World Journal of Dairy & Food Sciences 11 (1): 80-87, 2016
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2016.11.1.1157

Productivity, Stability and Anti-Recombinagenic Activity of *Cassia fistula* Pods Pulp Extract as a Source of Natural Food Color

[1]Hemat E. Elsheshetawy, [1]Samar M. Mahdy and [2]Naglaa M. Ebeed

[1]Department of Food Science, Faculty of Agriculture, Ain Shams University, Cairo, Egypt
[2]Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

Abstract: This study aims to take advantage of the aqueous extract of *Cassia fistula* pods pulp, which is used in folk medicine, in order to obtain a higher extractive value under different conditions of temperatures and pHs. The extract obtained from the significant highest extraction trials was subjected to test its stability under different conditions of temperatures and pHs. Thermal stability and brown substance determination on extracts were also studied. The mutagenic, recombinagenic, carcinogenic and anticarcinogenic potential of *Cassia fistula* pods extract was assessed using the epithelial tumor detection test (wts) in *D. melanogaster.* Results of the study showed that the highest extractive value was 67.47% at 90°C, pH 7 for 20 min. The extract exhibited high stability at different temperatures and pHs and in particular on the neutral and alkaline pH as well as high temperatures. Generally, the extract showed a significant high time and thermal stability at 80 and 90°C with the increase of extraction time at pH 7 or 9. The significant highest brown substance units after extraction for 30 min were at 90, 80 and 60°C. Studies on the effect of extracts on the genetic toxicology showed that descendants treated with extracts did not show any statistically significant changes in the frequency of tumors when compared to the negative control. Therefore, the results indicate that the *C. fistula* extracts, under the present experimental conditions, did not induce the occurrence of tumors in *D. melanogaster*.

Key words: *Cassia fistula* · Pods · Natural color · Antitumor activity

INTRODUCTION

Natural colors have always been part of the diet and it is receiving growing interest from both food manufactures and consumers in continuing replacement of synthetic colors. Chlorophylls, carotenoids and anthocyanins are consumed in the foods. Common natural colorings include annatto, saffron, paprika, grape skins, caramel, beetroot, cochineal and turmeric. Natural colors add a cachet to food products marketed as "natural" and "organic" that suit the shoppers demand and growing health-conscious of populations. Nevertheless, natural colors are perceived by the consumer as being less of a health hazard than the petroleum- (coal-tar) derived synthetics, with names like tartrazine, indigotine and erythrosine. The use of non-permitted colors is known to cause adverse effects in experimental animals [1, 2, 3] and in humans [4, 5]. Repeated exposure to even the permitted synthetic colors may be hazardous [6]. *Cassia fistula* Linn. is fast-growing, medium-sized, deciduous tree belongs to Fabaceae family and commonly known as Indian Laburnam, golden shower in English and Amultas in Hindi [7]**.** It is distributed in various regions including Asia, South Africa, China, West Indies and Brazil as well as in Egypt [8, 9]**.** The fruit is cylindrical pod containing many horizontal black seeds. The ripe pods are pendulous, cylindric, smooth, shining and of brown-black color [10]. *C. fistula* could be one of the alternative or complementary therapies particularly because of its low toxicity and its widespread use for its multiple medicinal effects. The pulp from pods is of great therapeutic value, it is a mild, pleasant and safe purgative due to presence of sennoside and rhein [11, 12], even for children and expectant mothers**.** Crude hydro-alcoholic extract of *Cassia fistula* fruit pulp has high antioxidant activity and antifungal action as a result of its high phenolic and flavonoid content [13-16].

Pulp of cassia fruits has high concentrations of soluble sugars, volatile oils, waxes, resinous substances and it contains several anthraquinones such as rhein,

Corresponding Author: Hemat E. Elsheshetawy, Department of Food Science, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. E-mail: sheshhem@gmail.com.

aloin, emodin and sennosides [17, 18]. Fistulic acid (an anthraquinone acid) was detected in *Cassia fistula* pods which are characterized as a structure of a new coloring matter [19].

