IN VITRO EVALUATION OF ANTIOXIDANT AND ANTHELMINTIC ACTIVITY OF DIFFERENT EXTRACTS OF *SOYMIDA FEBRIFUGA.*

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Summary

The objective of the present study is to evaluate *in vitro* antioxidant and *in vitro* anthelmintic activity of different extracts of *Soymida febrifuga. Soymida febrifuga* is lofty deciduous tree 22-25m in height and 2-5m in girth. Barks of *Soymida febrifuga* were colleted from Pune district and authenticated. The dried material was broken into a coarse powder in an electric grinder and subjected to extraction. The extraction of bark was carried out by successive extraction method with petroleum ether, chloroform and then with methanol. Antioxidant activity of these extracts was determined *in vitro* by DPPH and Nitric oxide method using ascorbic acid as standard. *In vitro* anthelmintic activity of these extracts was evaluated on earthworm with albendazole as standard. It was found that methanol extract of bark showed comparable antioxidant and anthelmintic activity.

Key words: *Soymida febrifuga*, evaluation, antioxidant, anthelmintic

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Introduction

The role of oxygen-derived free radicals in the pathogenesis of a number of degenerative diseases is well known [1]. Many plants contain substantial amounts of antioxidants including Vitamin C and E, carotenoids, flavonoids, tannins and thus can be utilized to scavenge the excess free radicals from the human body [2]. Epidemiological studies have suggested the association between consumption of antioxidant rich foods and beverages and the prevention of diseases [3]. The deleterious effects of ionizing radiation, especially those having low linear energy transfer (LET), in biological systems are mainly mediated through the generation of oxygen-derived intermediates in the form of free radicals and excited states [4]. Cellular membranes are among the most important targets of radiation damage [5]. Due to the presence of polyunsaturated fatty acids, membranes are highly susceptible for oxidative damage induced by reactive oxygen species (ROS), generated during radiation [6]. Adverse alterations in biomembranes can directly lead to cytotoxicity and / or indirectly to genotoxicity [7]. Hence compounds capable of protecting cellular membranes against ionizing radiation in particular and free radicals in general will have potential benefits as radioprotectors, antioxidants and antimutagens [8, 9]. There is sufficient evidence to suggest that adequate antioxidant defence by vitamin E and the other antioxidants can provide protection from the high levels of free radicals generated [10]. Currently, great interest centers on the possible protective value of a wide variety of plant-derived antioxidant compounds, particularly those from fruits and vegetables, against radiation damage [8, 11, 12]. Oxidative damage that results in lipid peroxidation can inactivate cellular components and can have serious effects on the cells probably leading to ageing as well as several diseases [6,7,10]. In recent years much attention has been focused on this subject, especially in the field of clinical medicine, due to its relevance in degenerative diseases, hyperoxia, ischemia-reperfusion, trauma, ageing, stroke and ethanol toxicity [13, 14]. Oxidative damage to proteins, as assessed by formation of carbonyl groups is also a highly damaging event, and may occur in the absence of lipid peroxidation. The modification of proteins by ROS is implicated in the etiology of a number of physiological disorders and diseases [15, 16]. Parasitic diseases caused by helminthes lead to significant health hazards to animals resulting in enormous economic impact. While a number of anthelmintics are currently available, all are encountering resistance and ones with a mode of action are needed. Several plants have been shown traditionally to have medicinal value in the control of helminthiasis and other ailments in man and animals [17, 18].

Soymida febrifuga is a lofty deciduous tree 22-25m in height and 2-5m in girth. Leaves are crowded towards the ends of the branches, 23-45 cm long, and leaflets 3 to 6 pairs paripinnate [19]. The average tannin content of bark is 17.41% on dry weight basis. It is acrid, refrigerant, anthelmintic, laxative and good for sore throat removes “vata”, cures “tridosha”, fever and cough. It removes blood impurities, good for ulcer, leprosy and dysentery [20]. Therefore, the present study was undertaken to evaluate antioxidant and anthelmintic activity of different extracts of *Soymida febrifuga*.

Materials and methods

All the chemicals used were purchased from E. Merck (India) Ltd., Mumbai. All the solvents used in the study are of analytical grade.

