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IN VITRO ANTIOXIDANT AND ANTHELMINTIC ACTIVITY OF EXTRACTS OF *JASMINUM ARBORESCENS* ROXB

BHAGATH K [1], PRASHITH KEKUDA TR [2*], RAGHAVENDRA HL [3], SWARNALATHA SP [2], PREETHI HR [2], SURABHI K S [2]

[1] DEPARTMENT OF CHEMISTRY, NATIONAL PRE-UNIVERSITY COLLEGE, BARKUR, UDUPI DIST., KARNATAKA, INDIA.

[*2] DEPARTMENT OF MICROBIOLOGY, S.R.N.M.N COLLEGE OF APPLIED SCIENCES, N.E.S CAMPUS, BALRAJ URS ROAD, SHIVAMOGGA-577201, KARNATAKA, INDIA.

[3] DEPARTMENT OF BIOCHEMISTRY, KUVEMPU UNIVERSITY, SHIVAGANGOTHRI, DAVANGERE-577002, KARNATAKA, INDIA.

ABSTRACT

The present study was carried out to study in vitro antioxidant and anthelmintic activity of ethanol, chloroform and petroleum ether extracts of leaves of Jasminum arborescens Roxb (Oleaceae). Different concentrations of solvent extracts were subjected to antioxidant activity by DPPH free radical scavenging and Fe^{+3} reducing power assays. A marked dose dependent antioxidant activity was observed in the trials. Antioxidant activity was higher in ethanol extract followed by chloroform and petroleum ether extract. Anthelmintic assay was performed on adult Indian earthworm Pheretima pasthuma. The time taken for paralysis and death of worms was found lesser in case of ethanol extract followed by chloroform and petroleum ether extract. The results of the study indicate the antioxidant and anthelmintic potential of solvent extracts. Further studies on isolation of constituents and antioxidant and anthelmintic activity in vivo are to be carried out.

Key words: *Jasminum arborescens Roxb., Soxhlet extraction, DPPH free radical scavenging assay, Fe^{+3} reducing power assay, Pheretima pasthuma*

Introduction

Free radicals are found to be a product of normal metabolism. Although oxygen is essential for aerobic forms of life, oxygen metabolites are highly toxic. As a consequence, reactive oxygen species (ROS) are known to be implicated in many cell disorders and in the development of many diseases including cardiovascular diseases, atherosclerosis, chronic inflammation etc [1,2]. Although organisms have endogenous antioxidant defences produced during normal cell aerobic respiration against ROS, other antioxidants are taken both from natural and synthetic origin [3]. Antioxidants that can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very important [4]. Synthetic antioxidants are widely used but their use is being restricted nowadays because of their toxic and carcinogenic effects. Thus, interest in finding natural antioxidants, without any undesirable effect, has increased greatly [3]. Helminthes are recognized as a major problem to livestock production throughout the tropics. Parasitic helminthes affect human being and animals by causing considerable hardship and stunted growth. Most diseases caused by helminthes are of a chronic and debilitating in nature [5]. The origin of many effective drugs is found in the traditional medicine practices and in view of this several workers have undertaken studies pertaining to testing of folklore medicinal plants for their proclaimed anthelmintic activity.

Correspondence: Dr.Prashith Kekuda TR, email: prashith_kekuda@rediffmail.com

The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. It is in the context that people consume several plants or plant derived preparations to cure helminthic infections [6]. The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents to the people, particularly in developing countries, including India. Indigenous system of medicine reports a number of plants for their anthelmintic efficacy. However, their scientific evaluation as compared to commercial anthelmintics is limited. Many plants have proven to possess anthelmintic activity *in vitro* and *in vivo*.

Jasminum arborescens Roxb. (syn: *J. roxburghianum* Wall) belonging to the family Oleaceae is distributed in Sub-Himalayan tract, Bengal, Central and South India. The plant is commonly called Tree Jasmine in English. It is known as Nava-mallikaa in Ayurveda and Nagamalli in Siddha. The leaves are astringent and stomachic. Juice of leaves, with pepper, garlic and other stimulants, is used as an emetic in obstruction of the bronchial tubes due to viscid phlegm [7]. The present study was carried out to study *in vitro* antioxidant and anthelmintic activity of ethanol, chloroform and petroleum ether extracts of leaves of *Jasminum arborescens* Roxb.

Materials and Methods

Collection and identification of plant material

The plant material was collected in December 2009 in and around Hosanagara (Tq), Shivamogga (Dt). The plant samples were identified by specialist and voucher specimen (KB/Chem/001-09) was deposited in the Department of Microbiology for future reference. Plants were cleaned off adhering soil/dust in field by shaking properly and using soft brush. Plants were placed in paper bags and brought to the laboratory. Remaining dust particles were removed by quick rinsing with distilled water.

