# The analytical and toxicological profiles of the red dye from the heart wood of *Caesalpinia sappan*

**Divakar Madhu C.[1]*, Susheel John V.[2] and Lakshmidevi S.[3]**

[1]Crescent college of Pharmaceutical sciences , Payangadi.P.O, Kannur, Kerala, India-670358,
[2]Sri Ramakrishna College of Pharmacy, SRIPMS, Coimbatore, Tamil Nadu, India, 641 044.
[3]Sri Ramakrishna College of Pharmacy, SRIPMS, Coimbatore, Tamil Nadu, India, 641 044.

## ABSTRACT

Three phenolic red pigments were observed in combination from the heart wood of *Caesalpinia sappan* (F: Caesalpiniaceae). Preliminary cytotoxic and acute toxicity studies indicated that these natural colour pigments were safe compared to the FDC grade coal tar dyes. The isolated colour extract (CSWC) was highly soluble in water and analysed by spectrophotometric and HPTLC methods.

**Keywords:** *C. sappan* heart wood, red dye, toxicity, UV and HPTLC analysis.
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***Author for Correspondence:** madhu.divakar@gmail.com

## INTRODUCTION

*Caesalpinia sappan* (F: Caesalpiniaceae) is a small spreading tree bearing orange red heart wood growing in South India, Srilanka and Malaysia. A decoction of the wood in water is used extensively for drinking purpose in Kerala. Literature survey (1, 2) indicated that the heart wood of *C.sappan* contains several constituents like amino acids, sugars, glycosides, steroids, flavonoids, saturated and unsaturated fatty acids, and phenolic compounds. The wood of *C.sappan* is also known as brazil wood, contains a red pigment called Brazilin (11) reported (3) to have anti-inflammatory activity, and also found useful in cosmetics. In the traditional system (4) of medicine the wood decoction is used for mild dysentery, diarrhoea, bronchitis, wounds, goitre, erysipelas, and also as an astringent & emmanogogue.

Nowadays, many different types of synthetic colours included in the class of coal tar dyes are used in pharmaceuticals and nutraceuticals. The production and processing of these synthetic dyes and colourants were estimated to release many hundred tonnes of unfixed and non-degradable waste which are hazardous to human health, and also might cause serious environmental pollution and ecological imbalance. The World Health Organisation (5) specified and set control limits for the use of these synthetic colours because of their toxic reactions towards eyes and skin.

In the light of the above findings an attempt has been made, to extract the colour pigments present in the heart wood of *C.sappan*, and to generate the analytical and toxicological profiles of these natural chromogens for use in industry as an alternative for the synthetic FDC colours.

## EXPERIMENTAL

### Plant material

*C.sappan* wood was collected in the month of March 2004 from North Parur, Cochin, Kerala and authenticated at the Department of Horticulture, The Tamil Nadu Agricultural university, Coimbatore, Tamil Nadu. The sample specimens (specimen no. PGSY/CCOPS/112) are preserved at the herbarium of the Dept. of Pharmacognosy, Crescent College of Pharmaceutical Sciences ,Payangadi, Kannur, Kerala -670358.

### Extraction procedure

1kg of *C.sappan* wood was ground to a fine powder form and treated with petroleum ether and then soxhleted with distilled water for 2 hrs. The aqueous extract obtained

was filtered and concentrated *in vacuum* and kept in a micro wave oven for 5 min to make the dry powder (% yield 10.5).

## HPTLC analysis of colour extracts CSWC

The HPTLC of the aqueous colour extract, (designated as CSWC: *C.sappan* wood colour) was done in a CAMAG WINCATS planar. Chromatography software using pre-prepared TLC plates (silica gel $60F_{254}$ MERK, Band width : 8mm, Slit dimension : 6x0.45mm, Wave length of scanning : 570nm) indicated the presence of three compounds (Rf value 0.91, 0.76, 0.61) using solvent system chloroform : methanol : water (64:50:10) and alcoholic ferric chloride as the detecting agent. The quantitative determination was done and the results are tabulated in table, Fig –3.

