dye reduction method and nitric oxide scavenging method

Methods

Extracts were prepared by reflux method using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH, NBT dye reduction method and nitric oxide scavenging methods and the correlation of their IC_{50} values with standards were carried out.

Results

The ethanolic herbs extract of J. sessiliflorum had the lowest IC_{50} values in all the anti-oxidant methods. Moreover, the ethanolic extracts showed the presence greatest amount of phytochemical constituents. The IC_{50} values were correlated with the IC_{50} values of standards in all the anti- oxidant activity determination methods.

Conclusions

The results of the present study indicate that the extracts of J.sessiliflorum exhibited strong antioxidant activity and thus it is a good source of antioxidant.

Keywords: Antioxidant, DPPH, NBT dye reduction, Nitric oxide scavenging, J. sessiliflorum

INTRODUCTION

Quality can be defined as the status of a drug that is determined by identity, purity, content and other chemical, physical, or biological properties, or by the manufacturing processes. Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product [1]. For the quality control of a traditional medicine, the traditional methods are procured and studied, and documents and the traditional information about the identity and quality assessment are interpreted in terms of modern assessment [2].

Jasminum sessiliflorum is a species of jasmine native to India, Sri Lanka and the Andaman Islands. It is a climbing shrub with a smooth stem and minutely pubescent branchlets. Natural antioxidants have a wide range of biochemical activities including inhibition of reactive oxygen species generation, direct or indirect scavenging of free radicals and alteration of intracellular redox potential [3]. Free radicals and other reactive oxygen species are generated continuously via normal physiological process, more so in pathological conditions. The use of natural antioxidants has gained much attention from consumers because they are considered safer than synthetic antioxidants. Recently there has been a worldwide trend towards the use of natural antioxidants present in different parts of plants due to their phytochemical constituents [4].

MATERIALS AND METHODS

Materials

DPPH, NBT, sodium nitroprusside were purchased from Merck, Mumbai. All the solvents used for this entire work were of analytical reagent grade.

Collection of plant materials

The plant J. sessiliflorum (Family: Oleaceae) were collected from Tirunelveli district, Tamilnadu, India during the month of March 2017. The plant was identified and authenticated by Mr. Chelladurai, Research officer-Botany, Central council for research in Ayurveda and Sidha, Government of India.

After authentication, the fresh healthy whole plant of *Jasminium sessiliflorum* dried properly in shade for 3 weeks, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight container, and used for further studies.

Preparation of extracts

About 1 kg of air-dried plant *Jasminium sessliflorum* was extracted in soxhlet assembly successively with petroleum ether, chloroform, ethyl acetate and ethanol (order of increasing polarity). Each time before extracting with the next solvent, the powdered material was dried at room temperature.

Each extract was concentrated by using rotary vacuum evaporator. The extract obtained with each solvent was weighed and the percentage yield was calculated in terms of dried weight of the plant material. The colour and consistency of the extract were also noted. All the solvents used for this entire work were of analytical reagent grade (Merck, Mumbai).

Preliminary Phytochemical Screening

Various standardized qualitative chemical tests were performed for qualitative determination of different phytoconstituents present in the different extracts of J. sessiliflorum [5].

Determination of anti-oxidant activity by different methods DPPH photometric assay

An ethanolic solution of 0.5ml of DPPH (0.4mM) was added to 1 ml of different concentrations of plant extract and allowed to react at room temperature for 30 minutes. Ethanol served as the blank and DPPH in ethanol without the extracts served as the positive control. After 30 min, the absorbance was measured at 518 nm and converted into percentage radical scavenging activity [5] as follows.

$$Scavenging\,activity(\%) = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

Where A518 control is the absorbance of DPPH radical+ ethanol; A518 sample is the absorbance of DPPH radical+ sample extract/ standard.

Superoxide radical scavenging method

The assay mixture contained sample with 0.1ml of Nitro blue tetrazolium (1.5M NBT) solution, 0.2 ml of EDTA (0.1M EDTA), 0.05 ml riboflavin (0.12 mM) and 2.55 ml of phosphate buffer (0.067 M phosphate buffer)[7]. The control tubes were also set up wherein DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and the absorbance at 560 nm was measured against the control samples. Ascorbate was used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of control and test samples.

Nitric oxide scavenging activity

Three ml of reaction solution containing 2ml of sodium nitroprusside (10 mM) and 0.5ml phosphate buffer saline (1M) were incubated at 25ºC for 2.5h. After incubation, 0.5mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulphanilic acid reagent (0.33%) and allowed to stand for 5min for completing diazotization. After that 1ml of naphthalene diamine dihydrochloride (1% NEDA) was added, mixed and allowed to stand for 0.5h. Sodium nitropruside in aqueous solution at physiological pH spontaneously generate nitric oxide, which interacts with oxygen to produce nitrite ions and is estimated at 540nm [8].

