ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(3):578-581

ORIGINAL RESEARCH ARTICLE

Phytochemical Screening and Evaluation of Various Extracts of *Thespesia populnea* for Antioxidant Activity

Laxmi Banjare*[1] and Pranita Kashyap[1]

[1]*Department of Pharmaceutical chemistry, Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, - 490042(C.G.), India*

Received 14 Feb 2012; Revised 17 May 2012; Accepted 26 May 2012

ABSTRACT

The antioxidant activity of Ethanolic extract of *Thespesia populnea* could be compared with that of hydroalcoholic extract. Further Study on the in-vivo antioxidant activity of the *Thespesia populnea* leaves extract is needed. It's antioxidant activity can be further correlated with other pharmacological activity like analgesic and anti-inflammatory activity using animal models as the generation of reactive oxygen species (ROS) and free radicals in human is suggested to contribute to the wide range of pathological condition. Thus the traditional uses of the seeds of *Thespesia populnea* used for curing different diseases can be confirmed and thus formulations of such extracts could be made .

Key words: Antioxidant Activity, Reactive oxygen species (ROS) and Free radicals.

INTRODUCTION

Thespesia populnea Milo's scientific name is *Thespesia populnea*[1,2] and it is also know as Portia tree . *Thespesia* is a genus of *Malvaceae* with basically tropical and subtropical world wide distribution [3]. Probably it originated in India, but is a common plant of costal strands across Old World tropics. Especially in south India *Thespesia populnea* is commonly named as medicinal plant and found to possess useful medicinal properties[3,4] such as antifertility , anti-inflammatory, antioxidant , purgative and hepto protective[5] activity etc and also the bark , leaves and flowers are useful in cutaneous infections such as scabes , psoriasis , eczema , ringworms , guinea worm , anti-inflammatory for poultice as a folk medicine etc. Literature survey revealed that the phytochemical screening of *Thespesia populnea* was carried out by isolating various phytoconstituents from different parts of the plant using various standard procedures[6] for extraction using the solvents by increasing polarity Petroleum ether, Ethyl acetate, chloroform, methanol etc.

MATERIALS AND METHODS

Plant Material

Plant material the of *Thespesia populnea* leaves were collected They were authenticated by Mrs swasti b.gaikwad Center for Pharmaceutical Sciences, Institute of Science and Technology, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad - 500 085, Andhra Pradesh, India. . Dried leaves samples were ground into a uniform powder using a blender and stored in polythene bags at room temperature.

Preparation of Extracts

The leaves were dried in shade for 12 days After complete drying of leaves , they were powdered coarsely using grinder and accurately weighed 50 gm of powder was defatted using Petroleum ether to remove the fats from the leaves The defatted powdered material were further used for extraction with different organic solvents, namely :Aqueous , Ethanol and Pet. Ether.

Extraction method

Solvents used for extraction were of Lab Grade. Extraction was carried out using Soxlet apparatus. It was originally designed for the extraction of a lipid from a solid material.However, a Soxhlet extractor is not limited to the extraction of lipids. It is continuous heating Extraction method .The solvent is heated to reflux. The solvent vapor travels up distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor cools, and drips back down into the chamber housing the Round bottom flask.

***Corresponding Author:** Laxmi Banjare**, Email:** banjarelaxmi24@gmail.com

Phytochemical Screening

Phytochemical screening of the extract was carried outto identify primary metabolites like carbohydrates (Molisch reagent test), Proteins (Biuret test) and of secondary metabolites such as alkaloids (Mayer's test), flavonoids , terpenoids (Salkowski test), tannins (Ferric chloride test), saponins (Frothing test), cardiac glycosides (Keller-Killiani test) and anthraquinones(Borntrager's test).