## UV spectrophotometric analysis of the colour extracts CSWC

The colour extract CSWC was dissolved in double distilled water and scanned at 400-800nm in Jasco V-530 UV Spectrophotometer and obtained a λmax value of 538nm. But the λmax values for CSWC in pH:4 and in pH 10 were 443nm and 537nm respectively. A standard curve was plotted by taking various concentrations of CSWC (100–1000 mcg/ml) against absorbance at a wavelength maximum of 538nm. The results are plotted in Fig –1, 2(a), 2(b) and 4.

## Preliminary Cytotoxicity Studies (8)

This study was conducted by Brine Shrimp Assay (BSA) method[8]. The brine shrimps eggs were hatched in a rectangular chamber and filled with artificial sea water and then ten numbers each of nauplii were transferred to vials using a pipette. The survival rate of the brine shrimps were observed after 24h for various concentrations of CSWC and a synthetic colour 'orange red' (Gold Camel FCF 15985, 14720, ISI no.5346). The $LC_{50}$ values for these two colours were found out from the dose response graph. The results are tabulated in Fig –5.

## Acute Toxicity Studies

The acute toxicity study (9, 10) was carried out using overnight fasted swiss albino mice. Both the colours CSWC and the synthetic colour ( FCF.15985, 14720) were dissolved in distilled water and administered intra peritonially in doses of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 g / kg body weight to different groups of mice of 6–10 each. Animals were observed at regular intervals of 1h for a period of 24h for death due to acute toxicity. Similarly the $LD_{50}$ value was determined by giving CSWC and the FDF colour through oral route to respective group of animals

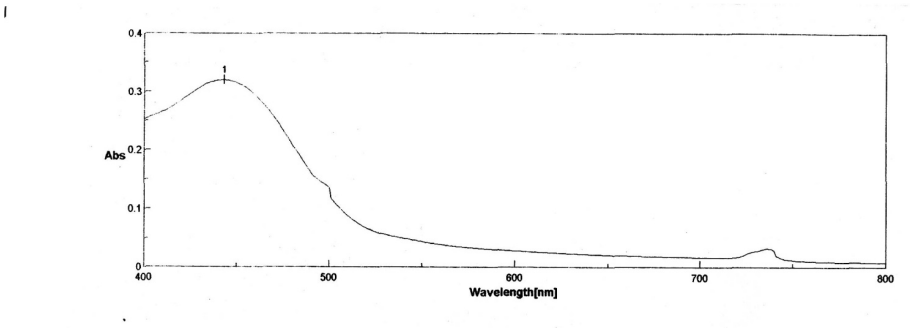



**Figure 1**- UV Spectrum of CSWC at pH4 (λ max 443nm)

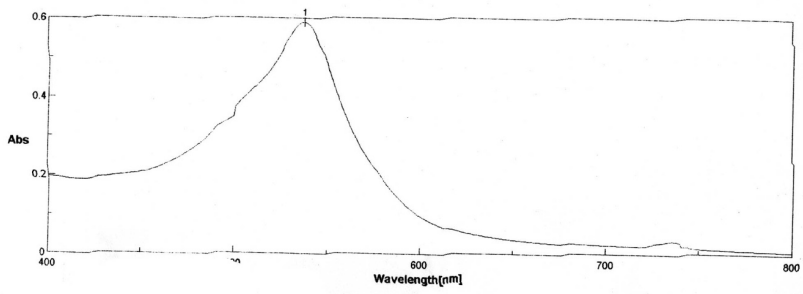



**Figure 2(a)**- UV Spectrum of CSWC at Normal pH (λ max 538nm)

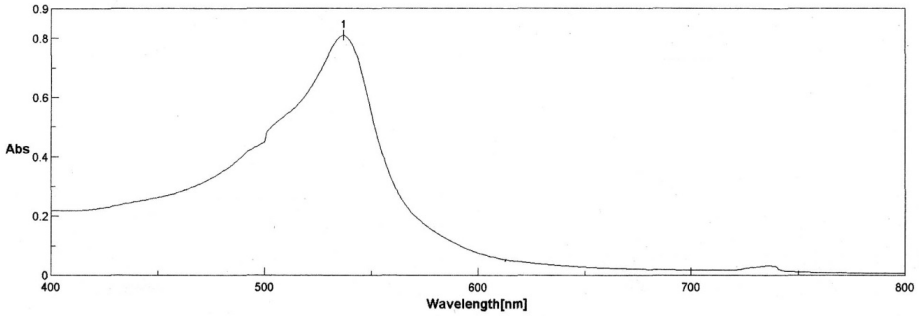
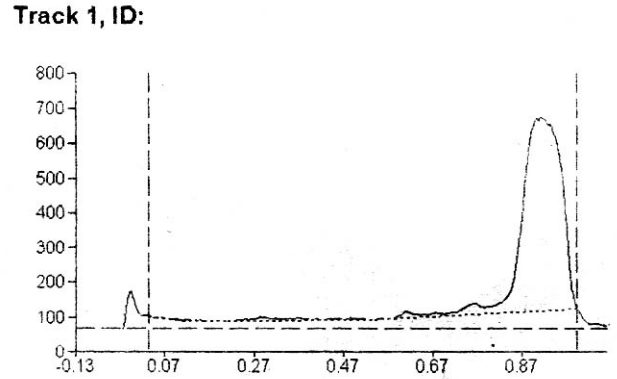



