EFFECT OF SUNLIGHT EXPOSURE ON ANTIOXIDANT PROPERTIES OF *CASSIA FISTULA* SEED AT VARIOUS LEVELS OF EXTRACTION AND ANALYSIS

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ABSTRACT

The study was focused on the influence of direct sunlight exposure on antioxidant composition of medicinally important plant materials. *Cassia fistula,* a well-known medicinal plant, was selected to study the time dependent response of antioxidant properties towards sunlight exposure at different levels of extraction and analysis. *Cassia fistula* seed flour and its liquid and dried extracts were exposed to sunlight for different durations (0, 2, 4, 6, 8 and 10 h) and subjected to antioxidant analysis. Regression analysis of experimental data showed a significant linear negative effect of sunlight exposure on total extractable components and hydroxyl radical scavenging activity. Significant exponential negative effects were observed on total phenolic acids, total antioxidant activity by phosphomolybdenum and DPPH assay, reducing power and DPPH radical scavenging capacities of sunlight exposed extracts. The time dependent effect of sunlight exposure on antioxidant properties of various extracts of *Cassia fistula* seed suggests the researchers and manufacturers of pharmaceutical products to assure protection of antioxidant materials from sunlight during extraction and analysis.

KEY WORDS: Antioxidant activity, *Cassia fistula* seed, Phosphomolybdenum assay, Radical scavenging activity, Regression analysis

No:of Tables: 3 **No: of Figures: 6** **No:of References:27**

INTRODUCTION

Antioxidants are the substances which play a significant role in preventing the oxidative stress caused by endogenous and exogenous free radicals in living and nonliving systems respectively. These substances protect lipids, proteins and nucleic acids from oxidative damage by reducing or scavenging free radicals due to their hydrogen donating ability(Frei, Stocker, & Ames, 1988; Gupta & Sharma, 2006). Plants are the good source of phytochemical compounds which possess antioxidant properties. The natural antioxidants are more effective and nontoxic as compared to synthetic antioxidants. However, there are several factors which may affect antioxidant activity of these compounds during extraction, isolation, purification and analysis. The processing conditions such as light intensity and temperature during soaking, boiling, steaming and drying are the major factors which have been found to affect antioxidant activities of plant material(Xu & Chang, 2008). High temperature drying of plant material has been observed to accelerate degradation of antioxidant compounds and decrease their activity(Burg & Fraile, 1995; Nicoli, Anese, & Parpinel, 1999). The intensity of direct sunlight during drying has been also found to decreases total antioxidant and phytochemical contents of plant materials (Chauhan & Kapfo, 2013; Hajimehdipoor *et al*., 2012; Mudau & Ngezimana, 2014).

Cassia fistula is a well known medicinal plant traditionally used as carminative and laxative. It is also used to cure leprosy, skin diseases and syphilis. It possesses antibacterial, antidiabetic, anti-fertility, anti-inflammatory antioxidant, antitumor, anti-hypercholesterolemic, antifungal, analgesic and hypatoprotective activities (El-Saadany, El-Massry, Labib, & Sitohy, 1991; Mazumder, Gupta, & Rath, 1998). The innumerable medicinal properties and therapeutic uses prove its importance as a valuable medicinal plant (Bhalerao & Kelkar, 2012; Danish *et al*., 2011). *C. fistula* seed is an important source of polyphenolic compounds including phenolic acids, polyphenols, tannins, flavonoids and other antioxidant compounds which act as free radical scavengers (Duh & Yen, 1997; Jayaraman, Sivaprakasam, Rajesh, Mathivanan, & Arumugam, 2014). Exposure to direct sunlight during drying, storage, extraction and analysis may affect the antioxidant activity of *C. fistula* seed. A careful literature survey showed that no work has been done on the effect of direct sunlight on antioxidant properties of *C. fistula*. Therefore, the present study was planned to evaluate the effect of sunlight exposure on the antioxidant properties of *C. fistula* seed at different stages of extraction and analysis.

MATERIALS AND METHODS

Dry pods of *C. fistula* were obtained from botanical garden of Bahauddin Zakariya University, Multan, Pakistan. Seeds were separated from pods, ground in an electric grinder and sieved through a fine muslin cloth. The seed flour thus obtained was then preserved in air tight container

and stored in dark at 25±5°C throughout the study period.

Experimental design for preparation of extracts

To study the effect of direct sunlight exposure on antioxidant properties of plant material at different stages of extraction and analysis seed flour was divided into four portions of equal weight (20 g) and processed for extraction and sunlight exposure according to the constructed experimental design (Fig. 1). All the samples were extracted in 70% Methanol, evaporated to dryness and processed for antioxidant analysis. The sunlight exposed samples were categorized and abbreviated as in Table 1.

Antioxidant Analysis

Total extractable components

The residue obtained after complete evaporation of solvent from each sample was weighed and total extractable component (TEC) was calculated as:

$$TEC\left(\frac{g}{100g}dry\ weight\right)=\frac{Weight\ of\ extract}{Weight\ of\ sample}\times 100$$

Total phenolic acids content

The total phenolic acids (TPA) content were determined by following the method described by (MUHAMMAD ASLAM Shad, Pervez, Zafar, Nawaz, & Khan, 2012).