Antioxidant Assay

The antioxidant activity of Plant extracts was determined by different in-vitro methods such as, theDPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered.

a. DPPH Radical scavenging activity

The antioxidant activity of different plant extracts were carried out using in-vitro method DPPH free radical scavenging assay .DPPH reagent was purchased fron Sigma company (Kolkata) . Four ml of 0.004 % of DPPH- methanolic solution was added in each of the testtubes containing 1000µl of extracts of different concentration. The mixture was shaken vigorously and incubated for 30min in room temperature. Absorbance of the resulting solution was measured at 519 nm UV-Visible Spectrophotometer.. Blank was prepared with 4 ml of DPPH –methanolic solution (without extract). Positive control was taken as Ascorbic acid (standard). Percentage of DPPH scavenging activity determined as follows:

% DPPH radical – scavenging =

$$\frac{\text{Absorbance of control – Absorbance of Sample) x 100}}{\text{Absorbance of control}}$$

b. Reducing Power

This was carried out as described previously12. 1 ml of extract solution (final concentration 50- 250 mg/l) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [K3Fe (CN6)] (10g/l), and then mixture was incubated at 50 degree C for 20 minutes. Two and one-half, 2.5 ml of trichloro acetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml Fecl3 (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectophotometer 117, INDIA). As a control, ascorbic acid was used (final concentration 10 mg/ml). Increased absorbance of the reaction mixture indicates stronger reducing power. In this study, petroleum ether, ethanol and extracts of leaves were used.

RESULTS

1. Extractive value

Soxlet apparatus was used for extraction of *Thespesia populnea* leaves using different solvents. Extractive value was found more in Ethanolic extract followed by aqueous and then by Petroleum ether (**Table 1**).

Table 1: Extractive value of *Thespesia populnea* leaves in different solvents

S. No	Extract	% Yield(w/w)
1	Pet .Ether	0.64
2	Ethanolic	3.2
3	Aqueous	2.56

2. Phytochemical screening

Preliminary phytochemical screening of the crude extracts revealed the presence of carbohydrates , Proteins etc and secondary metabolites like saponins , glycosides , alkaloids etc. in all the extracts but tannins were found absent in Ethanolic and Pet. Ether extract (**Table 2**) .

Table 2: Phytochemical screening result of different extracts of *Thespesia populnea*

S. No	Metabolites	Pet.Ether	Ethanolic	Aqueous
1	Carbohydrates	+	+	-
2	Proteins	+	+	+
3	Alkaloids	+	+	+
4	Glycosides			
5	Flavonoid	+	+	-
6	Saponin	+	+	-
7	Tanins	--	--	-

3. *In-vitro* antioxidant assay

The Scavenging effect of Petroleum ether , Ethanolic and aqueous extracts of and ascorbic acid on *Thespesia populnea* extract the DPPH radical is illustrated in (**Table 3.1 , 3.2,3.3 , 3.4 & 3.5**) ,respectively. The comparision of % inhibition concentration of different extracts with that of standard were presented .There was significant increase in percentage of Radical scavenging activity of ethanolic extract with increase in concentration, followed by aqueous then Pet. Ether extracts. But %RSA of Ethanolic extract was lesser than that of ascorbic acid.

Table 3.1: Absorbance of Petroleum Ether extract at λmax=519

S. No	Conc.(µg/ml)	Absorbance (Mean , n=3)
1	50	0.3550
2	100	0.3309
3	150	0.3093
4	200	o.2925
5	250	0.2409
	Blank	0.3669

Table 3.2: Absorbance of Ethanolic extract at λmax=519

S. No	Conc.(µg/ml)	Absorbance (Mean , n=3)
1	50	0.5614
2	100	0.4225
3	150	0.3195
4	200	0.2913
5	250	0.1062
	Blank	0.7141

Table 3.3: Absorbance of Aqueous extract at λmax=519

S. No	Conc.(µg/ml)	Absorbance (Mean , n=3)
1	50	0.6416
2	100	0.6106
3	150	0.4369
4	200	0.3078
5	250	0.1128
	Blank	0.7141

Table 3.4: Absorbance of Ascorbic acid at λmax=519

S. No	Conc.(µg/ml)	Absorbance (Mean , n=3)
1	50	0.0472
2	100	0.0421
3	150	0.0379
4	200	0.0320
5	250	0.0265
	Blank	0.7141

Table 3.5: Comparisionof % RSA of different extracts with Ascorbic acid

S.No	Conc. (µg/ml)	% RSA of Pet. Ether extract	% RSA of Ethanolic extract	% RSA of Aqueous extract	% RSA of Ascorbic acid
1	50	3.28	21.37	7.23	93.39
2	100	9.8	40.91	14.46	94.10
3	150	15.69	55.97	38.80	94.69
4	200	20.23	59.16	56.87	95.51
5	250	34.32	87.12	84.19	96.28

Data are expressed as mean ± SD of triplicate tests

4. Reducing power

Different extracts of Tribules terrestris exhibited good reducing power. The reducing power of the plant extract was determined by the method of Yildirim, A., et al. High absorbance indicates high reducing power. Reducing power of the Aqeous and Ethanolic extracts was dose dependent but the petroleum ether extract had shown negligible effect and is presented in (**Table 4**).

