Studies on Phytochemical Constituents, Quantification of Total Phenolic, Alkaloid Content and *In-vitro* Anti-oxidant Activity of *Thespesia populnea* Seeds

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ABSTRACT

Introduction: Anti-oxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress. There is an increasing interest in natural anti-oxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage. **Methods:** In this present study we investigated preliminary phytochemical, total phenolic, alkaloid content and *In-vitro* antioxidant activity of hexane, ethyl acetate, ethanol (70%) and methanol extracts of *Thespesia populnea* Seeds. **Results:** *Thespesia populnea* Seeds revealed the presence of steroids, flavonoids and alkaloids, glycosides, tannins, quinones and carbohydrates. The extracts do not contain the amino acids, oils and the hexane fraction do not contain triterpenes and tannins. The ethyl acetate extract have more phenolic content than other extracts and the methanolic extract has more alkaloidal than other extracts. The selected plant extracts were produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at a concentration of 160 µg and there after the percentage inhibition were raised gradually to its maximum level with higher concentrations. **Conclusion:** Among the four types of *T.populnea* seeds extracts, the methanolic extract showed better activity than aqueous extracts at 160 µg concentrations.

Keywords: *Thespesia populnea* Seeds, Total Phenolic content, Alkaloid content, Antioxidant activity.

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INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years and continue to provide mankind with new remedies. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. The search for eternal health and longevity and for remedies to relieve pain and discomfort drove early man to explore his immediate natural surroundings and led to the use of many plants, animal products, and minerals, etc. and the development of a variety of therapeutic agents. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. Natural products are known to play an important role in both drug discovery and chemical biology. In fact, many of the current drugs either mimic naturally occurring molecules or have structures that are fully or in part derived from natural motifs[1]. Recently, the biological and pharmacological properties of herbs have begun to receive more attention in the scientific community and have become a very important research focal point. This is attributed to the fact that they contain phytochemicals, such as anti-oxidants, and other bioactive compounds. We have been successful in evaluating the anti-oxidant activities of several traditional medicinal plants[2, 3].

Screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule and drug-like properties at the onset of drug discovery will pay off later in drug development. In recent years, considerable number of studies has indicated the important role of free radicals and reactive oxygen species (ROS) in the progress of many human diseases[4]. ROS production in excess of cellular anti-oxidant capacity may result in damage to lipids, proteins, and DNA[5, 6]. Many studies have indicated that anti-oxidant

nutrients and/or medicines have a protective role in human health[7]. Therefore, anti-oxidants are considered effective inhibitors pathogenetically associated with oxidative mechanisms. Anti-oxidants are vital substances which possess the ability to protect the body from damage caused by free radical induced oxidative stress[8]. There is an increasing interest in natural anti-oxidants, e.g., polyphenols, present in medicinal and dietary plants, which might help prevent oxidative damage[9]. Many synthetic anti-oxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and are used for industrial processing but they possess some side effects and toxic properties to human health[10]. Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases. Among the various medicinal and culinary herbs, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant anti-oxidant capacities and health benefits[11].

Thespesia populnea, commonly known as the Portia Tree is species of flowering plant in the mallow family, Malvaceae. According to Ayurveda, Paraspipal is astringent, acrid, cooling, depurative, vulnerary, alternative and useful in skin related troubles, leprosy, diseases of blood and urinary system, diarrhea, dysentery, Cholera, diabetes, ascites etc. It has been used many ways in traditional medicines in Polynesia and South Asia in treating coughs, headaches and intestinal diseases. In Tonga, a drink made from the leaves and bark is used to treat fevers in teething children. Various parts of the plants have high tannin contents and plant extracts have been shown to have anti-bacterial and anti-viral activity[12].

In present study we have extracted dried seeds of *Thespesia populnea* in hexane, ethyl acetate, ethanol (70%v/v) and methanol. These extracts were checked for their phyto chemical constituents, Quantification of Total Phenolic, Alkaloid Content and *In-vitro* antioxidant activity. The extracts were found to be a potent antioxidant effect.