**Figure 2(b)**- UV Spectrum of CSWC at pH 10 (λ max 537nm)




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.58 | 1.3 | 0.61 | 19.1 | 3.14 | 0.63 | 8.8 | 443.9 | 1.03 |
| 2 | 0.72 | 9.6 | 0.76 | 32.3 | 5.29 | 0.79 | 19.1 | 1140.1 | 2.64 |
| 3 | 0.80 | 19.7 | 0.91 | 557.9 | 91.57 | 0.99 | 3.5 | 41624.3 | 96.33 |

**Figure 3**- High Performance Thin Layer Chromatogram of CSWC

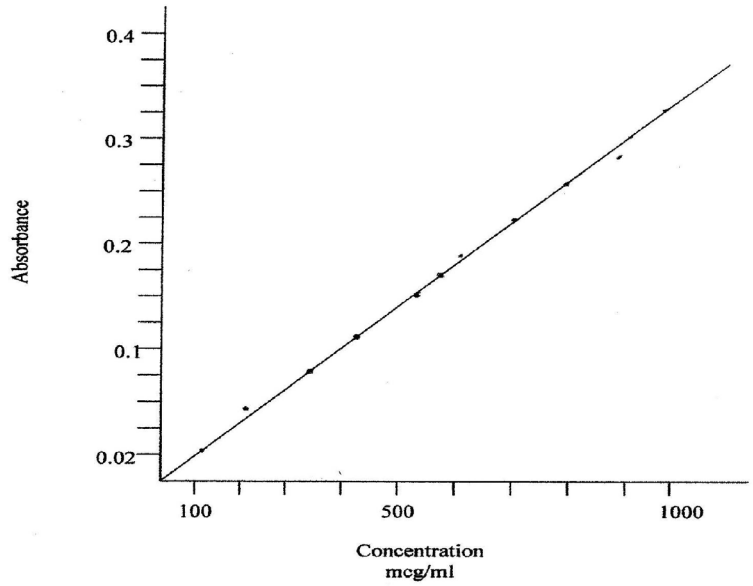



**Figure 4**- Standard curve of CSWC, concentration vs Absorbance

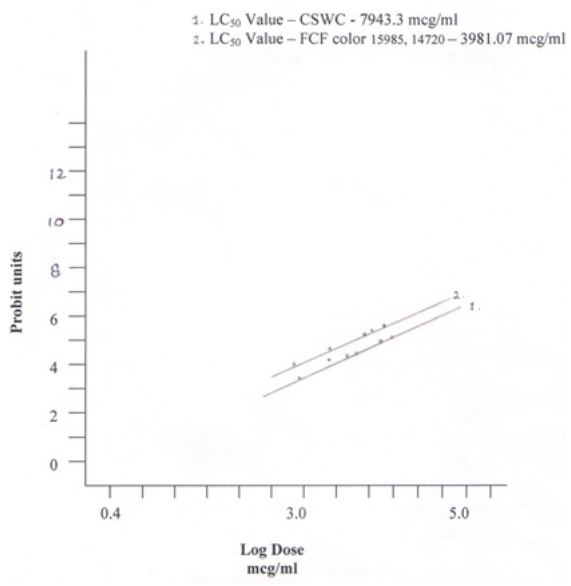



**Figure 5**- Dose Response curve$LC_{50}$ determination(Brine Shrimp Assay Method)

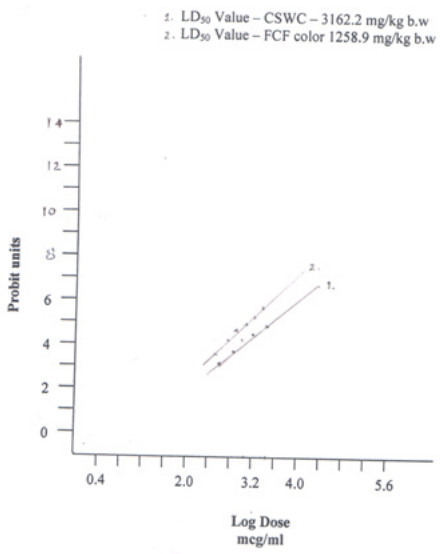



**Figure 7**- Dose Response curveRoute of Administratoion : Intra peritonial

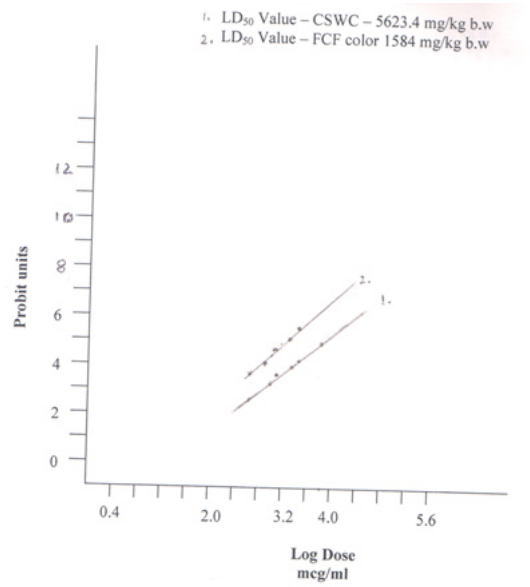



**Figure 6**- Dose Response curveRoute of Administration : Oral

by suspending the colours in 0.5% sodium carboxymethyl cellulose. The results are tabulated Fig –6 and 7.

All the animal experiments were conducted in accordance with National Institute of Health and Institutional Ethics Committee guidelines.

## RESULTS AND DISCUSSION

The colour extract CSWC obtained from *C.sappan* wood was found to be highly soluble in water, therefore this colour can be incorporated easily in any pharmaceutical formulations like syrups, suspensions etc and also in food materials and nutraceuticals.

The UV spectral data indicated that CSWC at 100 µg/ml can give measurable absorbance at a λmax of 538nm in normal pH 7 and 443 nm in pH 4 and 537 nm in pH 10. The bright yellow and carmine red colour variation of CSWC in acidic and alkaline pH can be of use to prepare many shades of colours from this red pigment.