The evaluation of toxic properties of *C. fistula* is crucial when considering public health protection because exposure to plant extracts can result in undesirable effects on consumers [20]. Various assays are used in field of genetic toxicology to assess the damage on the DNA, in the presence or absence of mass metabolism systems. These assays include the epithelial tumor clones test (*wts*) in *Drosophila melanogaster* developed by Eeken *et al.* [21]. The use of *wts* test can detect the carcinogenic of chemical or natural and synthetic agents that induced by genetic abnormalities such as mutation, deletion and recombination. This assay is based on warts tumor suppressor gene that encodes protein kinase depends on serine and threonine. Deletion or loss of *wts* gene leads to the formation of epithelial tumors that appears as homozygotic clones in heterozygotic flies which develop throughout the fly body cuticles [21-24]. The system identifies and characterizes the potential tumorgenesis compounds and scores for loss of heterozygosity (LOH). LOH occur due to different types of recombination, deletions, point mutations and loss of chromosomes or nondisjunction [22]. Heterozygous loss in the cells of the drosophila imaginable disks is associated with homozygous clone formation in the larvae, leading to visible phenotypes in the eyes and wings in the adult flies. Because consumers seek to take natural therapeutic substances and reduce synthetic materials in their food, so this work is a trial aims to obtain the highest crude pulp extract yield of *Cassia fistula* fruits with the optimum temperature and pH conditions. The effect of temperature and pH on selected crude extract stability was also evaluated. Also, Crude extract is evaluated as a source of brown color substance. Genetic toxicology of crude extract is determined to assess potential alterations in DNA associated with its using *in vivo* epithelial tumor detection test (wts) on *Drosophila melanogaster*.

MATERIALS AND METHODS

Pods of Cassia fistula were collected from the campus of Faculty of Agriculture, Ain Shams University, during June 2014. Caramel used in this study was purchased from Aarkay Food Products Ltd. Company, India. *Drosophila melanogaster* strains were provided by Bloomington Drosophila Stock Center of the University of Indiana, USA, under registry No. (Bloomington/7052). Two *D. melanogaster* strains were used for the experiments: (1) Oregon R: OR, wild type strain and (2) wts/TM3, sb^1 strain. Due to lethalness of the warts (wts) allele, wts^{MT4-1} allele on chromosome 3 is balanced over TM3 chromosome, with multiple inversions and marked by the dominant stubble (Sb) mutation, phenotypically identified by short bristles according to Eeken *et al.* [21]. The genetic structure of this strain is; st p in ri wtsMT4-1/TM3 Sb, which was abbreviated as wts/TM3. The genetic symbols about the various markers and the balancer chromosome can be found in web site (flybase.bio.indiana.edu) [25, 26].

Preparation of *C. fistula* Pods Pulp Extract: The pods pulp was separated and its moisture content was determined according to Reshmi *et al.* [28]. A 10g fresh pods pulp was extracted in 100 ml buffer solution at different pHs (3, 5, 7 and 9) and temperatures (10, room temperature (RT, 30°C±3), 60, 80 and 90°C) for 20 min and in addition, samples which extracted at 10°C and RT were allowed to stand overnight. The extracted samples were centrifuged at 4000 rpm for min to remove suspended particles then dried under vacuum. The extractive value was calculated according to the following equation:

Extractive value (%) = dry weight of extract / dry weight of fresh pulp x 100

A spectrophotometric scan was carried out in the range of 320 to 720 nm for the crude extract and the maximum absorbance was observed at 560 nm.

Crude Extracts Stability at Different Temperatures and pHs: For the stability studies of Cassia fistula pods pulp crude extract, the aforementioned extraction method applied at 90° C and pH 7 for 20 min were used to obtain the necessary extract for this study. The vacuum dried extract was dissolved in different pH buffers (3, 5, 7 and 9) at ratio 1:10 then incubated under different temperatures, 30 ±3 (RT), 40, 60, 80 and 90°C, for 30 min according to Reshmi *et al.* [28]. Samples were cooled and centrifuged at 4000 rpm for 5 min then the supernatant of each extract was volumetrically doubled with its buffer solution. The absorbance was measured at 560 nm and caramel color solutions (at the same conditions) were used as controls.