MATERIAL AND METHODS

Chemicals and Drugs

All chemicals and solvents were of the analytical grade obtained from S.D. Fine Chemical Pvt. Ltd., Mumbai, Sigma Chemical Company, U.S.A., Loba Chemic, Mumbai.

Preparation of extracts from Thespesia populnea seeds

The seeds of *Thespesia populnea* were collected from Amudalavalasa, Srikakulam Dist, Andhra Pradesh, India, during the month of December, 2009. The authentication of the above plant was done by Dr. P. Prayaga Murthy, Department of Botany, Andhra University, Visakhapatnam. Shade dried seed's powder of *T.populnea,* was separately extracted in a Soxhlet apparatus for 6 hrs successively with hexane, ethyl acetate, methanol and ethanol (70%v/v) were concentrated to dryness under vacuum. The weight of the powdered plant materials taken for extraction and the weight of the crude extractives obtained are given in Table 1.

Qualitative Phytochemical Screening

A systematic and complete study of crude drugs should include a thorough investigation of both primary and secondary metabolites derived as a result of plant metabolism. Different qualitative chemical tests and quantification of total phenolic and alkaloidal contents were performed for establishing the profile of a given extract for its nature of chemical composition.

Quantification of Total Phenolic content

Total phenolic content was determined using the Folin-Ciocalteau reagent Singleton *et al.,*[13]. Folin-Ciocalteau colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue absorption with a maximum at 765nm.The intensity of the light absorption at that wave length is proportional to the concentration of phenols. By using standard Gallic acid calibration curve, measure the concentration of phenolic content in Gallic acid total equivalents using unit's mg/gm. (GAE).

Table 1. Percentage of the extractives obtained from Thespesia populnea seeds

Name of the solvent	Weight of the powdered material (kg)	% of soluble extractives (Wt/Wt)
Hexane	2.5	6.08
Ethyl acetate	2.5	23.45
Methanol	2.5	29.25
Ethanol (70%v/v)	2.5	33.53

Quantification of Total Alkaloid Content

Total alkaloid content was determined by the Fazel *et al.,* method[14]. The plant extract (1mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of this solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extracts were collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. All experiments were performed thrice; the results were averaged and reported in the form of Mean ± S.E.M.

In-vitro anti oxidant activity

For the assessment of free radicals scavenging activity, the hexane, ethyl acetate, Ethanol (70%v/v) and methanol extracts were dissolved in water and 5% dimethyl sulphoxide (DMSO) respectively.

Superoxide radical Scavenging activity

Superoxide scavenging activity of the plant extract was determined by McCord & Fridovich method[15], which depends on light induced superoxide generation by riboflavin and the corresponding reduction of nitroblue tetrazolium.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is commonly used to evaluate the free radical scavenging effectiveness of various antioxidant substances[16]. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+}/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radical attacks deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (TBARS).

DPPH radical Scavenging activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by Braca *et al*.[17]. In DPPH assay method is based on the reduction of alcoholic DPPH solution (dark blue in colour) in the presence of a hydrogen donating antioxidant converted to the non radical form of yellow colored diphenyl-picrylhydrazine. Lower the absorbance higher the free radical scavenging activity[18].

RESULTS

Qualitative phytochemical screening and quantified total phenolic and alkaloidal contents of all extracts of *Thespesia populnea* seeds revealed the presence of steroids, flavanoids, alkaloids, glycosides, tannins, phenols, quinones and carbohydrates. The extracts do not contain the amino acids, oils and the hexane fraction do not contain triterpenes and tannins. Results of qualitative phytochemical screening of the extracts are shown in Table 2.

The Quantified phenolic contents of *Thespesia populnea* seeds extracts were ranging from 22.74±0.74 to 44.58±0.25 (mg/gm). The ethyl acetate extract have more phenolic content 44.58±0.25 (mg/gm) than other extracts and the alkaloidal content was ranging from 12.28±0.52 to 20.48±0.46 (mg/gm). The methanolic extract has more alkaloidal content 20.48±0.46 (mg/gm) than other extracts. Results of quantified phenolic and alkaloidal contents were showed in Table-3.

