Research Article [Siju *et al.*, 5(1): Jan., 2014:3213-3216]
CODEN (USA): IJPLCP ISSN: 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES
(Int. J. of Pharm. Life Sci.)

Antioxidant Activity of *Thespesia populnea* Linn Fruits

E. N. Siju[1]*, G. R. Rajalakshmi[2], V. S. Vipin[1], Jolly Samu[1] and Arun Shirwaikar [3]

1, Department of Pharmacology, Academy of Pharmaceutical Science, Pariyaram Medical College, Kannur,Kerala, India

2, College of Pharmaceutical Science, Government Medical College, Kozhikode,Kerala, India

3, College of Pharmacy, Gulf Medical University, Ajman, U. A. E.

Abstract

The present study was undertaken to find the antioxidant value of *Thespesia populnea*. Thus they may well be defined as the substances that are capable of quenching or stabilizing free radicals. Antioxidant potential of ethanolic and aqueous extract of *Thespesia populnea* fruit (TPF) was evaluated by *in- vitro* antioxidant studies like free radical scavenging activity by 1,1-diphenyl,2-picrylhydrazyl (DPPH) method, Reducing power assay, Superoxide anion scavenging activity , Hydroxyl radical scavenging activity and Nitric oxide method. The results suggest that the fruit of *Thespesia populnea* showed significant antioxidant property.

Key-Words: Antioxidant, *Thespesia populnea*, Radical scavenger, DPPH

Introduction

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Recently, there has been increased interest in oxygen containing free-radicals in biological systems and their implied roles as causative agents in the etiology of a variety of chronic disorders. Accordingly, attention is being focused on the protective biochemical functions of naturally occurring antioxidants in the cells of the organisms containing them[1].*Thespesia populnea (Linn.*) *So land ex Correa* (Family – Malvaceae) is very popular as a medicinal plant as mentioned in the ancient text of ethnic medicines distributed mainly along the coastal regions throughout India, It grows to a maximum height of 18 meters. Fruits are oblong brown capsules covered with minute peltate scales, pubescent, channelled along the back[2]. The bark is so often fibrous and fissured in nature with grey to brown in colours. The leaves are simple, alternate, long petiolate, cordate, entire, acuminate, prominent nerves 5 – 7 with peltate scales on one or both surfaces. The flowers are yellow with purple base, slowly changing to purple on withering[3].This plant is astringent, cooling and antidiahrroeal[4]. The bark and fruits posses more curative properties. The bark is astringent and is prescribed in the Philippines for the treatment of dysentery in the form of a decoction. It is used in folk medicine as a poultice for external applications for the treatment of scabies, psoriasis[5] and other skin ailments.

*** Corresponding Author**
E.mail: sijuellickal@rediffmail.com

The poultice prepared from fruits, flowers and leaves are also found to be useful in rheumatoid arthritis .Earlier the plant has been studied for its antibacterial[6], antiviral, anticancer[7], antisteroidogenic activity[8] and for dermatitis[9]. Aqueous extracts of fruits of this plant are reported for its wound healing activity[10].

Material and Methods

The fruit of *Thespesia populnea* were collected from Kottayam district in Kerala, India in March 2006. The same were authenticated by Mr. K G Sreekumar, Senior Research Officer, Pharmacognosy Unit, Govt Ayurveda Research Institute, Poojapura, Thiruvananthapuram, Kerala. A voucher specimen PC-03/2006 was submitted at Academy Of Pharmaceutical Science, Pariyaram Medical College, Kannur for future reference. Dried fruits were ground to coarse powder, passed through sieve no 24 and stored in air tight container and used for further extraction.

Preparation of Extracts

Ethyl alcohol extract (EETP): The shade dried powdered fruits (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The ethyl alcohol extract was concentrated in vacuum to a syrupy consistency. The percentage yield of extract was found to be 4.12 %.

Aqueous extract (AETP): The aqueous extract was prepared using fresh powder by maceration process. 100gm of the powdered drug were taken in a 2000ml conical flask with 500ml of distilled water and 10ml chloroform was added as preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered

through the muslin cloth and the marc is discarded and airtight container in its filtrate dried under hot air oven at 45ºC to semisolid mass which was stored in a refrigerator below 100C. The percentage yield of extract was found to be 6.19 %.