Time and Thermal Stability of Crude Extracts: The aforementioned crude extract was dissolved in buffer solutions (pH 7 and 9) with 1:10 ratio and held at 80 or 90° C for 180 min [29]. The samples were withdrawn at 30 min

intervals then cooled immediately in an ice bath followed by centrifugation at 4000 rpm for 5 min. The supernatant of each extract was volumetrically doubled with its buffer solution and the absorbance was measured against caramel as a control at 560 nm.

Determination of Brown Substances: Brown substances were determined in aqueous crude extracts of cassia pods pulp (10 mg/ml) at pH 7 with different extraction temperatures (30, 60, 80 and 90° C) for 10, 20 and 30 min according to Alberto [30]. The obtained extracts were centrifuged as mentioned before and the absorbance was measured spectrophotometrically at 365 and 456 nm and the concentration of brown substances was calculated by the following equation:

$$\text{Brown substances (units)} = 1000\ (6.50\ A_{365} - A_{456})/6.36\ C$$

where:
C = concentration of the sample in mg/ml.

Detection of Epithelial Tumor in *D. melanogaster*: This assay was conducted to identify and characterizes the potential tumorgenesis of *C. fistula* pods crude extract and scores for loss of heterozygosity (LOH) on *D. melanogaster*.

Cross and Experimental Procedure of Larval Treatment: Heterozygous larvae with genetic structure wts/+ were obtained by crossing virgin females wts/TM3, Sb1 with wild type males +/+. Eggs obtained from of the cross were collected during a 6 h period. After 72± 4h, Heterozygous larvae were cleaned from remaining feeding medium with a 20% glycerol solution and they were transferred to treatment vials containing Drosophila medium supplemented with 500 mg of *C. fistula* pods pulp extracts which were previously extracted at different temperatures and pHs. The extracts were dissolved in 2 ml of distilled water then mixed well in 98 ml of standard Drosophila medium at 50°C (5 mg/ml) for 24 h, then they were transferred to standard Drosophila medium [21, 23]. Doxorubicin, DXR (0.125 mg/ml) was used for the positive control and water for the negative control. Only adult flies, without the chromosome balancer (TM3, Sb1) were analyzed. All Drosophila stocks and crosses were maintained at 25° ±2°C and 65% humidity.

Scoring of Tumors: Adult males and females flies of the (wts/+) genotype, which had wild hairs (long and thin) were analyzed for tumor (wart) presence. The flies were observed using a Leica stereoscopic at a standard magnification of 25X. Only tumors that were large enough to be unequivocally classified were recorded. The tumor frequency was calculated as the number of tumors/number of wts/+ flies [21, 31].

Statistical Analysis: Each extraction and stability trial was carried out in triplicate. The experimental data were subjected to an analysis of variance for a completely random design using a Statistical Analysis System [32]**.** Duncan's multiple range tests were used to determine the difference among means at the level of 0.05.

RESULTS AND DISCUSSION

Extractive Value: *Cassia fistula* pods pulp was extracted by aqueous buffers with different temperatures and pHs. The yield percentages are shown in Table 1. From the point of temperature effect under the pH, an increase in extraction temperature increases the efficiency of the extraction yield. Since heat render the cell wall permeability, increase solubility and diffusion coefficients of the compounds to be extracted. The highest extractive values were 38.42, 43.14, 67.47 and 56.15% at 90°C for pH 3, 5, 7 and 9, respectively. According to the effect of pH on extractive value under the same temperature, the data showed that with the increase of extraction buffer alkalinity, the extractive value was increased except at

Table 1: Extractive value (%) of *C. fistula* pods pulp as affected by different temperatures and pHs.

