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Research Article

IN VITRO ANTIOXIDANT ACTIVITY OF SARACA ASOCA ROXB. DE WILDE STEM BARK EXTRACTS FROM VARIOUS EXTRACTION PROCESSES

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ABSTRACT

Antioxidant potential of various extracts i.e. ethanolic, hydroalcoholic and acetone prepared by different extraction methods (Soxhlet extraction, Ultrasonication extraction and Microwave assisted extraction) of stem bark of *Saraca asoca* Roxb. De Wilde was studied by using DPPH (1,1, diphenyl-2 picryl hydrazyl) *in-vitro* model. The highest IC_{50} value 193.88 µg/ml were showed by hydroalcoholic (ethanol 60%) extract prepared by soxhlet extraction method. The acetone extract prepared by ultrasonication extraction method exhibited the lowest IC_{50} value 97.82 µg/ml. The results indicate that the antioxidant property of the extract may be due to high content of phenolic compounds.

Key words: Antioxidant, Free radicals, Saraca asoca, DPPH radical scavenging.

INTRODUCTION

There is no doubt that plants are a good source of biologically active natural products. In the investigation of bioactive natural compounds, it is essential to have access to simple biological tests to locate required activities[1]. Free radicals, powerful oxidants are species that contain unpaired electrons. They are capable of randomly damaging all components of the body (lipid, proteins, DNA and saccharides) and are involved in mutations. Radical reactions are also important in the development of chronic diseases that are life limiting like Cancers, hypertensions and cardiac infraction, atherosclerosis, rheumatism and also in cataract[2]. Reactive oxygen species (ROS) are generated continuously in the body by both endogenous and exogenous factors like normal aerobic respiration, by stimulated polymorpho-nuclear leukocytes, macrophages and exposure to various pollutants like tobacco smoke, ionizing radiation, organic solvents and pesticides[3, 4].

Many degenerative human diseases have been recognized as being a consequence of free radical damage. There have been many studies undertaken on how to delay or prevent the onset of these diseases[5]. The most likely and practical way to fight against degenerative diseases is to improve body antioxidant status which could be achieved by higher consumption of vegetables and fruits. Foods from plant origin usually contain natural antioxidants that can scavenge free radical[6]. The antioxidant may mediate their effect by directly reacting with ROS, quenching them and/or chelating the catalytic metal ions[7].

Phenolic compounds are naturally occurring substances in fruit, vegetables, nuts, seeds, flowers and some herb beverages and are an integral part of the human diet. Several studies have indicated that the antioxidant activities of some fruits and vegetables were highly correlated with their total phenolic contents. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions[8, 9]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[10]. Flavonoids, phenolic acids and phenolic diterpenes, lignans are the examples of phenolic compotents with antioxidant properties[11, 12].

Saraca asoca Roxb. De Wilde – Ashoka is a Sanskrit word which means"without sorrow" or that gives no grief. Ashoka tree, universally known by its binomial latin name *Saraca asoca* (Roxb.), De wild or *Saraca indica* belonging family Caesalpinacea[13,14]. It is a small evergreen tree 7-10cm high. It occurs up to the altitude 750 m[15]. Five lignin glycosides, lyoniside, nudiposide, 5-methoxy-9-β-xylopyranosyl-(-)-isolariciresinol, icariside E_3 and schizanriside, three flavonoids, (-)-epicatechin, epiafzelechin-(4β-8)-epicatechin and procyanidine B_2, together with β-sitosterol glucoside, were isolated from dried bark[16]. *Saraca asoca* exhibited antibacterial activity[17], anticancer activity[18], antimenorrhagic activity[19], antioxytocic activity[20]. It have many uses like to treat skin infections, CNS function, genitor-urinary functions, uterus pain painful periods, clots and ammenorhea[21,22].

MATERIALS AND METHODS

Collection and identification of plant material

The stem bark of *Saraca asoca* Roxb.was collected from Bhupal Nobles Institute Campus, Udaipur (Raj.) India in the month of Sep.-Oct. 2008. It was dried under shade. Drug sample was identified by Dr. SS Katewa (Department of Botany), College of Science, MLSU, Udaipur (Raj.) India.

Materials

Chemicals 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and Gallic acid were perchased from M/S Sigma Chemical Co. Other chemicals and reagents used were of analytical grade. UV-visible spectrophotometer, Shimadzu 1800 was used for recording the spectra.