In vitro Determination of Antioxidant activity:

DPPH assay:

The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and up on reaction with a hydrogen donor changes to yellow in color. The free radical scavenging activity was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) using the method of Blois[11.] 0.1 mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3 ml of various concentrations of EETP and AETP the reference compound (10, 25, 50 and 100 µg). After 30 min, absorbance was measured at 517 nm. BHT (25µg) was used as the reference material. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples[12.]

Reducing power:

The reducing power was determined according to the method of Oyaizu[13]. Different concentrations of EETP and AETP (10, 25, 50 and 100 µg) in 1ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 ml, 1%).The mixture was incubated at 500 $^{\circ}$C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm. Sodium metabisulphite was used as the reference material. All the tests were performed in triplicate and the results averaged. Increased absorbance of the reaction mixture indicates increase in reducing power. The % reducing power was calculated by using the formula:

% increase in absorbance = Control OD – Test OD/ Control OD X 100

Superoxide anion scavenging activity:

Oxygen is essential for the survival of aerobic cells, but it has long been known to be toxic to them when supplied at concentrations greater than those in normal air. The biochemical mechanisms responsible for oxygen toxicity include lipid peroxidation and the generation of $H_2O_2^+$ the superoxide radical, $O_2^{\bullet}$. This superoxide radical can inhibit or propagate the process of lipid peroxidation. Measurement of superoxide anion scavenging activity was done by using the method explained by Nishimiki (Nishimiki et al., 1972)[14] and modified by Ilhami et al. About 1 ml of nitro blue tetrazolium (NBT) solution containing 156 µM NBT which is dissolved in 1.0ml of phosphate buffer (100 mM, pH 7.4), 1 ml of NADH solution containing 468 µM of NADH which is dissolved in 1 ml of phosphate buffer (100 mM, pH 7.4) and 0.1 ml of various concentration of EETP and AETP and the reference compounds (10, 25, 50 and 100 µg) were mixed and the reaction started by adding 100 µl of phenazine methosulphate (PMS) solution containing 60 µM of PMS 100 µl of phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 250 ^{0} C for 5 min and the absorbance at 560 nm was measured against the control samples. Vitamin C used as the reference compound. All the tests were performed in triplicate and the results averaged. The percentage decrease in absorbance was calculated.

Hydroxyl radical scavenging activity:

In biochemical systems, superoxide radical and H_2O_2 react together to form the hydroxyl radical, $OH^{\bullet}$, this can attack and destroy almost all known biochemical system[15]. Phenylhydrazine when added to erythrocyte hosts cause peroxidation of endogeneous lipids and alteration of membrane fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like $O_2^{\bullet}$, $OH^{\bullet}$ and H_2O_2 which are generated in solution from auto-oxidation of phenyl hydrazine. This forms the basis of this experiment. Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radical generated by Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao[16]. The reaction mixture contained in a final volume of 1.0 ml, 100 µl of 2-deoxy-2-ribose (28 mM in KH_2PO_4-KOH buffer, 20 mM, pH 7.4), various concentrations of EETP and AETP(10, 25, 50 and 100 µg) and the reference compound sodium metabisulphate (25 µg) in KH_2PO_4-KOH buffer (20 mM, pH 7.4), 200 µl of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1 v/v), 100 µl of 1.0 mM H_2O_2 and 100 µl of 1.0 mM ascorbic acid was incubated at 370 $^{\circ}$C for 1h. 1.0 ml of thiobarbituric acid (1%) and 1.0 ml of trichloroacetic acid (2.8%) were added to the test tubes and incubated at 100°C for 20min. After cooling, absorbance was measured at 532 nm.

Nitric oxide radical scavenging activity:

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with

the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. This forms the basis of this experiment[17]. Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by the Griess reaction [18,19]. The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS),EETP,AETP and the reference compound in different concentrations (10, 25, 50 and 100 µg) were incubated at 25°C for 150 min. Each 30 min, 0.5 ml of the incubated sample was removed and 0.5 ml of the Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2%H_3PO_4) was added. The absorbance of the chromophore formed was measured at 546nm.