	Temperature (°C)							
pH	10	10/O	RT	RT/O	60	80	90	Rate of increase (% /°C)
3	11.23^{Df}	29.55^{Be}	31.91^{Cd}	33.69^{Bc}	35.46^{Cb}	36.64^{Cb}	38.42^{Da}	$2.519x10^{-3}$
5	26.00^{Ce}	28.96^{Be}	30.15^{Cd}	35.46^{Bc}	36.05^{Cbc}	39.60^{Cb}	43.14^{Ca}	$3.883x10^{-3}$
7	38.42^{Bd}	41.37^{Ac}	38.42^{Ad}	39.01^{Ad}	41.96^{Bc}	43.74^{Bb}	67.47^{Aa}	$1.187x10^{-2}$
9	41.37^{Ac}	39.60^{Ad}	35.46^{Be}	39.01^{Ad}	43.14^{Ac}	46.69^{Ab}	56.15^{Ba}	$6.585x10^{-3}$

Means with the same capital letter in the same column (pH) or the same small letter in the same row (temperature) are significantly different at (p = 0.05). RT: room temperature, 30±3°C, O: overnight.

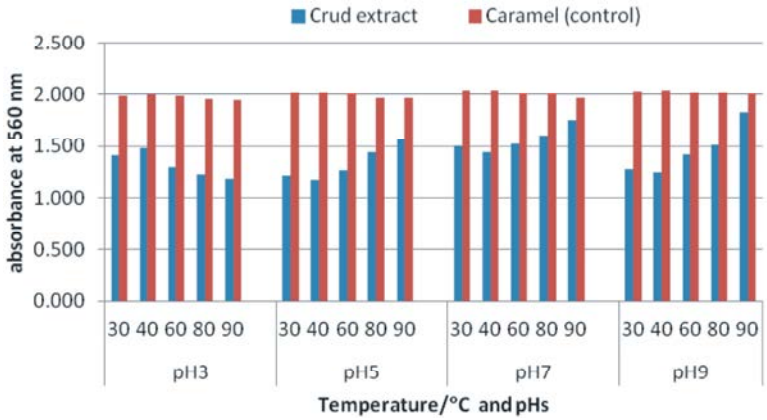

Fig. 1: Stability of aqueous crude extract of *Cassia fistula* pods pulp at different temperatures and pHs compared with caramel color.

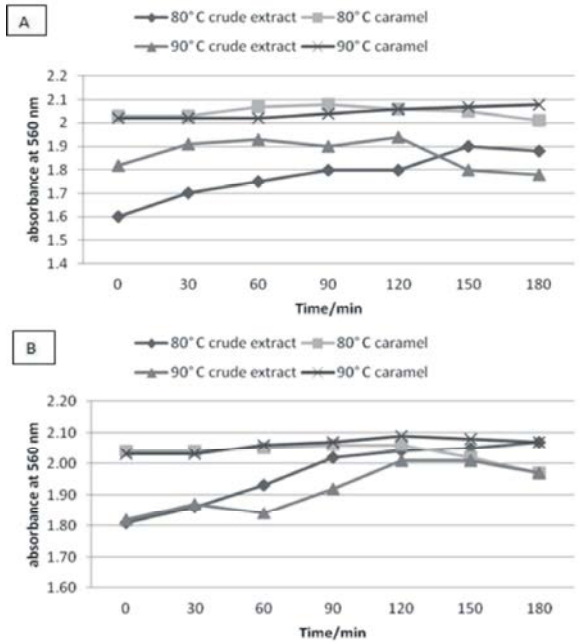

Fig. 2: Time and thermal stability of aqueous crude extracts of *Cassia fistula* pods pulp at pH 7 (A) and pH 9 (B) with compared to caramel color (control).

90°C where the significant highest extractive value was 67.47% recorded at pH 7. The highest rates of increase (% /°C) were $1.187x10^{-2}$ and $6.585x10^{-3}$ for pH 7 and 9, respectively.

Thermal and pH Stability of Crude Extracts: The results presented in Fig. 1 showed that the thermal and pH-stability of crude extracts of *Cassia fistula* pods pulp and solutions of caramel were prepared and subjected to the same pH, temperature and time conditions to be used as control. As seen, the stability of crude extracts was decreased at pH 3 with increasing of heat temperature up to 90°C. The highest stability of the crude extract was achieved at the alkaline pH 7, 9 and it was more stable than at pH 3 and 5. The stability of crude extract was increased by 17.33 and 42.97% with the increase of temperature from 30 to 90°C at pH 7 and 9, respectively. Moreover, a significant increase in absorbance of the crude extract was observed between pH 5, 7 and 9 with elevated temperature. These results are probably due to the occurrence of Maillard reaction in crude extract with high temperatures. On the other hand, caramel color solutions showed significantly higher stability than the aqueous crude extracts of *C. fistula* pods pulp at the different tested temperatures and pHs. Agrawal *et al.* [33] reported that *C. fistula* pods pulp showed the presence of alkaloid, glycosides, steroids, phenolic compounds, tannins, proteins, amino acids and sugars.