Trolox equivalent total antioxidant activity by phosphomolybdenum assay

The total antioxidant activity (TAOA) by Phosphomolybdenum assay was determined by using the reported method (Prieto, Pineda, & Aguilar, 1999). An aliquot (1 ml) of each extract (0.1mg/ml) was mixed with 3 ml of the reagent solution (0.6M Sulphuric acid, 28mM Sodium phosphate solution and 4mM Ammonium molybdate solution 1:1:1 V/V). The reaction mixture was incubated at 95°C for 90 minutes followed by cooling to room temperature and the absorbance was measured at 695 nm against a blank. Trolox equivalent total antioxidant activity was measured using the regression equation obtained from the standard curve of Trolox (R^2=0.9865).

Trolox equivalent total antioxidant activity by 2,2-Diphenyl-2-picryl hydrazyl (DPPH·) assay

Total antioxidant content in methanolic extracts was determined by method as described earlier (Muhammad Aslam Shad, Nawaz, Yaqoob, & Yousuf, 2012). Methanolic extract (1 ml) was mixed with 40 µM Methanolic solution (3 mL) of stable 2, 2-dipheny-1-picrylyhydrazyl (DPPH) radical. The solution was allowed to stand for 30 minutes at room temperature and absorbance was recorded at 517 nm. Trolox equivalent total antioxidant activity was calculated as applying the linear regression equation obtained from a calibration curve of Trolox (R^2 = 0.9844).

Reducing power

The reducing power (RP) was determined by following the developed method (Oyaizu, 1986). An aliquot (2.5 ml) of each extract was mixed with 0.2M phosphate buffer solution of pH 6.6 (2.5 ml) and 1% potassium ferricyanide solution (2.5 ml). The mixture was incubated at 50 °C for 20 minutes followed by the addition of 10% trichloroacetic acid (2.5 ml). The mixture was centrifuged at 3000 rpm for 10 min and upper layer (5 ml) was mixed with distilled water (5 ml) and 1% ferric chloride

(1 ml). The absorbance was measured at 700 nm. A higher absorbance indicates a higher reducing power.

DPPH radical scavenging capacity

The DPPH radical scavenging capacity (DPPH RSC) was assayed by reported method (Sánchez-Moreno, Larrauri, & Saura-Calixto, 1999). The Methanolic extract (1 ml) was mixed with 40 µM Methanolic DPPH· solution (3 ml) and the mixture was allowed to react at room temperature (25±5 °C) for 30 minutes. After 30 minutes of incubation, the decolourisation of the purple colour was recorded at 517 nm spectrophotometrically. Methanol was taken as blank and DPPH solution, without the plant extracts, was used as positive control. The radical scavenging capacity was calculated as follows:

DPPH radical scavenging capacity (%) $= \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity (HRSC) was assayed by the method of (Smirnoff & Cumbes, 1989). An aliquot (1 ml) of each extract (0.1 mg/ml) was mixed with 9mM ferrous sulphate solution (1 ml) and 9mM salicylic acid solution (1 ml) followed by the addition of 8.8mM hydrogen peroxide solution (1 ml). The reaction mixture was incubated at 37 ºC for 30 minutes and the absorbance of the hydroxylated salicylate complex was measured at 510 nm against a blank. The scavenging activity of hydroxyl radical effect was calculated as:

$$\text{Hydroxyl radical scavenging activity } (\%) = [1 - (\frac{A_1 - A_2}{A_0})] \times 100$$

where, A_1 is the absorbance of sample containing salicylic acid, A_2 is the absorbance of sample without salicylic acid and A_o is the absorbance of blank.

Statistical analysis

The results were expressed as mean ± standard deviation of three parallel replicates and the data were analyzed by regression analysis to investigate the effect of sunlight exposure on the antioxidant properties.

RESULTS AND DISCUSSION

Polyphenolic compounds are commonly found in both edible and inedible plants and have been reported to possess multiple biological activities. Phenolic acids and polyphenols are known to act as antioxidants owing to their ability to scavenge free radicals. Antioxidants perform their action by donating electron to reactive oxygen and nitrogen species (Podsędek, 2007). The in vitro determination of antioxidant activities of plant materials can be determined as antioxidant content, radical scavenging and reducing abilities (Rana *et al*., 2010). However, each of these methods is sensitive towards direct sunlight (Chauhan & Kapfo, 2013; Ponmari, Sathishkumar, Lakshmi, & Annamalai, 2011b). Sun drying not only deactivates the enzymes due to increased temperature but also causes photo degradation of phytochemicals (Mueller-Harvey, 2001). In present study, effect of direct sunlight exposure on antioxidant properties of *C. fistula* seed at different stages of extraction was investigated. The in vitro antioxidant activity of extracts was determined in terms

of total phenolic acid contents, total antioxidant activity, reducing power and radical scavenging capacity. The experimental results for antioxidant activity of unexposed samples of *C. fistula* seeds are presented in Table 2. Total phenolic content and DPPH radical scavenging capacity shown by methanolic extracts for the unexposed sample were 0.389±0.052 g GAE/100 g of dry weight and 49.16±2.57 % respectively (Table 2) and are in high agreement with those reported earlier for the antioxidant potential of *C. fistula* seeds (Jothy, Zuraini, & Sasidharan, 2011). The sunlight induced variations in antioxidant parameters of various samples of *C. fistula* seeds are presented in Figures 2-6. The regression analysis of experimental data (Table 3) showed a time dependant linear negative effect of duration of sunlight exposure on TEC and HRSC as shown in Figure 2 and 6A-C respectively following regression equation was obtained from suggested regression model.