Results and Discussion

The present study shows that the flavonoid rich fraction of *Thespesia populnea* possess a good *in vitro* antioxidant activity. DPPH assay observed that the EETP and AETP have demonstrated dose dependent increase in the DPPH radical scavenging activity. Whereas 25µg butylated hydroxy toluene (BHT) (std.) has 86.01% activity, 100 µg of EETP has shown maximum scavenging activity i.e. 68.49 %. (Table No.1&2).Ethanolic extract showed better activity than aqueous extract.

Reducing power activity observed that the EETP and AETP have exhibited dose dependent increase in the reducing property. Whereas 25µg sodium metabisulphate (std.) has 75.74% reducing property, 100µg of EETP have shown maximum reducing power i.e. 73.92% and 100µg of AETP have shown maximum reducing power i.e. 70.96%. (Table No.1& 2)

Superoxide anion scavenging activity observed that EETP and AETP demonstrated dose dependent increase in the superoxide anion scavenging activity. 25µg vitamin C (std.) has 77.64% activity, whereas, EETP at 100µg has shown 68.18%. (Table No. 1&2)

Hydroxyl ion radical scavenging activity observed that the EETP and AETP demonstrated dose dependent increase in the hydroxical radical scavenging activity. Thus 25µg sodium metabisulphate (std.) has 75.22 % scavenging activity; However, EETP and AETP at 100µg have shown significant scavenging activity. (Table no 1&2)

Nitric oxide radical scavenging activity observed that the EETP and, AETP demonstrated dose dependent increase in the nitric oxide anion scavenging property. Whereas 25µg BHT (std.) has 74.32% nitric oxide anion scavenging property, 100µg of the EETP has shown maximum nitric oxide anion scavenging activity i.e. 64.84%.(Table No 1&2) .In the present study, the nitrite produced by the incubation of solution of sodium nitroprusside in standard phosphate buffer at 25º was reduced by the ethanolic and aqueous extract of *Thespesia populnea*.This may be due to the antioxidant principles in the extract, which complete with oxygen to react with nitric oxide thereby inhibiting the generation of nitrite[19].

Conclusion

The findings of the present study suggested that fruits of *Thespesia populnea* could be explored as potential natural antioxidant.Further study is needed to identify the compounds present in the fruits of *Thespesia populnea* that have antioxidant activities.

References

1. Vinay R. Patel, Prakash R. Patel and Sushil S. Kajal (2010). Antioxidant Activity of Some Selected Medicinal Plants in Western Region of India, *Advances in Biological Research*, 4 (1): 23-26.
2. Arun Shirwaikar, Sarala Devi, Siju E.N. (2011). Anti-Inflammatory activity of *Thespesia populnea* fruits by Membrane Stabilization, *International Journal of Pharm Tech Research*, 3(4):(Oct-Dec) :2060-2063.
3. Warrier P.K., Nambiar V.P.K. and Ramankutty C. (2001). *Indian Medicinal Plants, Orient Longman Ltd., Madras*, Vol:5, 281.
4. Gollapalle Lakshminaraya Shastry Viswanatha, Shylaja Hanumanthappa, Nandakumar Krishnadas and Srinath Rangappa (2011). Antidiarrheal effect of fractions from stem bark of Thespesia populnea in rodents: Possible antimotility and antisecretory mechanisms, *Asian Pac J Trop Med*, 4(6): (Jun): 451-456.
5. Siddharth Shrivastav, Rakesh K. Sindhu, Sanjeev Kumar and Pradeep Kumar (2009). Anti-psoriatic and phytochemical evaluation of thespesia Populnea bark extracts, *International Journal of Pharmacy and Pharmaceutical Sciences*, 1(1): (Nov-Dec): 176-185.
6. Archana Moon, Aqueel Khan and Bharat Wadher (2010). Antibacterial potential of *Thespesia populnea* (Linn) sol.ex Corr. leaves and its corresponding callus against drug resistant isolates, *Indian J of Natural Products and Resources*, 1(4): (Dec) : 444-449.
7. Johnson Inbaraj J., Gandhidasan R. and Murugesan R. (1999). Cytotoxicity and superoxide anion generation by some naturally occuring quinines, *Free Radic Biol Med.*, 26(9-10): (May): 1072-1078.
8. Kavimani S., Ilango R., Karpagam S., Suryaprabha K., and Jaykar B. (1999). Anti-steroidogenic acivity

of floral extract of *Thespesia populnea* corr. in mouse ovary, *Indian J Exp Biol.,* 37(12): (Dec): 1241-1242.