Time and Thermal Stability of Crude Extracts: Time and thermal stability of aqueous crude extracts of *C. fistula* pods pulp as affected by two temperatures (80 and 90°C) and pH-values (7 and 9) over a period of 180 min was presented in Fig. 2. As seen, the absorbance of control samples (caramel) tested at pH 7 or 9 was almost stable at 80 and 90°C for 180 min. On other side, the crude extracts of *Cassia fistula* pods pulp showed significant lower absorbance level compared with their control samples at the tested pH, temperature and time periods. The absorbance of crude extracts at 90°C being higher than those measured at 80°C at pH 7 till 120 min. However, a non-significant drop in the absorbance of crude extract (8.24%) was observed after 120 min of heating at 90°C and

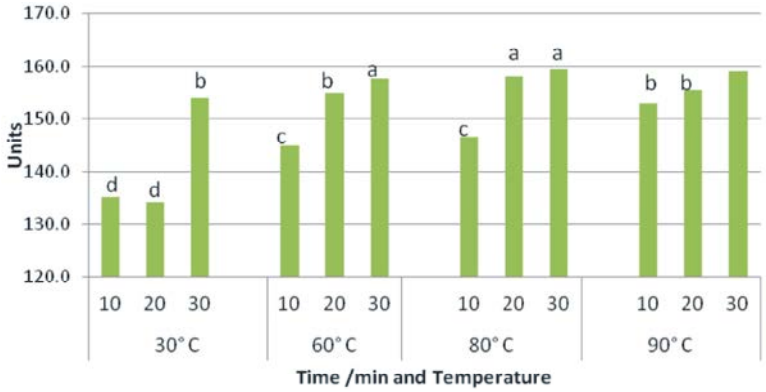

Fig. 3: Brown substances (units) of *Cassia fistula* pods pulp at different temperatures and extraction times. Means with the same letter are significantly different at (p = 0.05)

Table 2: Tumor clone frequency observed *Drosophila melanogaster* in heterozygous (wts/+) larvae using *in vivo* epithelial tumor in detection test (wts) after treatment with aqueous extracts of *Cassia fistula* pods pulp

Treatments		No. of F1 files scored	Number of wts tumors scored	Frequency (No. of wts tumors/fly)
Negative control		2376	60	0.025
DXR (0.125 mg/ml)		450	410	0.911***
pH 5	80°C	1001	32	0.032
	90°C	963	35	0.036
pH 7	10°C	1325	37	0.028
	RT	1557	35	0.022
	90°C	1150	20	0.017
pH 9	RT	1594	38	0.027
	80°C	1981	56	0.028
	90°C	1141	31	0.027

*The value is considerably different from the negative control (P<0.05).

pH 7 compared with that tested at 80°C. At pH 9, the crude extract showed significant higher absorbance level at 80°C rather than at 90°C during the heating period and the drop reached 4.79% after 180 min. The stability of crude extract with the increase of temperature and pH values may be due to the formation of complexes by Maillard reaction in crude extracts which helps to support its absorption stability at 560 nm. These results are in agreement with those obtained by Martins *et al.* [34] and Nie *et al.* [35], who reported that the Maillard reaction is lower at lower pH values than at neutral and alkaline pH values.