RESULLTS AND DISCUSSIONS

Phytochemical screening

The phytochemical analysis of the different extracts of J. sessiliflorum revealed the presence of phytochemicals. An adequate amount of tannins, phenols, flavonoids glycosides, terpenoids, and alkaloids were found in all the extract. The results obtained are depicted in table 1.

Table 1: Phytochemical evaluation of extracts

Sl.No.	Test	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
1.	Alkaloids	-	+	-	+
2.	Carbohydrates	-	-	+	+
3.	Glycosides	-	-	-	+
4.	Terpenoids	+	+	+	+
5.	Proteins	-	-	+	-
6.	Amino acids	-	+	-	-
7.	Steroids	+	+	+	+
8.	Flavonoids	+	+	+	+
9.	Phenols	+	+	+	+

10.	Tannins	+	+	+	+
11.	Quinones	-	-	+	-
12	Anthraquinones	-	+	-	-
13	Saponins	+	+	+	-

DPPH photometric assay

The percentage of DPPH radical scavenging activity of various extracts of plant is presented in Figure 1. The DPPH radical scavenging activity of the extract increases with increasing concentration. The ethanolic extract exhibited highest activity compared to all the other extracts. The IC50 of the ethanolic extract of plant and Rutin were found to be 455µg/ml and 581µg/ml respectively.

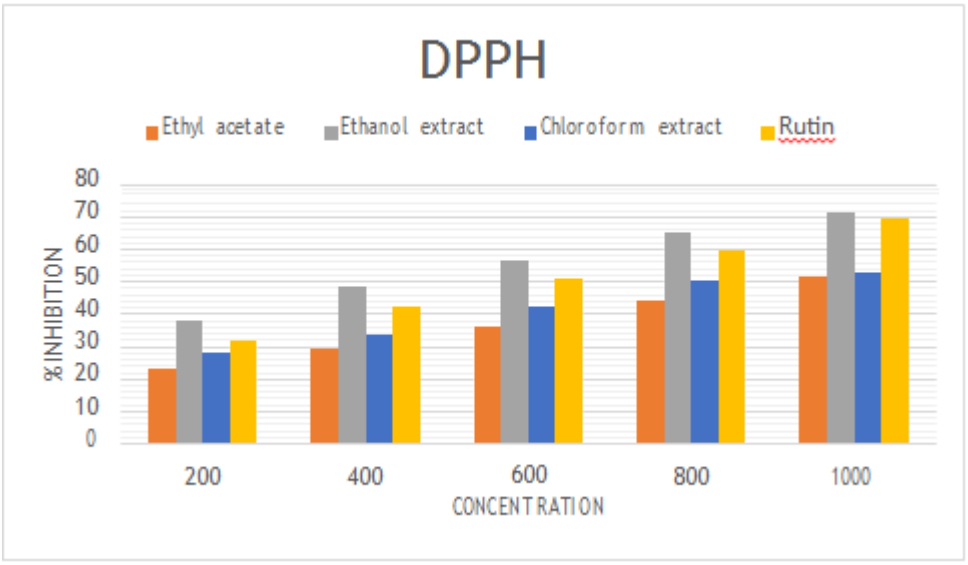

Figure 1: Effect of various extracts of plant on DPPH assay

Superoxide radical scavenging method

The percentage scavenging of superoxide anion examined at different concentrations of various extracts of plant (200, 400, 600, 800, 1000 µg/ml) were presented in Figure 2. The ethanolic extract of plant were found to have strong superoxide radical scavenging activity; whereas, ethyl acetate showed weak activity when compared to that of standard ascorbate. The IC50 of the ethanolic extract of plant and ascorbate were found to be 428µg/ml and 509µg/ml respectively.