9. Housen B.M., Knight T.E. and Milbrodt M. (1997). *Thespesia populnea* dermatitis *Am J Contact Dermat.,* 8(4): (Dec) : 225-228.
10. Nagappa A.N. and Binu Cheriyan (2001).Wound healing activity of the aqueous extract of *Thespesia populnea* fruit, *Fitoterapia*, 72(5): (Jun): 503-506.
11. Blois M.S. (1958). Antioxidant determinations by the use of stable free radical, *Nature*, 181: (April): 1199-2000.
12. Malaya Gupta U., Kanti Mazumdar P., Gomathi R. and Sambath Kumar (2004). Antioxidant and Free Radical Scavenging Activities of Ervatamia coronaria Stapf. Leaves, *Iranian Journal of Pharmaceutical Research*, 2: 119-126.
13. Oyaizu M. (1986). Studies on products of browning reaction prepared from glucosamine, *The Japanese Journal of Nutrition and Dietetics*, 44(6): 307–315.
14. Nishimiki M., Rao N.A. and Yagi K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen, *Biochemical and Biophysical Research Communications*, 46(2): (Jan): 849- 853.
15. Sasanka Chakrabart, Asha Naik S. and Gali Reddy R. (1990). Phenylhydrazine mediated degradation of bovine serum albumin and membrane proteins of human erythrocytes, *Biochim Biophys Acta.,* 1028(1): (Sep): 89-94.
16. Kunchandy E. and Rao M.N.A. (1990). Oxygen radical scavenging activity of curcumin, *International Journal of Pharmaceutics*, 58(3): (Feb): 237-240.
17. Susanta Kumar Mondal, Goutam Chatraborty, Gupta M. and Mazumder U.K. (2004). In-vitro antioxidant activity of Dispyros malabarica Kostal bark, *Indian J Exp Biol.* 44(1): (Jan): 39-44.
18. Green L.C., Wagner D.A., Glogowski J., Skipper P.L., Wishnok J.S., Tannenbaum S.R. (1982). Analysis of nitrate, nitrite and [15 N] in biological fluids, *Anal Biochem.,* 126(1): (Oct): 131-138.
19. Marcocci L., Maguire J.J., Droy-Lefaix M.T. and Packer L. (1994). The nitric oxide scavenging properties of Ginkgo biloba extract EGb, *Biochem Biophys Res. Commun.* 201(2): (Jun): 748-755.

Table 1: Effect of Ethanolic extract of *Thespesia populnea* on free radical Scavenging activity using different models

S/No.	Conc. µg/ml	%Scavenging				
		DPPH	Reducing power	Superoxide anion	Hydroxyl radical	Nitric oxide radical
1.	Std 25µg	86.01	75.74	77.64	75.22	74.32
2.	10	26.23	28.21	44.49	33.84	22.64
3.	25	34.14	42.08	53.32	48.56	41.14
4.	50	54.63	58.74	63.91	59.22	51.46
5.	100	68.49	73.92	68.18	68.96	64.84

Table 2: Effect of Aqueous extract of *Thespesia populnea* on free radical Scavenging activity using different models

S/No.	Conc. µg/ml	%Scavenging				
		DPPH	Reducing power	Superoxide anion	Hydroxyl radical	Nitric oxide radical
1.	Std 25µg	86.01	75.74	77.64	75.22	74.32
2.	10	23.01	26.24	42.34	40.39	19.62
3.	25	30.03	38.04	51.56	48.57	36.14
4.	50	51.02	58.76	60.23	54.26	46.42
5.	100	64.04	70.96	66.86	61.84	61.82

How to cite this article

Siju E. N., Rajalakshmi G. R., Vipin V. S., Jolly Samu and Arun Shirwaikar (2014). Antioxidant activity of *Thespesia populnea* Linn fruits. *Int. J. Pharm. Life Sci.*, 5(1):3213-3216.

Source of Support: Nil; Conflict of Interest: None declared

Received: 23.11.13; Revised: 10.12.13; Accepted:12.12.13