$$\mathrm{TEC}(\%) = \mathrm{E_{SC}} \times \mathrm{DE} + \mathrm{TEC_o}$$

$$\mathrm{HRSC}\ (\%) = \mathrm{E_{SC}} \times \mathrm{DE} + \mathrm{RSC_o}$$

where, $\mathrm{TEC_o}$ and $\mathrm{RSC_o}$ are pre-exponential factors which indicate TEC and OH radical scavenging capacity of antioxidants at negligible time respectively, E_{SC} is light exposure sensitivity coefficient and DE (0, 2, 4, 6, 8, 10) is the duration of sunlight exposure. The regression analysis of the experimental data (Table 3) also showed an exponential decrease in TPA, TAOA, RP and DPPH RSC in response to an increase in the duration of sunlight exposure with high values of coefficient of determination, as shown in Figures 3A-C, 4A-C, 5A-C and 6A-C.

$$TPA\ (Gallic\ acid\ Eqv.\frac{g}{100g\ dry\ weight}) = TPA_o\ e^{Esc.DE}$$

$$TAOA\ \left(Trolox\ Eqv.\frac{g}{100g\ dry\ extract}\right) = A_o\ e^{Esc.DE}$$

$$23\ bnhDPPH\ RSC\ (\%) = RSC_o\ e^{Esc.DE}$$

where, $\mathrm{TPA_o}$, $\mathrm{A_o}$, $\mathrm{R_o}$, $\mathrm{RSC_o}$ are pre exponential factors, which indicate the degree of extraction of phenolic compounds, the antioxidant activity, reducing power and radical scavenging capacity respectively, at negligible time. Thus, using the values of intercept and slope of Figures 3A-C, 4A-C, 5A-C and 6A-C the regression equations were written in Table 3.The predicted values were calculated by putting the values of input variables, pre exponential factors and light exposure sensitivity coefficient in above regression equations and regression models were plotted against the experimental values to test the validity of the suggested model as shown in Figures 2B, 3D-F, 4D-F, 5D-F and 6D-F. A good agreement between the experimental and predicted values of TEC, TPA, TAOA by Phosphomolybdenum assay, TAOA by DPPH assay, RP, DPPH RSC and HRSC was observed with high values of co-efficient of determination (R^2 = 0.8777, 0.9475-0.9858, 0.9542-0.974, 0.9085-0.9667, 0.842-0.8534, 0.8475-0.9409 and 0.9435-0.9764 respectively). The relatively low degree of scattering and high values of regression coefficient of the observed values about the regression line favors the applicability of the suggested model to study the time dependent effect of sunlight exposure on antioxidant extraction.

Table 1. Categorization and abbreviation of various samples as prepared by experimental design

Sr. No.	Sample	Abbreviation	Duration of sunlight exposure (h)
1.	Control	-	0
2.	Dry sample exposed to direct sunlight	DSEL	2 - 10
3.	Dried extract exposed to direct sunlight	DEEL	2 - 10
4.	Liquid extract exposed to direct sunlight	LEEL	2 - 10

Table 2. Antioxidant properties of unexposed flour of *C. Fistula* seed

Parameters	Values
Total extractable components (%)	14.14±1.314
Total phenolic acids (GAE g/100g dry weight)	0.389±0.052
TAOA by Phosphomolybdenum assay (Trolox Eqv. g/100g dry extract)	6.242±0.91
TAOA by DPPH assay (Trolox Eqv. g/100g dry extract)	13.946±1.09
Reducing power (Absorbance at 700 nm)	0.33±0.028
DPPH radical scavenging capacity (%)	49.16±2.57
Hydroxyl radical scavenging capacity (%)	59.206±3.54

Table 3. Regression analysis of experimental data on antioxidant properties of *C. Fistula* flour and its extracts exposed to direct sunlight