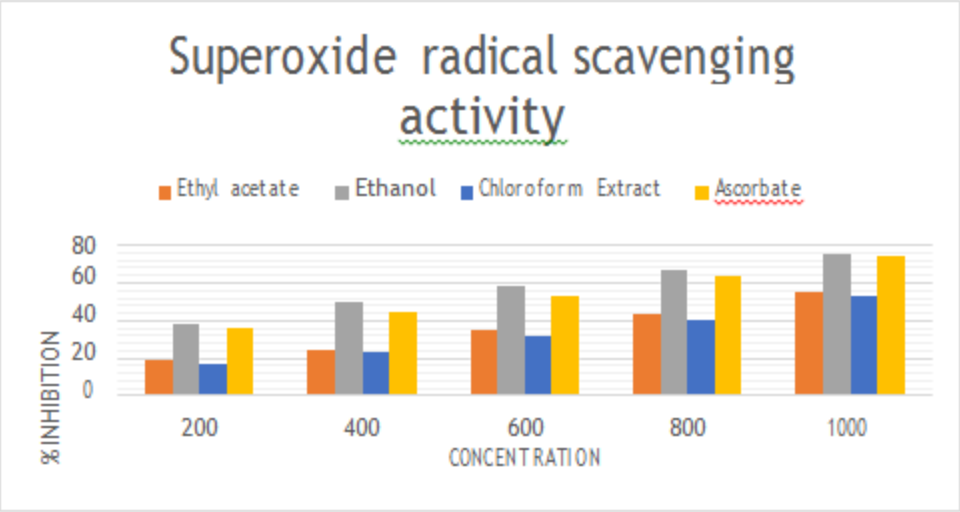

Figure 2: Effect of various extracts of plant on Superoxide anion scavenging activity

Nitric oxide scavenging activity

The reduction of nitric oxide radical by the various extracts of plant and ascorbate was noted to be concentration dependent and was illustrated in Figure 3. The ethanolic extract of plant was found to be most effective in scavenging nitric oxide radical activity. The IC50 of the ethanolic extract of plant and ascorbate were found to be 496µg/ml and 566µg/ml respectively.

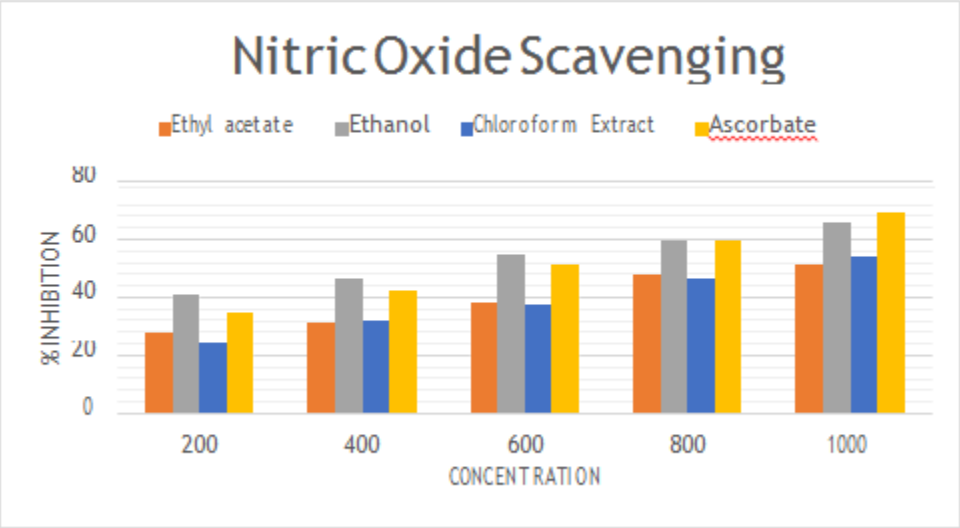

Figure 3: Effect of various extracts of plant on Nitric oxide scavenging activity

Rising concern in the investigate for natural alternatives in favor of synthetic antioxidant has led to the evaluation of plant sources. In this study, leaf extract of J. sessiliflorum exhibited outstanding scavenging effects on DPPH radical scavenging activity, superoxide anion scavenging activity and nitric oxide scavenging.[9] Along with the good number widely used procedures for measurement of antioxidant activity capacity, the DPPH radical scavenging analysis is one of the top known, correct, and regularly employed to measure the electron donating ability of the plant.[10]

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity

[11]. Hydroxyl radical is one of the potent reactive oxygen species in the biological system. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and cause damage to cell [12]. Over production of nitric oxide manifest in various pathological conditions mainly by formation of peroxynitrites [13]. The plant extract evaluated were found to decrease the quantity of nitrite ions *in vitro* which can be attributed to the antioxidant constituents present in the extracts.

CONCLUSION

J. sessiliflorum was quantified for the main phytochemicals present in the extract. The presence of various phenolics and non-phenolics phytocompounds concluded that the plant might be of medicinal importance. The varying antioxidant (free radical scavenging) activities of extracts when compared to standard antioxidant i.e. Vitamin C, suggested the possibility that the antioxidant activity of this medicinal plant may contribute to play their role against various reactive oxygen species (ROS) mediated disorders such as cellular aging and cancer, becoming an alternative in the fight against skin aging and cancer cells [14]. Antioxidant activity of sample should be determined using different methods in parallel, because various methods could give different results. The ethanolic extracts showed potential anti-oxidant activity. J. sessiliflorum may be exploited as natural antioxidant source to prevent oxidative stress. Therefore, further research is needed for the isolation and identification of the active components in the extracts. [15]

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