Brown Substances of Crude Extracts: Brown substance units of *Cassia fistula* pods pulp extracts at pH 7 with different temperature (30, 60, 80 and 90°C) for 10, 20 and 30 min extraction time were determined and the results are presented in Fig. 3. As seen, brown substance units were significantly increased from135, 145, 146.6 and 153 for 10 min extracts obtained at 30, 60 and 80°C, respectively to 154, 158, 159.4 and 159 units for the corresponding extracts obtained after 30 min of heating. After 10 min of extraction time, the significant highest brown substance units were 153 for extract obtained at 90°C. There was no significant difference in brown substance units after 30 min at 60, 80 and 90°C. These data were in agreement with those of Nie *et al.* [35].

Genetic Toxicology of *Cassia fistula* Pods Pulp Extract: The potential tumorgenesis of *C. fistula* aqueous extracts were tested using *in vivo* epithelial tumor detection test (wts) on *drosophila melanogaster*. The wts-epithelial tumor detection test results for the chronic treatment of larvae treated with 5mg/ml *C. fistula* aqueous extract are shown in Table 2. Warts tumors were induced in wts/+ adult flies in frequencies of 0.911 per fly by doxorubicin (DXR 0.125 mg/ml) as positive control, extremely statistically significant above the control according to $x2$ -test. In the 410 flies analyzed and treated with DXR, 450 tumors were identified. These tumors arose in every part of the fly analyzed (eyes, head, wings legs and halters).

Meanwhile, the frequency of tumors in the wts/+ control flies was low (0.02–0.03; i.e. 2–3 flies with one wart in 100 flies scored). In the negative control experiment, the

frequency of spontaneous tumors was low, where 60 small tumors were scored among 2376 flies with an average of 0.025tumor /fly, indicating a spontaneous frequency. Descendants treated with different *C. fistula* aqueous extracts obtained under different levels of pH and temperatures, did not show any significant changes in the frequency of tumors, when compared to the corresponding negative control. Therefore, no significant carcinogenic effect of *C. fistula* aqueous extract was found. On the other hand, DXR (used as a positive control, enhanced the frequency of tumors ($P<0.05$). Carcinogenicity for extracts were carried out to confirm quality criteria 3for food quality and safety and their potential utilization in multicomponent biological/food systems as well as their possible use in the clinical treatment of several human malignancies. Induction of tumors using wts- epithelial tumor detection test allows one to draw closer analogy between the activity of a substance in Drosophila and its potential carcinogenic hazard to humans [22]. Gupta *et al.* [36] reported the antitumor activity of methanolic extract (ME) of *cassia fistula* seeds on the growth of Ehrich Ascites Carcinoma (EAC) and on the life span of tumor bearing mice. ME treatment showed an increase of life span and a decrease in the tumor volume and viable tumor cell count in the EAC tumor host. The same results were obtained by Bahorun *et al.* [17], Jothy *et al.* [20], Duraipandiyana *et al.* [37] and Luximon *et al.* [38]. Anti-tumour activity of *C. fistula* seed extract based on cytological studies reveal that a reduction in the mitotic activity can be the leading mechanism of action against tumorigenesis. Indeed the appearance of membrane blebbing and intracytoplasmic vacuoles in the treated tumour cells suggest that these pathways may account for the reduction in tumor volume [36, 39].

From the previous data it can be deduced that the high therapeutic crude extract of *C. fistula* pods pulp may be used as a source of natural brown color with antitumor activity.