Parameter	Regression equation	Regression coefficient (R^2)
TEC	$TEC(\%) = -0.1349DE^{*} + 14.363$	0.8777
TPA	$TPA\ of\ DSEL\left(Gallic\ acid\ Eqv.\frac{g}{100g\ dry\ weight}\right) = 0.356\ e^{-0.107DE}$	0.948
	$TPA\ of\ DEEL\left(Gallic\ acid\ Eqv.\frac{g}{100g\ dry\ weight}\right) = 0.3464e^{-0.171DE}$	0.9801
	$TPA\ of\ LEEL\left(Gallic\ acid\ Eqv.\frac{g}{100g\ dry\ weight}\right) = 0.3554e^{-0.167DE}$	0.9764
TAOA by Phospho-molybdenum assay	$TAOA\ of\ DSEL\left(Trolox\ Eqv.\frac{g}{100g\ dry\ extract}\right) = 6.2711e^{-0.026DE}$	0.9748
	$TAOA\ of\ DEEL\ (Trolox\ Eqv.\frac{g}{100g\ dry\ extract}) = 5.7417e^{-0.101DE}$	0. 9578
	$TAOA\ of\ LEEL\ (Trolox\ Eqv.\frac{g}{100g\ dry\ extract}) = 5.8552e^{-0.075DE}$	0.9515
TAOA by DPPH assay	$TAOA\ of\ DSEL\ (Trolox\ Eqv.\frac{g}{100g\ dry\ extract}) = 13.165e^{-0.05DE}$	0.9043
	$TAOA\ of\ DEEL\ (Trolox\ Eqv.\frac{g}{100g\ dry\ extract}) = 12.607e^{-0.111DE}$	0.9523
	$TAOA\ of\ LEEL\ (Trolox\ Eqv.\frac{g}{100g\ dry\ extract}) = 14.274e^{-0.137DE}$	0.9891
RP	$RP\ of\ DSEL\ (Abs.at\ 700nm) = 0.2866\ e^{-0.061DE}$	0.8387
	$RP\ of\ DEEL\ (Abs.at\ 700nm) = 0.279\ e^{-0.076DE}$	0.8692
	$RP\ of\ LEEL\ (Abs.at\ 700nm) = 0.2939\ e^{-0.053DE}$	0.8484
DPPH RSC	$DPPH\ RSC\ of\ DSEL\ (\%) = 47.637e^{-0.045DE}$	0.9313

	$DPPH\ RSC\ of\ DEEL\ (\%) = 44.034e^{-0.063DE}$	0.9042
	$DPPH\ RSC\ of\ LEEL\ (\%) = 42.53e^{-0.065DE}$	0.8613
	HRSC of DSEL (%) = -1.7315DE + 58.292	0.9435
HRSC	$HRSC\ of\ DEEL\ (\%)$ = -0.8806DE + 59.553	0.9764
	$HRSC\ of\ LEEL\ (\%)$ = -0.5575DE + 58.878	0.972

*DE= Duration of sunlight exposure

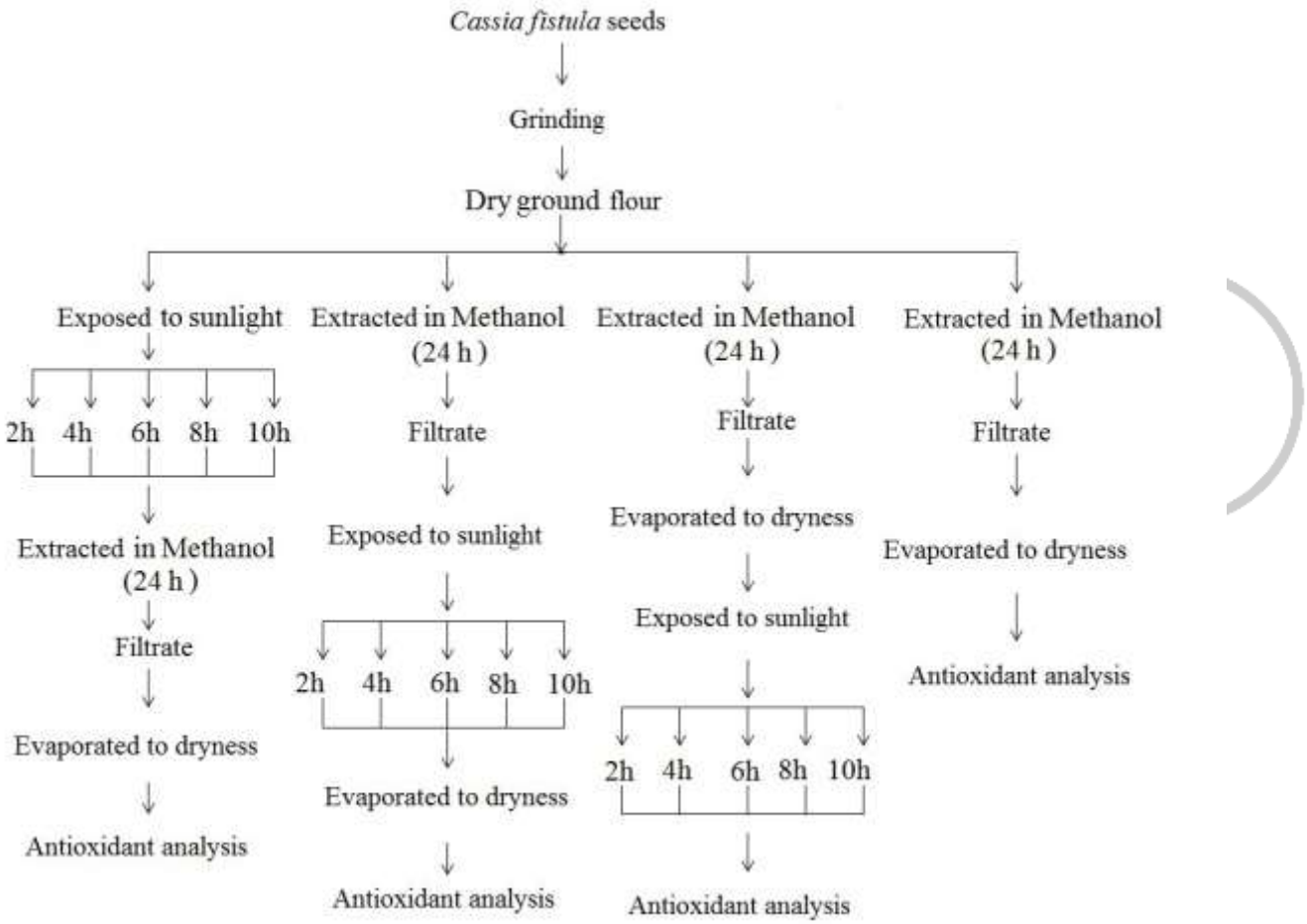

Fig. 1: Experimental design for the sunlight exposure and preparation of extracts from *C. fistula* seed