Extraction and Qualitative phytochemical analysis

Leaf material was shade dried and powdered mechanically. About 250g of powdered material was subjected to soxhlet extraction and exhaustively extracted with solvents namely petroleum ether, chloroform and ethanol for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator. The solvent extracts were subjected to preliminary analysis for screening the presence of various phytoconstituents namely alkaloids, steroids, flavonoids, saponins, tannins, steroids and glycosides by chemical tests [8].

DPPH radical scavenging assay

Different concentrations of solvent extracts and ascorbic acid (standard) namely 25, 50, 100, 200 and 400 mcg/ml were prepared in methanol. DPPH (0.002% in methanol) was used as free radical. Equal volume of different concentrations of solvent extracts and DPPH were mixed in clean and labeled test tubes separately and the tubes were incubated at room temperature in dark for 30 minutes. The optical density was measured at 517nm using UV-Vis Spectrophotometer. The degree of stable $DPPH^{\cdot}$ decolorization to DPPHH (reduced form of DPPH) yellow indicated the scavenging efficiency of the extract. The scavenging activity of the extract against the stable $DPPH^{\cdot}$ was calculated using the following equation.

Scavenging activity (%) = A – B / A X 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination [9, 10].

Antioxidant activity of solvent extracts by Fe^{+3} reducing power assay

Different concentrations of solvent extracts and tannic acid (25, 50, 100, 200 and 400 mcg/ml) in 1ml of methanol were mixed with 2.5ml of phosphate buffer (200mM, pH 6.6) and 2.5ml of 1% potassium ferricyanide separately. The mixtures were placed in a water bath for 20 min at 50°C, cooled rapidly, mixed with 2.5ml of 10% trichloroacetic acid and 0.5ml of 0.1% Ferric chloride. The intensity of iron (II) - ferricyanide complex was determined by measuring the formation of Perl's Prussian blue at 700nm after 10min. The higher absorbance of the reaction mixture indicates increased reducing power [11].

Anthelmintic activity of solvent extracts

The anthelmintic assay was performed on adult Indian earthworm *Pheretima pasthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings. Standard drug (Piperazine citrate, 1%) and solvent extracts (20mg/ml) were prepared in normal saline (0.85%) and poured into respective labeled petriplates. Six worms of nearly equal size were introduced into each of the plates. Observations were made for the time taken to paralysis and death of individual worm. Paralysis was said to occur when the worms were not able to move even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colors. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased [6, 12].

Results and Discussion

Qualitative Phytochemical analysis

Preliminary phytochemical analysis of solvent extracts showed the presence of various phytoconstituents. Ethanol extract revealed the presence of all except alkaloids. Phytoconstituents namely terpenoids, flavonoids, steroids and glycosides were detected in petroleum ether extract. Chloroform extract showed the presence of steroids, glycosides, tannins and saponins (Table-1).

Table 1: Preliminary phytochemical analysis of solvent extracts

Phytochemical group	Ethanol extract	Petroleum ether extract	Chloroform extract
Alkaloids	-	-	-
Terpenoids	+	+	-
Flavonoids	+	+	-
Steroids	+	+	+
Glycosides	+	+	+
Tannins	+	-	+
Saponins	+	-	+

DPPH free radical scavenging activity

DPPH free radical scavenging activity of different concentrations of solvent extracts and Ascorbic acid is presented in Figure-1. The extracts exhibited marked antioxidant activity by scavenging DPPH* (free radical) and converting into DPPHH. A dose dependent radical scavenging activity was observed. The scavenging activity of ascorbic acid was greater than that of solvent extracts. Overall, the scavenging potential of ethanol extract was higher followed by chloroform and petroleum ether extracts. Free radicals are chemical species containing one or more unpaired electrons that makes them highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability [13]. Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS [14, 15]. In recent years much attention has been

devoted to natural antioxidant and their association with health benefits [13]. There are several methods available to assess antioxidant activity of compounds. An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1, diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases [16]. In this study, the scavenging activity of methanol extract was found to be dose dependent i.e., higher the concentration, more was the scavenging activity. Though the DPPH radical scavenging abilities of the extracts were less than that of ascorbic acid, the study showed that the extracts have the proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