REFERENCES

1. Prasad, M., P. and B. Rastogi, 1982. Effect of feeding a commonly used non-permitted colour orange II on the hematological values of *Mus musculus*. Journal of Food Science and Technology, 19: 150-153.
2. Wess, J.A. and D.C. Archer, 1982. Disparate in vivo and *in vitro* immuno-modulatory activities of Rhodamine B. Food and Chemical Toxicology, 20: 9-14.
3. Singh, R.L., S.K. Khanna and G.B. Singh, 1987. Acute and short-term toxicity studies in orange II. Veterinary Human Toxicology, 29: 300-304.
4. Chandra, S.S. and T. Nagaraja, 1987. A food poisoning outbreak with chemical dye: an investigation report. Medical Journal of Armed Forces India, 43: 291-293.
5. Sachadeva, S.M., K.S. Mani, S.K. Adaval, Y.P. Jalpota, K.C. Rasela and D.S. Chadha, 1992. Acquired toxic methaemoglobinaemia. Journal of Association of Physicians India, 40: 239-240.
6. Griffiths, J.C., 2005. Coloring foods and beverages. Food Technology, 59(5): 38-45.
7. Shukla, R.K., A. Porval, D. Painuly and A. Shukla, 2013. Physico-chemical characteristics, proximate analysis and total phenolic content of *Cassia fistula* bark. Natural Products an Indian Journal, 9(4): 133-137.
8. Prashanth, K.V., N.S. Chauhan, H. Padh and M. Rajani, 2006. Search for antibacterial antifungal agents from selected Indian medicinal plants. J. Ethnopharmacol., 107: 182-188.
9. Thirumal, M., S. Srimanthula and G. Kishore, 2012. *Cassia fistula* Linn - Pharmacognostical, Phytochemical and Pharmacological Review. Critical Review in Pharmaceutical Sciences, Issue (1): 43-65.www.earthjournals.org
10. Deshpande, H.A. and S.R. Bhalsing, 2013. Recent Advances in the Phytochemistry of Some Medicinally Important *Cassia* Species: A Review. Int. J. Pharm. Med. Bio. Sci., 2(3): 60-78.
11. Van, O.F.H.L., 1976. Some aspects of the pharmacology of anthraquinone drugs. Pharmacology, 14(1): 18-29.
12. Moshahid, M.A.R., M. Irshad, G. El Hassadi and S. Ben Younis, 2009. Bioefficacies of *Cassia fistula:* An Indian labrum (review). African Journal of Pharmacy and Pharmacology, 3(6): 287-292.
13. Raja, N., S. Albert and S. Ignacimuthu, 2000. Effect of solvent residues of *Vitex negundo* Linn. and Cassia fistula Linn. On pulse beetle, *Callosobruchus maculates* Fab. and its larval parasitoid, *Dinarmus vagabundus* (Timberlake). Indian J. Exp. Biol., 38: 290-292.
14. Bhatnagar, M., V. Sunil, V. Yogesh, S. Durgesh and S. Kanika, 2010. Antioxidant activity of fruit pulp of *Cassia fistula*. Pharmacognosy Journal, 2(8): 219-228.