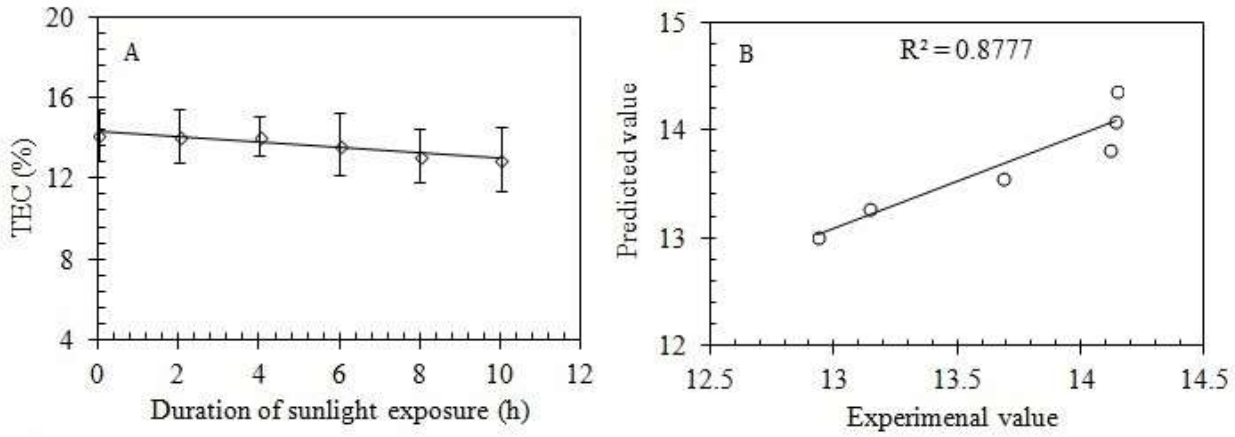

Fig. 2 A: Time dependant variation in TEC of *C. fistula* seed flour exposed to direct sunlight

B: Agreement between the experimental and predicted values of TEC of *C. fistula* seed flour exposed to direct sunlight

