Arabian Journal of Chemistry (2013) **xxx**, xxx–xxx

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

In-vitro antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of *Cassia* species

Jignasu P. Mehta [a,*], Pravin H. Parmar [a], Sohil H. Vadia [a], Mahendra K. Patel [b], Chandrabhanu B. Tripathi [b]

[a] *Analytical Chemistry Division, Department of Chemistry, DST-FIST Sponsored Department, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364002, India*
[b] *Department of Pharmacology, Government Medical College, Bhavnagar 364001, India*

Received 1 January 2013; accepted 9 June 2013

KEYWORDS

Cassia siamea (Lam.);
Cassia javanica (Linn.);
95% Ethanolic extracts;
In vitro antioxidant activity;
In vivo anti-inflammatory activity

Abstract *Cassia* species are native to Southeast Asia and sub-Saharan Africa and are commonly used as food and in a broad variety of medicinal applications. *Cassia* species are widely used in India, but there are few reports in the literature of studies on its chemical compositions and biological properties. In this study, the antioxidant and anti-inflammatory activities of two plants of *Cassia* species namely *Cassia siamea* (Lam.) and *Cassia javanica* (Linn.) were evaluated and the total phenolic compounds and flavonoid contents were determined.

The antioxidant activity of the extracts was measured using scavenging of 2,2′-Diphenyl-1-picrylhydrazyl hydrate (DPPH), bleaching of β-carotene and % inhibition of H_2O_2. The anti-inflammatory activity was evaluated using carrageenan induced paw edema method on *Wistar* albino rats. The etahnolic extracts of aerial parts of *C. siamea* and *C. javanica* were evaluated for *in vivo* anti-inflammatory activity against the animal model of female *Wistar* albino rats. Ethanol extracts showed significant and dose-dependent anti-inflammatory effects. The contents of flavonoids and total phenolic compounds could be correlated with the antioxidant and anti-inflammatory activities observed for *C. siamea* and *C. javanica*. Our findings suggest that aerial parts of *C. siamea* and *C. javanica* contain potential antioxidant and anti-inflammatory compounds, which could be tested as drug candidates against oxidative and inflammation-related pathological processes in medicinal chemistry studies.

1. Introduction

The use of traditional medicine is widespread and plants still represent a large source of natural secondary metabolites that might serve as leads for the development of novel drugs. Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have

* Corresponding author. Tel.: +91 2782439852 (O), mobile: +91 9498706147.
E-mail address: jpm1569@yahoo.co.in (J.P. Mehta).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1878-5352 © 2013 Production and hosting by Elsevier B.V. on behalf of King Saud University.
http://dx.doi.org/10.1016/j.arabjc.2013.06.010

an antioxidant and/or radical scavenging mechanism as part of their activity (Perry et al., 1999; Lin and Huang, 2002; Repetto and Llesuy, 2002).

Inflammation and oxidative stress play an important role in various diseases. Inflammation is an immunological defense mechanism elicited in response to mechanical injuries, burns, microbial infections, allergens and other noxious stimulus (Yoon and Baek, 2005; Winrow et al., 1993; Gutteridge, 1995; Menichini et al., 2009; Mueller et al., 2010).

Use of anti-inflammatory agents may therefore, be helpful in the treatment of inflammatory disorders (Sosa et al., 2002). Non-steroidal anti-inflammatory drugs (NSAID) or steroidal anti-inflammatory drugs (SAID) are commonly used to treat different inflammatory diseases. The adverse effects of the currently available anti-inflammatory drugs however, pose a major problem in their clinical use therefore; naturally, originated agents with very little side effects are desirable to substitute chemical therapeutics (Yonathan et al., 2006; Boakye-Gyasi et al., 2008). There has been a growing interest in phenolic components of fruits and vegetables, which may promote human health or lower the risk of disease. Recent studies have focused on health functions of phenolics including flavonoids from fruit and vegetables (Saleem et al., 2002; You-dim et al., 2002; Qian et al., 2004; Chen et al., 2006). In search for sources of natural antioxidants, some medicinal plants have been extensively studied for their antioxidant activity and radical scavenging activity (De las Heras et al., 1998; Desmarchelier et al., 2000; Schinella et al., 2002; VanderJagt et al., 2002).