In the present study, the Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts *T.populnea* seeds were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin and the results are given in fig-1. The mean IC_{50} values for superoxide radical of Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts *T.populnea* seeds were found to be 169.22µg, 147.25µg, 289.04 µg and 420.31µg respectively. The mean IC_{50} value of ascorbic acid was found to be 53.5µg. The results were given in Table-4.

The Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts *T.populnea* seeds were found to possess concentration dependent scavenging activity on hydroxyl radicals and the results were given fig-2. The mean IC_{50} values for hydroxyl radical of alcoholic crude extract, methanolic, ethyl acetate and hexane extracts *T.populnea* seeds were found to be 203.22µg, 161.02µg, 268.42µg and 526.24µg respectively. The mean IC_{50} value of ascorbic acid was found to be 67.8µg. The results were given in Table-4.

The Ethanol (70%v/v), methanolic, ethyl acetate and hexane extracts *T.populnea* seeds were found to possess concentration dependent scavenging activity on DPPH radicals and the results were given fig-3. The mean IC_{50} values for DPPH radical of alcoholic crude extract,

Table 2. Nature of phytoconstituents presents in different extracts of *Thespesia populnea* seeds

Name of the Phytochemicals	*Thespesia populnea* seeds			
	Hexane extract	Ethyl acetate extract	Methanol extract	Ethanol (70%v/v)
Phytosterols	+	+	+	+
Triterpenes	–	+	+	+
Glycosides	+	+	+	+
Saponins	–	–	–	–
Flavonoids	–	+	+	+
Phenols	–	+	+	+
Tannins	_	+	+	+
Carbohydrates	+	+	+	+
Alkaloids	+	+	+	+
Amino acids	–	–	–	–
Oils	–	–	–	–
Quinones	+	+	+	+

+ = Present, – = Absent

Table 3. Total phenolic and alkaloid content (mg/gm) of *Thespesia populnea seeds* extracts

Name of the extract	Total Phenolic content (mg/gm)	Total alkaloid content (mg/gm)
Hexane	22.74±0.74	12.28±0.52
Ethyl acetate	44.58±0.25	16.32±0.28
Methanol	32.82±0.45	20.48±0.46
Ethanol (70%v/v)	27.22±0.57	14.53±0.22

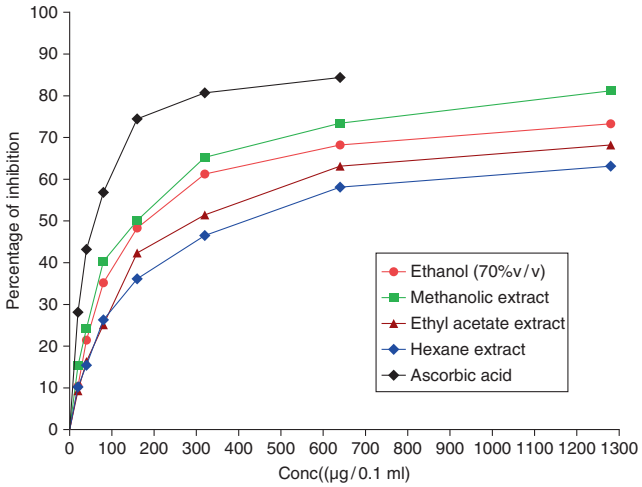

Figure 1. *In-vitro* concentration dependent percentage inhibition of Superoxide radical by different extracts of *Thespesia populnea* seeds.

methanolic, ethyl acetate and hexane extracts *T.populnea* seeds were found to be 151.42µg, 114.25µg, 157.32µg and 225.25µg respectively. The mean IC_{50} value of ascorbic acid was found to be 18.5µg. The results were given in Table-4.