1) **Preparation of extracts**: Barks of *Soymida febrifuga* were colleted from Pune district, dried, powdered and subjected to extraction. The extraction of bark was carried out by successive extraction method using soxhlet extractor with petroleum ether, chloroform and then with methanol.

2) **Antioxidant activity:**

Instrument: Schimadzu UV/VIS spectrophotometer (Japan)

DPPH scavenging activity [21,22] **:** Antiradical activity of extracts was performed by DPPH model. Stock solution of DPPH (1.3mg/ml) in methanol was prepared. 75µl of stock solution of DPPH was added in 3ml of methanol and absorbance was measured at 517 nm. The various concentrations of extracts (25, 50, 75, 100, 125 µg/ml) were prepared. In all diluted solutions, 75µl of stock solution of DPPH was added than absorbance was recorded at 516nm and EC_{50} was calculated against methanol as a blank. The capacity to scavange the DPPH radicle was calculated by following equation:
Scavenging effect (%) = [(Abs. Blank − Abs. sample)/ Abs. Blank] × 100
Each assay was repeated thrice and the results, recorded as mean of the triplicate experiments.

Table 1: DPPH scavenging activity:

Extracts	% Scavenging				
Concentration	25µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	125 µg/ml
Petroleum Ether	7.85	9.56	18.1	15.71	16.6
Chloroform	9.56	10.13	11.5	13.43	15.14
Methanol	34.05	57.28	69.47	87.47	89.74
Ascorbic acid	38.27	61.48	74.45	93.05	95.24

All tests were performed three times and averaged.

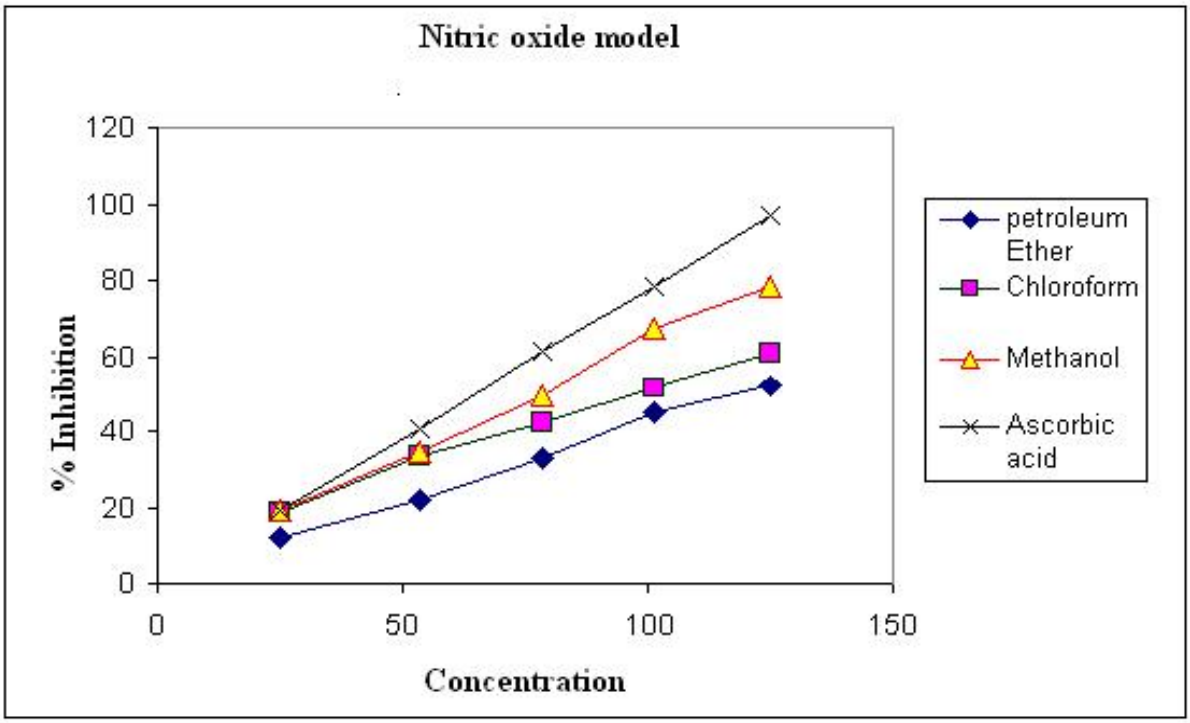
Nitric oxide scavenging activity [23, 24:] Sodium nitroprusside solution (10mM) in phosphate buffer saline was mixed with different concentrations of bark extracts (Petroleum ether, Chloroform, Methanol extracts) of *Soymida febrifuga* and incubated at 25°C for 150 minutes. The same reaction mixture without extract but equivalent amount of phosphate buffer served as control. At regular interval, sample (1.5 ml) of incubated solution was removed and diluted with 1.5 ml of Griess reagent [1 part of 0.1 % Naphthylethylenediamine (NEDA) dihydrochloride in distilled water +1 part of 1 % sulphanilic acid in 5 % concentrated hydrophosphoric acid]. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with NEDA was read at 564 nm. Ascorbic acid was used as positive control. The capacity to scavange the nitric oxide radicle was calculated by following equation:
Scavenging effect (%) = [(Abs. Blank − Abs. sample)/ Abs. Blank] × 100
Each assay was repeated trice and the results, recorded as mean of the triplicate experiments.