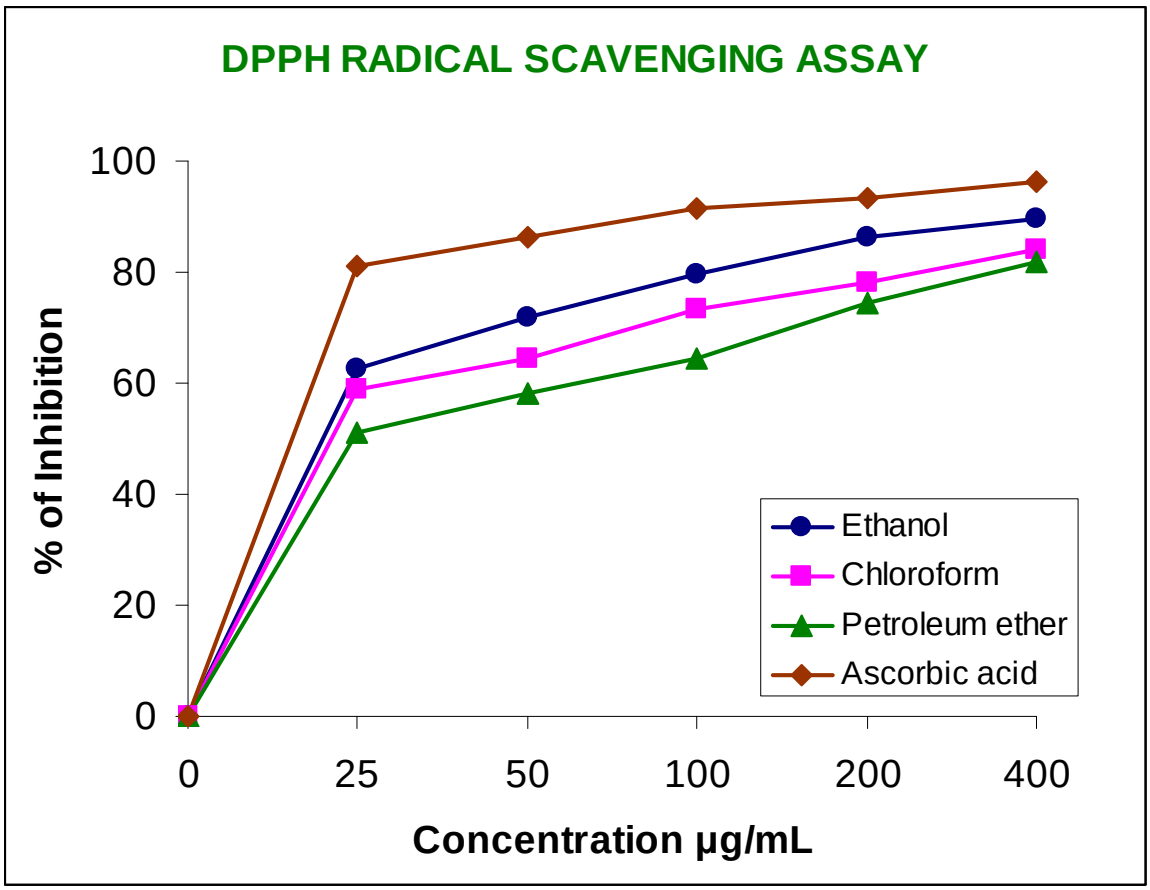

Figure 1: DPPH free radical scavenging activity of different concentrations of solvent extracts and Ascorbic acid was used as standard

Reducing Power Assay

The result of reducing power of different concentrations of solvent extracts and tannic acid is represented in Figure 2. In this study, the absorbance was found to increase with the dose of extracts and standard which is suggestive of reducing power. In the Fe^{+3} reducing assay, the reducing power of crude solvent extracts was found to increase with the dose. The presence of reductants such as antioxidant substances in the antioxidant samples causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm [17]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant

activity [18]. However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging [19].