Cassia siamea (Lam.) is a very widespread medicinal and food plant cultivated in Southeast Asia and sub-Saharan Africa. Many traditional claims are reported as medical treatments on various diseases like constipation, malaria and associated diseases such as fevers and jaundice (Ahn et al., 1978; Nsonde-Ntandou et al., 2005; Kaur et al., 2006). Aerial parts of *C. siamea* are useful in ringworm and related skin diseases because of presence of anthraquinone derivatives (Ahn et al., 1978). Ethno-botanical surveys also suggest antinociceptive and antiviral activities of aerial parts of *C. siamea* (Gbeassor et al., 1989; Mbatchi et al., 2006), antioxidant and antihypertensive activity (Kaur et al., 2006). A laxative activity (Elujoba et al., 1989), sedative activity (Thongsaard et al., 2001; Sukma et al., 2002) and anti-inflammatory of stem bark extract of *C. siamea* (Abatan, 1990; Nsonde Ntandou et al., 2010) were also reported. Such activities can be due to the presence of barakol, which has anxiolytic and CNS inhibitory effects (Sukma et al., 2002). *Cassia javanica* (Linn.) is a tree that belongs to family *Leguminosae*. Previous literature provides meager information about therapeutic uses of this plant. Aerial parts of *C. javanica* are used traditionally in herbal drug formulation for diabetics and are active against *Herpes simplex* infection (Kaur and Arora, 2011). These activities are attributed to the fact that aerial parts of *C. javanica* contain a variety of secondary metabolites, such as, flavones, sterols, several hydrocarbons, anthraquinones, glycosides etc. Among these flavones, glycosides and sterols are considered to be useful as anti-inflammatory and antioxidant agents.

Based in ethanopharmacological relevance of *C. siamea* and *C. javanica*, the present study was aimed to evaluate antioxidant activity through free radical scavenging of DPPH, bleaching of β-carotene and % inhibition of H_2O_2. Moreover, considering that antioxidants and free radical scavengers can exert an anti-inflammatory effect and hence, extracts were also evaluated for *in vivo* anti-inflammatory activity (Li et al., 2003; Lee et al., 2006; Geronikaki and Gavalas, 2006).

2. Experimental

2.1. Plant material

Leaves, bark, seeds and flowers of *C. siamea* were collected from the University campus of Maharaja Krishnakumarsinhji Bhavnagar University Bhavnagar, Gujarat, India. Leaves, bark, flowers and seeds of *C. javanica* were collected from Gandhinagar City, Gujarat, India. Voucher specimens of *C. siamea* (BU/PG/CHHERBS/01/11) and *C. javanica* (BU/PG/CH/HERBS/02/11) were deposited. Both species were authenticated at Botany Department, Sir P. P. Institute of Science, Bhavnagar, India.

2.2. Preparations of extracts

The aerial parts of *C. siamea*, and *C. javanica* were cleaned with deionized water, oven dried at 50 °C for 48 h and powdered in a grinder. The plant material (100 g) was extracted with different solvents (1500 mL) using Soxhlet apparatus for 24 h at a temperature not exceeding the boiling point of the respective solvents. The obtained extracts were filtered using Whatmann filter paper No. 1 and concentrated under vacuum at 40 °C using a rotary vacuum evaporator (Büchi Laboratoriums, Switzerland) to dryness. The extractive values of the extracts were calculated.

2.3. Chemicals and reagents

2,2′-Diphenyl-1-picrylhydrazyl hydrate (DPPH), β-carotene, linoleic acid, butylatedhydroxytoluene (BHT), ferrous chloride and Folin–Ciocalteu reagent were purchased from Hi-Media Lab. Pvt. Ltd., Mumbai, India. Sodium dihydrogen orthophosphate, di-sodium hydrogen orthophosphate dehydrate and sodium acetate was purchased from E-Merck India Ltd. Other chemicals and solvents were procured from SD Fine-Chem Ltd., Mumbai, India and were of analytical grade, while Hydrogen peroxide (H_2O_2) was purchased from RFCL Limited (*Rankem*), Mumbai, India. λ-Carrageenan was purchased from Sigma–Aldrich Chemicals, India. Reference standard diclofenac sodium was obtained as gift sample from Unique Pharmaceuticals, Ankleshwer, India.