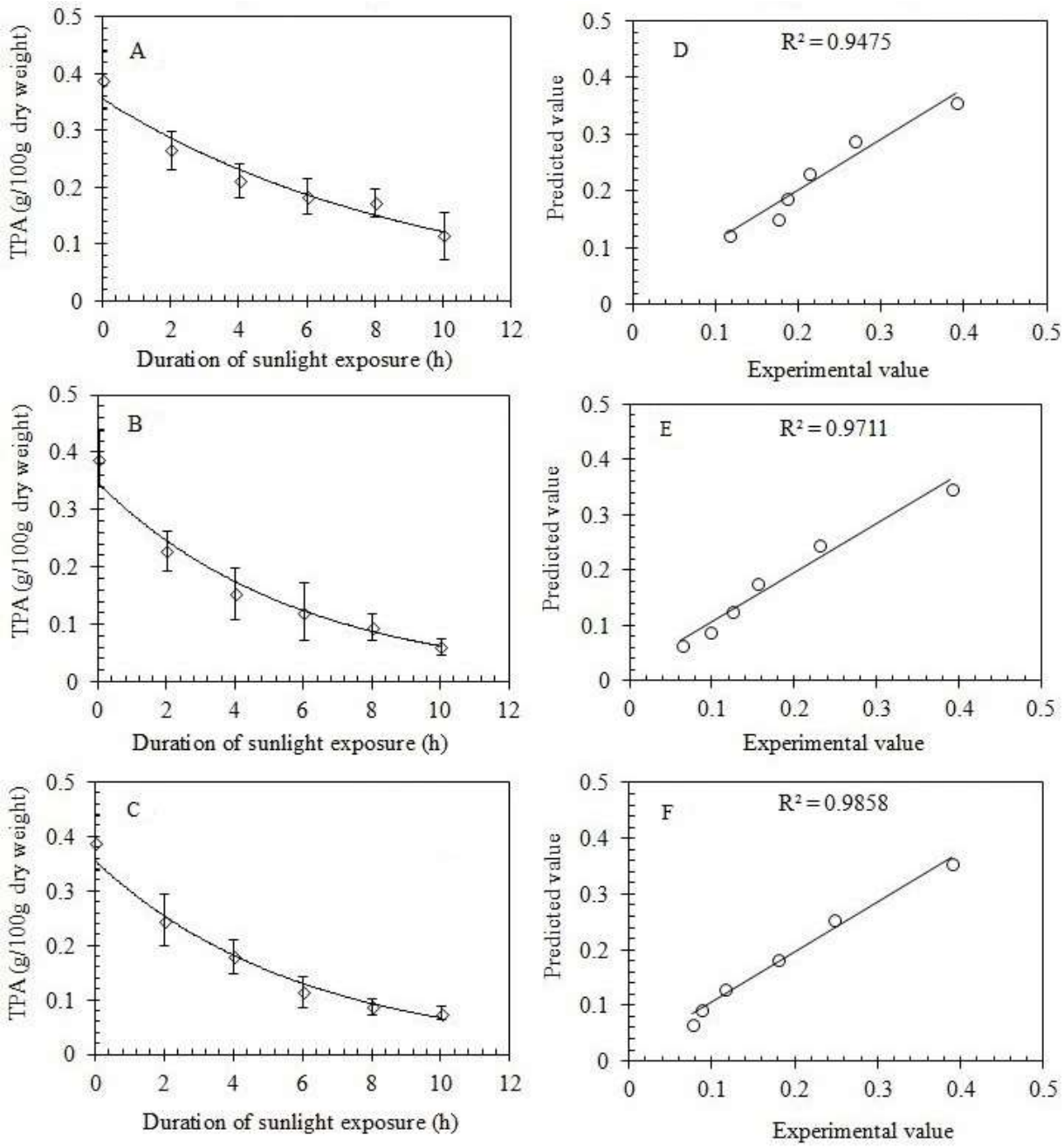

Fig. 3A-C: Time dependant variation in TPA of *C. fistula* seed flour and its extracts exposed to direct sunlight

D-F: Agreement between the experimental and predicted values of TPA of *C. fistula* seed flour and its extracts exposed to direct sunlight

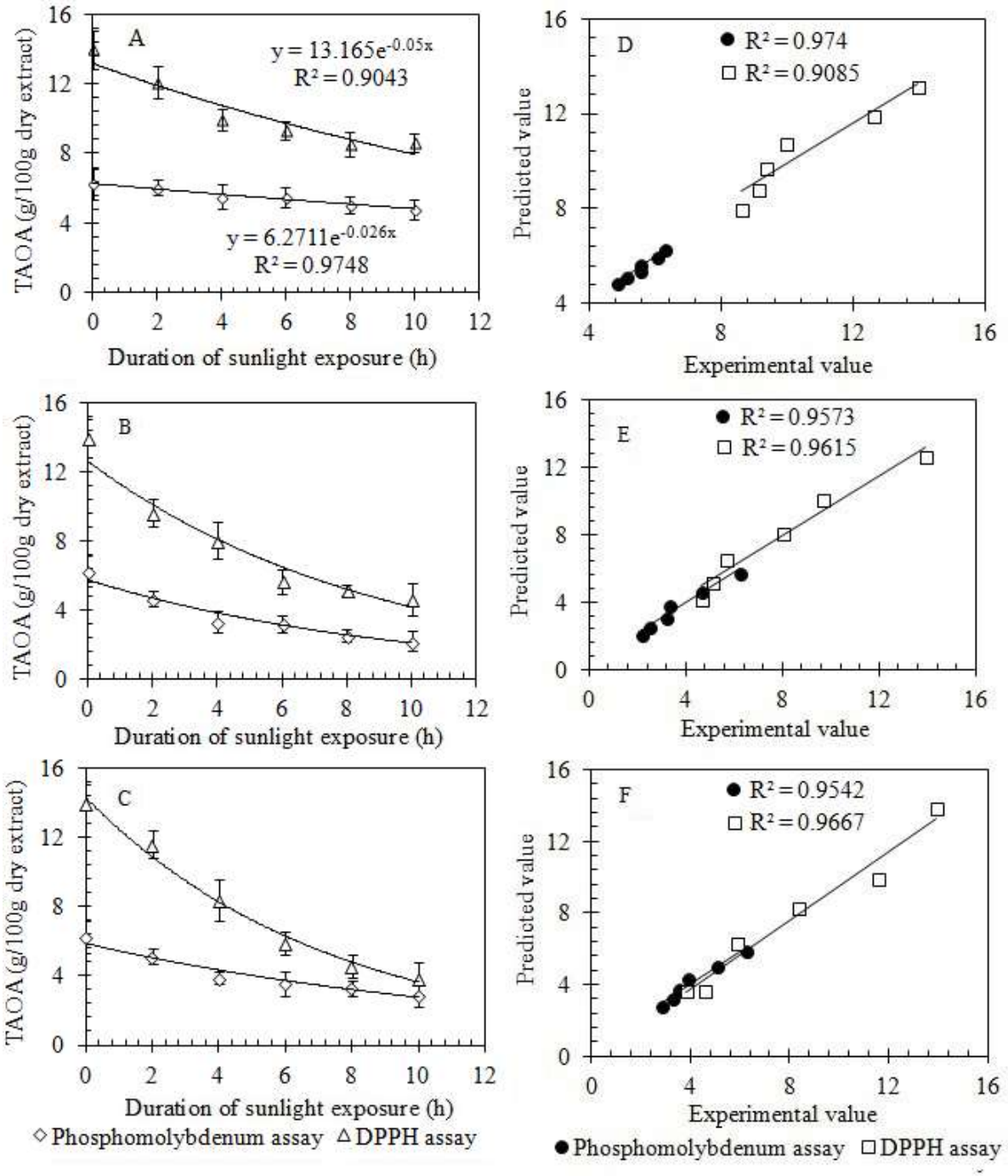

Fig. 4A-C: Time dependant variation in TAOA of *C. fistula* seed flour and its extracts exposed to direct sunlight