The colour extract CSWC and the synthetic red colour (FCF 15985, 14720) showed an $LC_{50}$ value of 7943.3 and 3981.07 mcg/ml in the brine shrimp assay method. CSWC showed an $LD_{50}$ value of 5623.4 mg/kg body weight and 3162.2 mg /kg b.w respectively in oral and intraperitonial routes of administration in acute toxicity studies. While the synthetic colour (FCF.15985, 14720) showed an $LD_{50}$ value of 1584 mg/kg b.w and 1258.9 mg/kg b.w respectively in oral and intraperitonial routes of administration. These values indicated that the natural colour pigment CSWC is much safer compared to its synthetic counter part. HPTLC evaluation indicated the presence of three phenolic compounds in the aqueous extract CSWC with Rf values 0.91, 0.76 and 0.61 and quantified as 963, 26.4 and 10.3 mg/g of CSWC respectively. Reports (2, 4) indicated the anti-inflammatory, antiulcer and astringent properties of this natural colour pigments, therefore these herbal colours can be utilized in pharmaceuticals, cosmeceuticals and food materials not only as a colouring agent, but can impart many useful therapeutic activities also.

## CONCLUSIONS

The results indicated that the colour extract CSWC obtained from *C.sappan* wood is very safe to use as a colouring agent in pharmaceutical and cosmeceuticals instead of synthetic FDC grade colours. The percentage yield (35.5%) of CSWC was found satisfactory for any commercial purposes. Also the results showed that the routine analysis and validation of CSWC can be done effectively by UV and HPTLC techniques.

## ACKNOWLEDGEMENT

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