DISCUSSION

T.populnea has number of medicinal properties[19-22] and many reports are available regarding its role in different pharmacological activities[23-25]. Different parts of the plant are effective in some activities[26-29] but there are fewer reports on seeds of *T.populnea*. The present study was carried out to evaluate the *In-vitro* antioxidant activity of *T.populnea* seeds. The *T.populnea* seed extracts produced concentration dependent percentage inhibition of superoxide radical and produced maximum activity at a concentration of 160 µg and there after the percentage inhibition gradually rose to its maximum level with higher concentrations. Among the four types of *T.populnea* seeds extracts, the methanolic extract showed better activity than alcoholic extracts at 160 µg concentrations. Among the samples, better free radical scavenging activity was found in methanolic extract of *T.populnea*, the order of activity was found to be in the following order: ascorbic acid >methanolic extract >alcoholic crude extract > ethyl acetate extract> hexane extract. These results were encouraging as the *T.populnea* seeds appeared to be containing pharmacologically active substances like different parts of the *T.populnea*. So, these findings can form the basis of further studies to isolate active compounds with goal to find new therapeutic problems.

CONCLUSION

In conclusion, the results of present study indicate that the Ethanol (70%v/v), methanolic, ethyl acetate and

Table 4. 50% Inhibition concentrations (ic_{50}) of different extracts of *thespesia populnea* seeds against superoxide, hydroxyl and dpph radicals.

Extracts	50% Inhibition Conc (IC_{50})		
	Superoxide radical	Hydroxyl radical	DPPH radical
Ethanol (70%)	169.22	203.22	151.42
Methanolic extract	147.25	161.02	114.25
Ethyl acetate extract	289.04	268.42	157.32
Hexane extract	420.31	526.24	225.25
Ascorbic acid	53.5	67.8	18.5

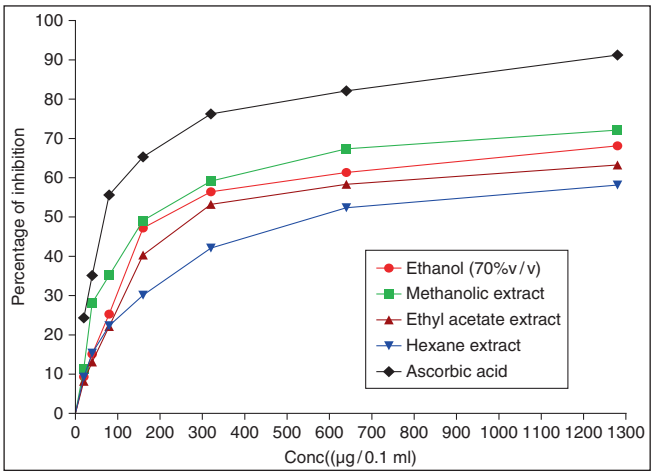

Figure 2. *In-vitro* concentration dependent percentage inhibition of Hydroxyl radical by different extracts of *Thespesia populnea* seeds.

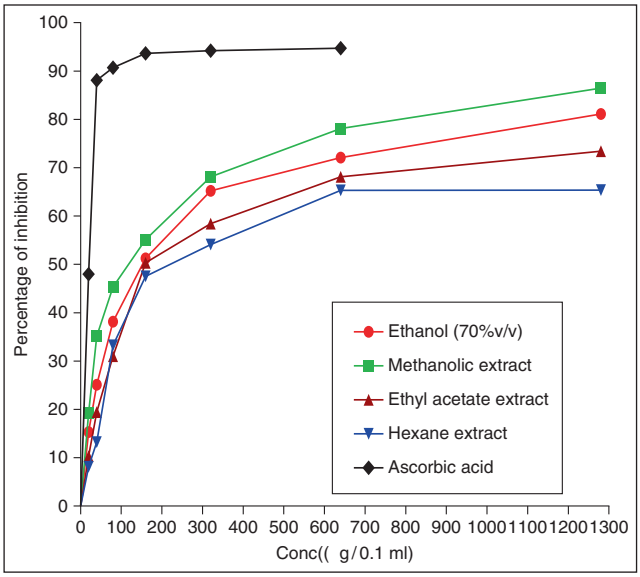

Figure 3. *In-vitro* concentration dependent percentage inhibition of DPPH radical by different extracts of *Thespesia populnea* seeds.

hexane extracts *T.populnea* seeds were found to possess interesting antioxidant activity may be due to the presence of phenolic and alkaloids in the extracts. Now, our next step is to isolate the lead bio-active molecules from this plant seeds.

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