Table 2: Nitric oxide scavenging activity: IC_{50} Values:

Sr. No.	Extract	IC_{50}
1	Petroleum ether	125.52
2	Chloroform	92.23
3	Methanol	76.54
4	Ascorbic acid	63.84

All tests were performed three times and averaged.

Figure 1: Antioxidant activity (Nitric oxide model):



3) *In vitro* anthelmintic activity

Worms: Earthworms (*Pherotima posthuma*) of about 5-7 cm long were used for anthelmintic activity, collected from Vermiculture Plant, Niphad, Dist-Nashik (MS).

Standard used for the activity:
Albendazole suspension (Micronized albendazole suspension in the concentration of 20 mg/ml, manufactured by Intas Pharma.).

Preparation of Plant extracts:
Petroleum ether 60-80^{0}C extract (PEL), Chloroform (CHL), and Methanol (MEL) in the concentration of 80 mg/ml suspensions prepared in 1% gum acacia suspension (suspension prepared in normal saline solution).

Method:

The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [25-28]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* [29-32]. Earthworms (*Pherotima posthuma*) were used for anthelmintic activity, and grouped in to control, standard and Petroleum ether 60-80^{0}C extract (PEL), Chloroform extract (CHL), and Methanol extract (MEL); such as six worms in each group taken into Petri dishes.10 ml suspension were taken into each Petri dish. Albendazole was used as standard where normal saline solution as control. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50 ˚C) [33-34].

Table 3: *In vitro* evaluation of anthelmintic activity:

Group	Sample	* Time for paralysis (min)	* Time for Death (min)
I	Control	No paralysis (Up to 25min.)	No death (Up to 25min.)
II	Albendazole (20mg/ml)	8.45	18.25
III	PEL (40mg/ml)	18.45	No death (up to 25min)
IV	CHL (40mg/ml)	6.20	19.00
V	MEL (40mg/ml)	4.30	6.30

*Averages of three readings
PEL - Petroleum ether Extract
CHL - Chloroform Extract
MEL- Methanol Extract

Results and Conclusions

DPPH is a stable nitrogen-centered free radical, and its color changes from violet to yellow when are reduced by either the process of hydrogen- or electron donation. Substances to perform this above reaction can be considered as antioxidants and therefore radical scavengers [35]. The DPPH radical scavenging activity was known to correlate well with the inhibitory capacity of lipid peroxidation of a test compound [36].
Antioxidant activity of the extracts was compared with the standard drug ascorbic acid. From DPPH scavenging method (Table 1) and Nitric oxide scavenging method (Table 2, Figure 1), shows that methanol extract has greater antioxidant activity and is comparable with standard drug ascorbic acid. *In vitro* Anthelmintic activity of extracts was also carried out on earthworms with similar biochemical composition and functions as that of worms present in human body. Albendazole (20mg/ml) was used as standard for activity and methanol extracts shows comparable anthelmintic activity (Table 3).

In conclusion methanolic extract of bark of *Soymida febrifuga* shows comparable antioxidant and *in vitro* anthelmintic activity. In future aspects efforts can be put to prepare more potent and chemically defined extract. Also, development of analytical methods to standardize this type of extract should be tried.

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