Table 4: Reducing Power of various extracts of *Thespesia populnea*

S. No	Conc.(µg/ml)	Reducing power of Pet. Ether extract	Reducing power of Ethenolic extract	Reducing power of Aqueous extract	Reducing power of Ascorbic acid
1	50	4.32	19.10	9.12	20.33
2	100	11.13	22.53	24.82	34.73
3	150	23.10	34.29	47.22	53.45
4	200	25.12	48.61	51.29	58.81
5	250	29.11	56.71	62.18	65.67

Data are expressed as mean ± SD of triplicate tests

DISCUSSION

The Phytochemical screening of different extracts of *Thespesia populnea* namely: Petroleum Ether , Ethanolic and water ,revealed that they contained chemicals like flavanoid which is claimed to have antioxidant response .The in-vitro antioxidant activity study of these extracts was carried out using DPPH radical scavenging method. Out of these three extracts, Ethanolic extract showed more significant antioxidant activity followed by water then Pet. Ether extract. The DPPH assay has been largely used as a quick, reliable and reproducible parameter to search for the in vitro general antioxidant activity of pure compounds as well as plant extracts. The decrease in absorbance by the DPPH radical with increase in concentration of the extract which manifested in the rapid discoloration of the purple DPPH, suggest that Ethanolic extract of *Thespesia populnea* has antioxidant activity due to it's proton donating ability.

CONCLUSION

It is concluded from this study that *Thespesia populnea* extracts possessed antioxidant activity. . The antioxidant potential extracts of it may attribute to the presence of saponins and flavanoids. Based on the results, it was found Ethanolic extract gave more antioxidant activity than Petroleum Ether and aqueous extracts. But when compared, the antioxidant activity of seeds were found to be lesser than that of fruits Therefore it is necessary to exploit its maximum potential in the field of Medicinal and pharmaceutical sciences for novel and fruitful application as antioxidant activity can be further related with other pharmacological activities.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Pranita Kashyap, Principal, Shri Rawatpura Sarkar Institute of

pharmacy for permitting this research work. The author's acknowledges the staff members and friends of PG department of pharmacy for their kind co-operation for complete this research work successfully.

REFRENCES

1. Fryxell, P.A. 1966, Australian Plants: Thespesia in Australia Sept.1966 vol.3 pp 366-367.
2. Morton, J.F. 1966, Australian Plants: Seaside mahoe (*Thespesia populnea* Soland.) Sept.1966 vol.3 pp 367-369.
3. Uses of Plants. Bishop Museum Press, Honolulu.. Allen, J.A. 2002. *Cordia subcordata*. Tropical Tree Seed Manual pp. 18–419. Agriculture Handbook 721. USDA Forest Service, Washington, DC. In: Vozzo,J.A. (ed.)
4. Fosberg, F.R, and M.H. Sachet. 1972. Thespesia populnea (L.)Solander ex Correa and Thespesia populneoides (Roxburgh) Kosteletsky (Malvaceae). Smithsonian Contributions to Botany 7: 1–13.
5. Shirwaikarkumar A, Krishnan AV, Sreenivasan KK. Chemical investigation and antihepatotoxic activity of *Thespesia populnea* . Int J Pharmacog. 1995; 33: 305-10.
6. Bevans,N. Alford,M. Guanches,F. Aregullin,M. Rodriguez,E. 2001 "Preliminary Phytochemical Study of Two Caribbean Malvaceae Used in the Treatment of Conjunctivitis" J USAIR Issue 2, 20-24 , 2001.
7. Eavens WC and Treese Pharmacognosy.London ,Ballitere, Tindall ,1996,pp388-43.