2.4. Instrumentation

GC–MS analysis was performed on Shimadzu GC–MS-QP2010 Plus system equipped with RTX-5 m.s. capillary column (0.25 mm × 30 m × 0.25 μm). The interface temperature and the MS scan range of the instrument were set at 230 °C and 35–450 atomic mass units (AMU), respectively. Helium was used as the carrier gas at a flow rate of 10 mL/min. Column temperature was started at 60 °C, held for 2 min, ramped up to 170 °C at a rate of 2 °C/min, held for 3 min, ramped to 250 °C at a rate of 3 °C/min and finally held for 120 min. The samples (1 μL) were injected in split mode at 220 °C and compounds identified using NIST98 MS data library. A total of 15

Please cite this article in press as: Mehta, J.P. et al., *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of *Cassia* speciesCassia species –>. Arabian Journal of Chemistry (2013), http://dx.doi.org/10.1016/j.arabjc.2013.06.010

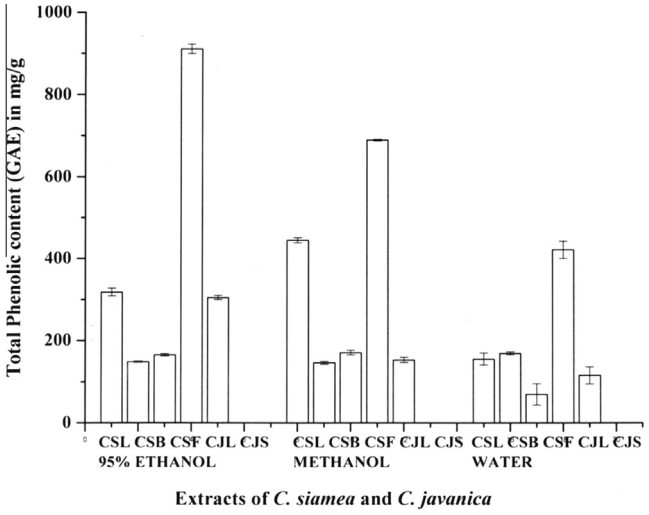

Figure 1 Total phenolic contents of 95% ethanol, methanol and water extracts of *C. siamea* leaf, *C. siamea* bark, *C. siamea* flowers, *C. javanica* leaf and *C. javanica* seeds. Results of total phenolic contents were calculated as average ± S.D for each group.

compounds were identified based on GC–MS results in which few were known and two to three compounds were not previously reported in both species.

2.5. Animals

An inbred colony of female *Swiss* albino mice (25–60 g) were used for acute toxicity study, while adult female *Wistar* albino rats (170–300 g) were used for anti-inflammatory study. They were kept in polypropylene cages at 25 ± 2 °C, with relative humidity 45–55% under 12 h light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed (Government Medical College, Bhavnagar) and water *ad libitum*. The test extracts and the standard drugs were administered in the form of a suspension in water using 1% carboxymethyl cellulose as suspending agent. All pharmacological activities were carried out as per CPCSEA (Committee for the purpose of control and supervision of experiments on animals) norms. All the pharmacological experimental protocols were approved by the Institutional animal ethics committee (IAEC No. 24/2011, pharmacology No. 24/2011, date: 13/09/2011), Government Medical College, Bhavnagar.

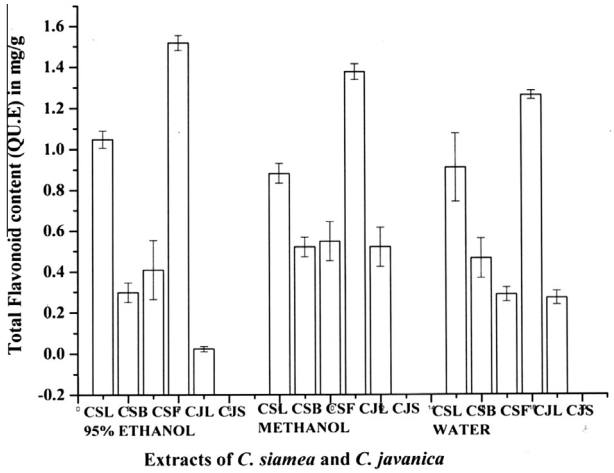

Figure 2 Total flavonoid contents of 95% ethanol, methanol and water extracts of *C. siamea* leaf, *C. siamea* bark, *C. siamea* flowers, *C. javanica* leaf and *C. javanica* seeds. Results of total flavonoid contents were calculated as average ± S.D for each group.