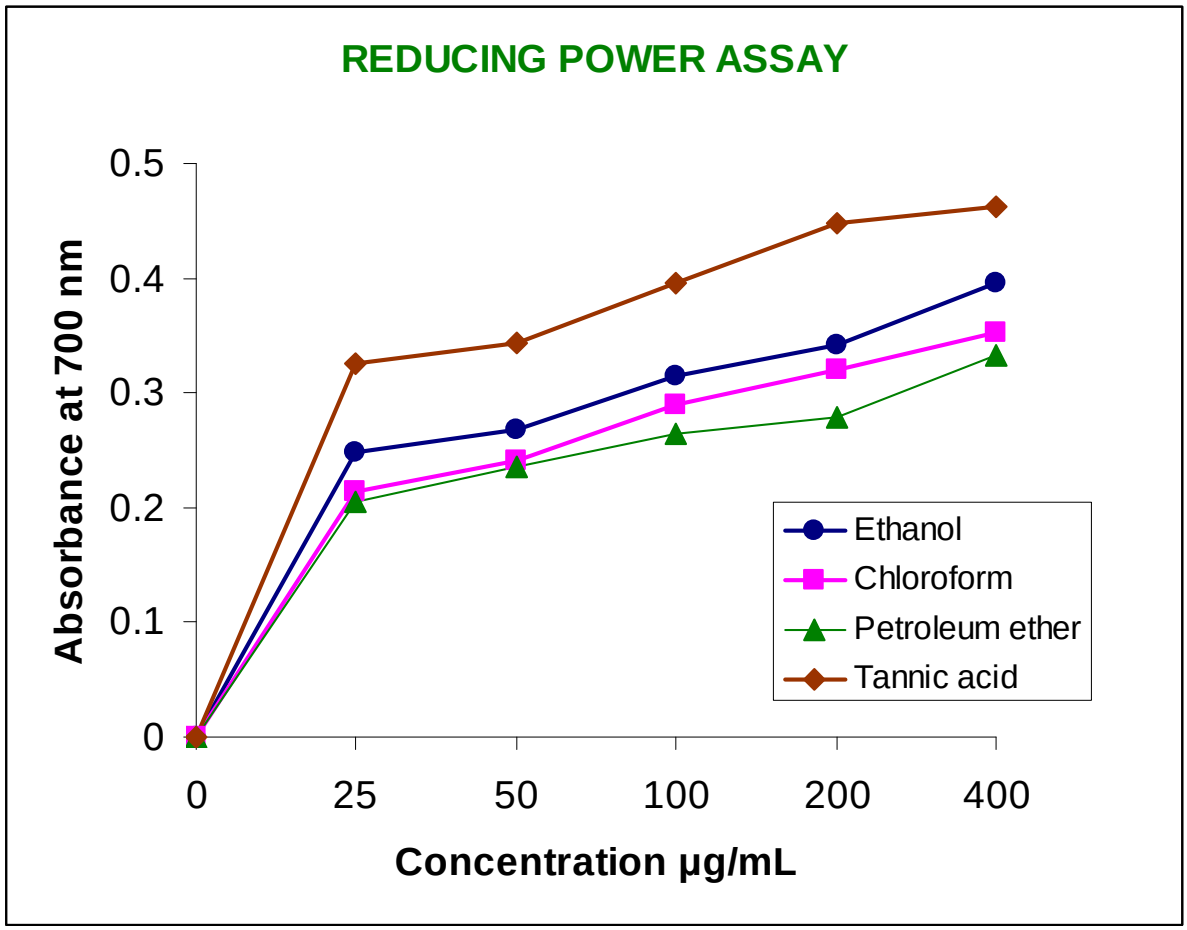

Figure 2: Fe^{+3} reducing activity of different concentrations of solvent extracts. Tannic acid was used as standard.

Anthelmintic activity

The different extracts of the plant material were evaluated for anthelmintic activity using adult Indian earthworm model. Among extracts, ethanol extract caused paralysis and death of worms in less time when compared to chloroform and petroleum ether extracts. In control, no paralysis and mortality of worms was observed. The anthelmintic potency of standard drug Piperazine citrate was found to be higher than extracts (Table-2). During the past few decades, despite numerous advances made in understanding the mode of transmission of the parasites and the treatment of diseases, there are still no efficient products to control certain helminthes and the indiscriminate use of some drugs has generated several cases of resistance. Furthermore, it has been recognized recently that anthelmintic substances having considerable toxicity to human beings are present in foods derived from livestock, posing a serious threat to human health. Consequently, the discovery and development of new chemical substances for helminth control is greatly needed and has promoted studies of traditionally used anthelmintic plants, which are generally considered to be very important sources of bioactive substances [20].

Table 2: Anthelmintic activity of Standard drug and solvent extracts

Treatment	Time in minutes *	
	For paralysis	For death
Control (normal saline 0.85%)	-	-
Standard (Piperizine citrate)	78	105
Ethanol extract	137	159
Chloroform extract	168	196
Petroleum ether extract	189	215

Conclusion

Free radicals contribute to more than one hundred disorders in humans. Due to negative effects of synthetic antioxidants, nowadays, much attention has been placed on phytoconstituents. Many of the phytoconstituents are beneficial and many of them are acting as natural antioxidants. The results of the present investigation were suggestive of the potential of solvent extracts in scavenging free radical. According to our study, the presence of phytoconstituents such as flavonoids and phenolic compounds in the solvent extracts may be responsible for the high radical scavenging activity. Helminth infections are among the most common infections in man, affecting a large proportion of the world's population. Parasitoses have been of concern to the medical field for centuries and the helminths still cause considerable problems for human beings and animals. The result of the present study revealed the potential of extracts of the plant selected as the worms were found to be susceptible and thus could be used as anthelmintic. Further studies on isolation of constituents and antioxidant and anthelmintic activity in vivo are to be carried out.

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Author Information: Dr. Prashith Kekuda Tr Is Working In Department Of Microbiology, S.R.N.M.N College Of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India

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