D-F: Agreement between the experimental and predicted values of TAOA of *C. fistula* seed flour and its extracts exposed to direct sunlight

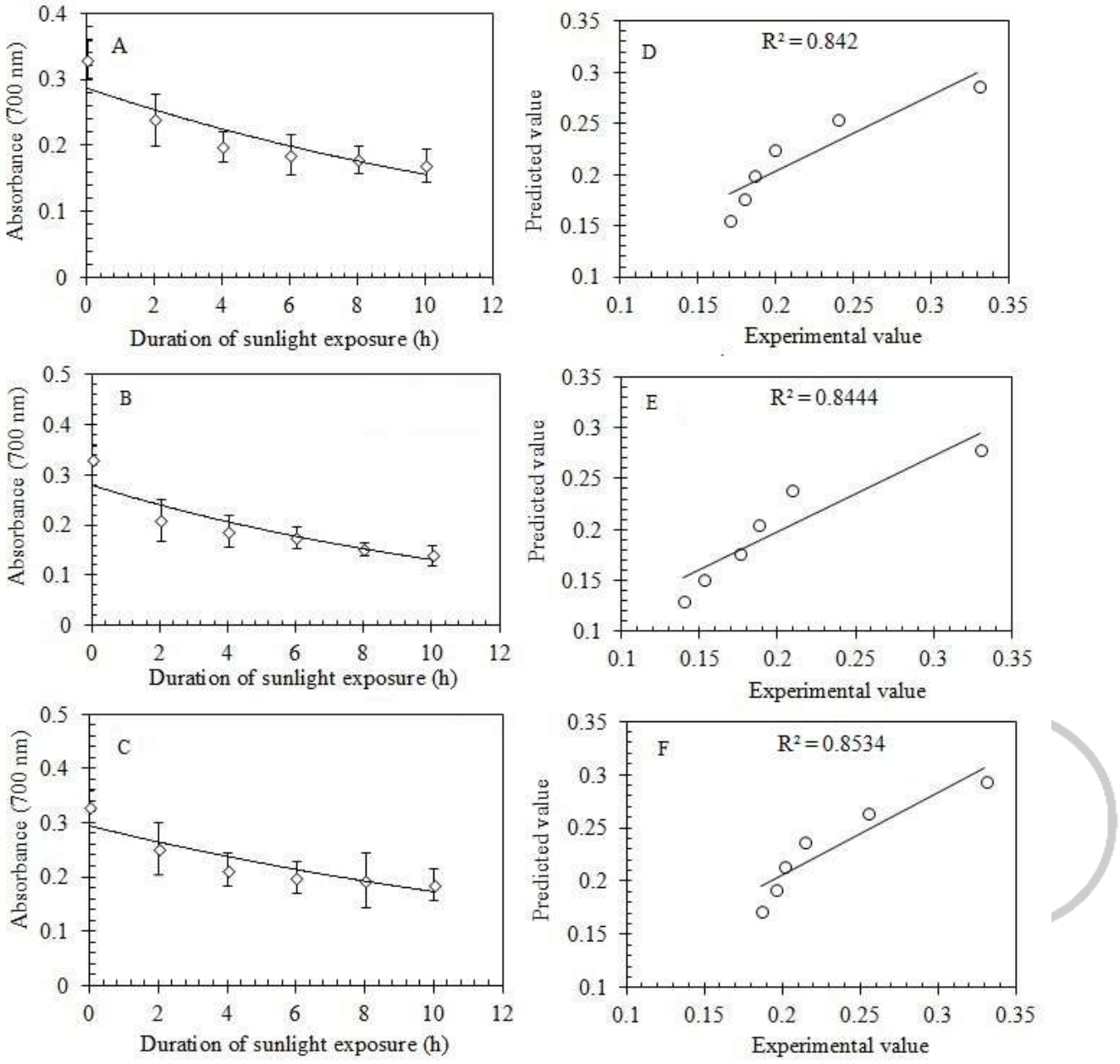

Fig. 5A-C: Time dependant variation in reducing power of *C. fistula* seed flour and its extracts exposed to direct sunlight

D-F: Agreement between the experimental and predicted values of reducing power of *C. fistula* seed flour and its extracts exposed to direct sunlight