Preparation of extracts

Dried stem bark was ground in to moderately coarse powder (≠22) in a grinder. Extracts were prepared by various extraction methods (soxhlet, ultrasonication and microwave assisted extraction) by using three solvents (ethanol 90%, ethanol 60% and acetone). The parameters used for soxhlet is time-48 hrs., temperature-50^{0}C, for ultrasonication method is time-90 min., temperature-50^{0}C and for microwave assisted extraction method, time- 3 min., temperature 60^{0}C, power- 480 htz.

In-vitro antioxidant studies

The prepared extracts were tested for its free radical scavenging property using DPPH method. All experiments were performed thrice. In-vitro DPPH (1,1-diphenyl-2-picryl hydrozyl) radical scavenging activity was carried out by adopting the methopd of Blois[23], Cotelle[24], spectrophotometric method[25]. To a methanolic extract of DPPH (200µM), 0.05ml of test extracts dissolved in ethanol were added at different concentrations (100-500 µg/ml). An equal amount of ethanol was added to the control. After 20 min., the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula[26]. The antioxidant activity is expressed as IC_{50}.

$$\text{Inhibition (\%)} = \text{Control-Test} \setminus \text{Control} \times 100$$

Statistical Analysis

Linear regression analysis was used to calculate the IC_{50} values.

RESULTS

Table 1: Effect of Ethanolic (90%), Ethanolic (60%), Acetone extract of *Saraca asoca* Roxb.(stem bark) prepared by Soxhlet method (at 60 °C, 48 hrs.) on DPPH radical scavenging model (Values are mean of three replicates)

Concentration (µg)	Percent Reduction Ethanol 90%	IC_{50} (µg/ml)	Percent Reduction Ethanol 60%	IC_{50} (µg/ml)	Percent Reduction Acetone	IC_{50} (µg/ml)
50	15.48		18.11		16.01	
60	18.37		21.25		20.20	
70	20.73	187.70	23.35	193.88	23.35	168.40
80	21.52		24.40		24.93	
90	24.40		26.77		28.34	
100	27.55		30.44		29.39	

Table 2: Effect of Ethanolic (90%), Ethanolic (60%), Acetone extract of *Saraca asoca* Roxb. (stem bark) prepared by microwave method (at 60°C, 480Htz, 3 min.) on DPPH radical scavenging model. (Values are mean of three replicates)

Concentration (µg)	Percent Reduction Ethanol 90%	IC_{50} (µg/ml)	Percent Reduction Ethanol 60%	IC_{50} (µg/ml)	Percent Reduction Acetone	IC_{50} (µg/ml)
50	17.58		18.63		20.73	
60	21.25		21.78		23.35	
70	22.83	166.80	24.14	165.84	24.67	160.09
80	25.98		27.03		28.08	
90	28.34		29.92		30.97	
100	29.92		32.02		34.38	

Table 3: Effect of Ethanolic (90%), Ethanolic (60%), Acetone extract of *Saraca asoca* Roxb. (stem bark) prepared by ultrasonication method (at 50°C, 90 min.) on DPPH radical scavenging model. [Values are mean of three replicates]

Concentration (µg)	Percent Reduction Ethanol 90%	IC_{50} (µg/ml)	Percent Reduction Ethanol 60%	IC_{50} (µg/ml)	Percent Reduction Acetone	IC_{50} (µg/ml)
50	21.25		22.57		26.50	
60	23.35		27.03		29.65	
70	27.82	139.78	30.44	126.54	35.17	97.82
80	29.65		34.38		40.15	
90	34.12		37.27		47.50	
100	37.27		40.15		50.91	

Several concentrations ranging from (50-100 µg/ml) of the prepared extracts by various methods using different parameters were tested for their antioxidant activity in DPPH- radical scavenging, *In-vitro* model. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner. From the results given in Tables 1-3, it was inferred that, with respect to maximum percentage inhibition, in DPPH model, the extracts prepared by ultrasonication method showed maximum % inhibition respectively at 100 µg/ml concentration. However the IC_{50} value of ethanolic (90%), ethanolic (60%) and acetone extracts prepared by ultrasonication method were shown to be 139.78, 126.54, 97.82 µg/ml respectively. The extracts prepared by soxhlet extraction method showed least inhibition at 100 µg/ml concentration in this model.

DISCUSSION

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals. The 1, 1, diphenyl-2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds such as phenolic and anthocyanins or crude mixtures (extracts) of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. When DPPH encounter proton radical scavengers, its purple colour fads rapidly. This assay determines the scavenging of stable radical species of DPPH by antioxidants. The literature supports that phytoconstituents such as polyphenolic compounds in drugs are responsible for the antioxidant potential[27, 28, 29]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant[30].

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