15. Rajagopal, P.L., K. Premaletha, S.S. Kiron and K.R. Sreejith, 2013. Phytochemical and pharmacological review on *Cassia fistula* Linn. "the golden shower". International Journal of Pharmaceutical, Chemical and Biological Sciences, 3(3): 672-679.
16. Jasani, M.D., R. Kolhe, T.N. Pandya, R.N. Acharya and V.J. Shukla, 2014. Experimental study on collection methods of *cassia fistula* fruit pulp. Journal of Herbal Science, 3(1): 15-19.
17. Bahorun, T., S.N. Vidushi and A. Okezie, 2005. Phytochemical constituents of *Cassia fistula*. African Journal of Biotechnology, 4(13) 1530-1540. http://www.academicjournals.org/AJB
18. Sakulpanich, A. and W. Gritsanapan, 2008. Extraction method for high content of anthraquinones from *cassia fistula* pods. J Health Res., 22(4): 167-172
19. Agrawal, G.D., S.A.I. Rizvi, P.C. Gupta and J.D. Tewari, 1972. Structure of fistulic acid a new coloring matter from the pods of Cassia fistula. Planta Med., 2: 150-155.
20. Jothy, S.L., Z. Zuraini, C. Yeng, L.L. Yee, Y.L. Lachimanan, N.S. Lai and S. Sreenivasan, 2011. Bioassay-Directed Isolation of Active Compounds with Antiyeast Activity from a Cassia fistula Seed Extract. Molecules, 16(9): 7583-7592, doi:10.3390/ molecules16097583
21. Eeken, J.C.J., I. Klink, B.L.V. Veen and W. Ferro, 2002. Induction of epithelial tumors in Drosophila melanogaster heterozygous for the tumor suppressor gene wts. Environ. Mol. Mutagen., 40: 277-282.
22. Sidorov, R.A., E.G. Ugnivenko, E.M. Khovanova and G.A. Belitsky, 2001. Induction of tumor clones in *D. melanogaster* wts/+ heterozygotes with chemical carcinogens. Muta. Res., 498: 181-191.
23. Costa, W.F., A. B. Oliveira and J.C. Nepomuceno, 2012. Lapachol as an epithelial tumor inhibitor agent in *Drosophila melanogaster* heterozygote for tumor suppressor gene wts. Genetics and Molecular Research, 10: 3236-3245.
24. Orsolin, P.C., R.G. Silva-Oliveira and J.C. Nepomuceno, 2012. Assessment of the mutagenic, recombinagenic and carcinogenic potential of orlistat in somatic cells of *Drosophila melanogaster*. Food Chem. Toxicol., 50: 2598-2604.
25. FlyBase, C., 2008. Insertion Identifiers and Alleles Based on Genomic Location of Insertions With Respect to Gene Annotations. (http//flybase.org/).
26. Lindsley, D.L. and G.G. Zimm, 1992. The Genome of *Drosophila melanogaster* Academic Press, San Diego, CA.
27. AOAC, 1990. Official Method of Analysis Washington, DC: Association of Official Analytical Chemists (No. 934.06).
28. Reshmi, S.K., K.M. Aravindhan and P.S. Devi, 2012. The effect of light, temperature, pH on stability of betacyanin pigments in *Basella alba* fruit. Asian Journal of Pharmaceutical and Clinical Research, 5(4): 107-110.
29. Attia, G.Y., M.E.M. Moussa and E.R. Sheashea, 2013. Characterization of red pigments extracted from red beet (*Beta vulgaris* L.) and its potential uses as antioxidant and natural food colorants. Egypt. J. Agric. Res., 91(3): 1095-1110.
30. Alberto L., 1985. Determination of colored substances in soybean lecithin. J. Am. Oil Chem. Soc., 62(5): 883-887.
31. Nepomuceno, J.C., 2015. Using the *Drosophila melanogaster* to assessment carcinogenic agents through the test for detection of epithelial tumor clones (Warts). Adv. Tech. Biol. Med. 3: 149. doi: 10.4172/2379-1764.1000149
32. SAS Program, 2000. SAS / STAT User's Guide Release 6.12 Edition. Cary NC, USA: SAS Inst. Inc.
33. Agrawal, K., A. Joshi, S. Ghildiyal, M.K. Gautam, M. Gangwar, R.K. Goel and V.K. Joshi1, 2014. Qualitative phytochemical and physiochemical analysis of *Cassia fistula* L. fruit. Medicinal Plants, 6(2): 138-142.
34. Martins, S.I.F.S., W.M.F. Jongen and M.A.J.S. van Boekel, 2001. A review of maillard reaction in food and implications to kinetic modeling. Trendsin Food Science and Technology, 11: 364-373.
35. Nie, S., H. Jungen, H. Jielun, Z. Yanan, W. Sunan, L. Chang, M. Massimo and X. Mingyong, 2013. Effect of pH, temperature and heating time on the formation of furan in sugar-glycine model systems. Food Science and Human Wellness, 2: 87-92.
36. Gupta, M., U.K. Mazumder, N. Rath and D.K. Mukhopadhyay, 2000. Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma; J Ethnopharmacol., 72(1-2): 151-156.
37. Duraipandiyan, V., A.B. Albert, S. Ignacimuthu, C. Muthukumar and N.A. Al-Harbi, 2012. Anticancer activity of Rhein isolated from *Cassia fistula* L. flower. Asian Pacific Journal of Tropical Disease, 2(1): S517-S523.

38. Luximon, R.A., T. Bahorun, M.A. Soobrattee and O.I. Aruoma, 2002. Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in extracts of Cassia fistula; J Agric Food Chem., 50(18): 5042-5047.

39. Irshad, M., S.J. Mehdi, A.A. Al-Fatlawi, M. Zafaryab, A. Ali, I. Ahmad, M. Singh and M.M.A. Rizvi, 2014. Phytochemical composition of *Cassia fistula* fruit extracts and its anticancer activity against human cancer cell lines. J. Biol. Active. Prod. Nat., 4(3): 158-170.