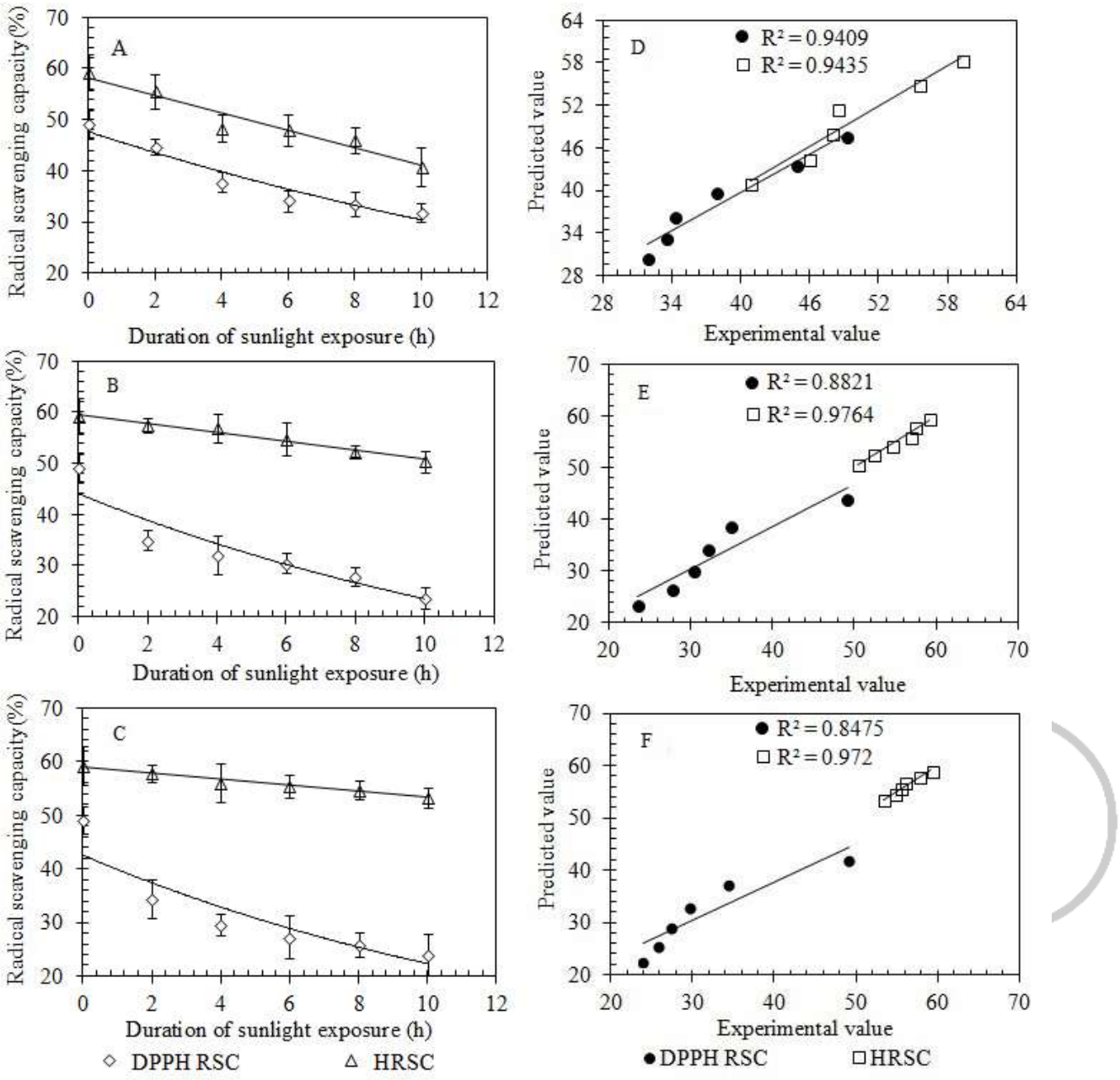

Fig. 6A-C: Time dependant variation in radical scavenging capacity of *C. fistula* seed flour and its extracts exposed to direct sunlight

D-F: Agreement between the experimental and predicted values of radical scavenging capacity of *C. fistula* seed flour and its extracts exposed to direct sunlight

A significant exponential decrease (R^2 = 0.9891, 0.9042, 0.8613) was observed in case of TAOA by DPPH assay of LEEL of seed extract and DPPH RSC of DEEL and LEEL as compared to other samples and parameters while a significant linear decrease (R^2 = 0.9435) was observed in case of HRSC of DSEL as compared to other samples.

The present findings showed that methanolic seed extracts of *C. fistula* strongly scavenged the free radicals which indicated that the extract had good potential as a source for natural antioxidant to prevent free radical mediated oxidative damage. Of course, there could be few explanations for the loss of phenolics and antioxidant activity due to sun drying that attributes the deactivation of the polyphenol oxidases by absorbing the water molecule. Data in the effects of sun drying, antioxidant activity and TPC of fruits and vegetables are conflicting due to several factors like different drying conditions, type of extraction solvents, and antioxidant assays used. In conclusion, this indicated that the traditional sun drying method had an adverse effect in the TPC and TAOA resulting in all the extracts of the dried plant material possessing lower antioxidant properties than fresh plant material. Sun drying of plant materials has been reported to significantly decrease phenolic antioxidants by 23-88% (Chauhan & Kapfo, 2013; Ponmari, Sathishkumar, Lakshmi, & Annamalai, 2011a). However some studies reported an increase in both TPC and TAA of samples after processing (Mejia-Meza *et al*., 2010; Tseng & Zhao, 2012).

Conclusion

In conclusion *C. fistula* contains significant amount of antioxidant compounds which makes this plant an important consideration for pharmaceutical applications. The present data suggests that researchers should avoid their samples being exposed to sunlight for long duration because it affects the antioxidant properties of samples even in dry or liquid form.

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