2.6. Determination of total phenols and flavonoids

Total phenolic content was determined according to Folin–ciocalteu method (Sabir and Rocha, 2008). The results are expressed as grams of gallic acid equivalents per 100 g of dry extract. The concentration of phenolic compounds was calculated according to the following Eq. (1) obtained from the standard Gallic acid (5–50 μg) curve.

$$\text{Absorbance} = 0.016 \times \text{GAE in } \mu g + 0.029\,(R^2 = 0.996) \quad (1)$$

Flavonoid content in the various extracts was determined by a colorimetric method (Moreno et al., 2000). The results are expressed as grams of quercetin equivalents per 100 g of dry extract. The concentration of flavonoid compounds was calculated according to the following Eq. (2) obtained from the standard quercetin (20–100 μg) curve.

$$\text{Absorbance} = 0.00001 \times \text{Quercetin in } \mu g + 0.011\,(R2 = 0.994) \quad (2)$$

2.7. DPPH radical scavenging assay

The radical-scavenging activity was evaluated using an improved DPPH assay (Huang et al., 2011). For 0.2 mM DPPH, 2.7 mL was added to 0.3 mL of the extract solution at various concentrations. The mixture was shaken vigorously and incubated at room temperature for 1 h before the absorbance was measured at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the Eq. (3).

$$\%\,\text{RSA} = [(A_0 - A_S)/A0] \times 100 \quad (3)$$

where A_0 and A_S are the absorbance of the control (containing all reagents, except the test compound) and test compound, respectively.

2.8. β-Carotene bleaching inhibition assay

Antioxidant activity was also assessed using the β-carotene linoleate model system (Shon et al., 2003) Briefly, 6 mg of β-carotene was dissolved in 20 mL of chloroform, and 4 mL of this solution was pipetted into a round-bottomed 500 mL flask containing 80 mg linoleic acid and 800 mg Tween-80. After removing chloroform using a rotary evaporator, 200 mL of distilled water was added slowly to the mixture with vigorous agitation to form a stable emulsion. Then, 3 mL aliquots of the emulsion were transferred into different test tubes containing 0.2 mL of the samples and were incubated in a water bath at 50 °C for 2 h. Ascorbic acid was used as standards for compar-

Please cite this article in press as: Mehta, J.P. et al., *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of *Cassia* speciesCassia species –>. Arabian Journal of Chemistry (2013), http://dx.doi.org/10.1016/j.arabjc.2013.06.010

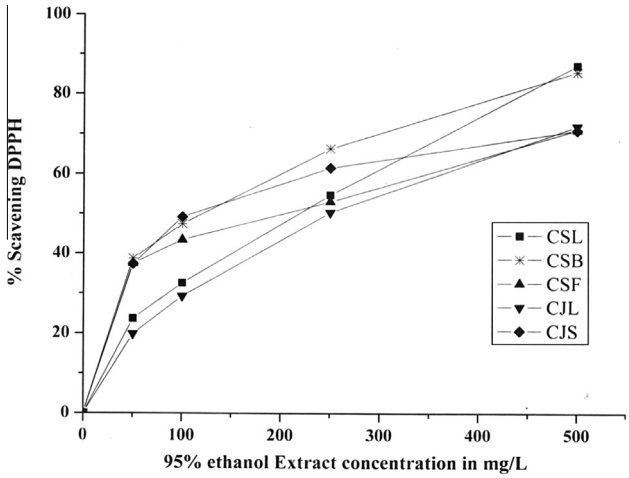

Figure 3 Percentage scavenging of DPPH in different dosages of aerial parts of *C. siamea* and *C. javanica* in ethanolic extract. (i) –■– CSL (ii) –*– CSB (iii) –▲– CSF (iv) –▼– CJL (v) –♦– CJS.

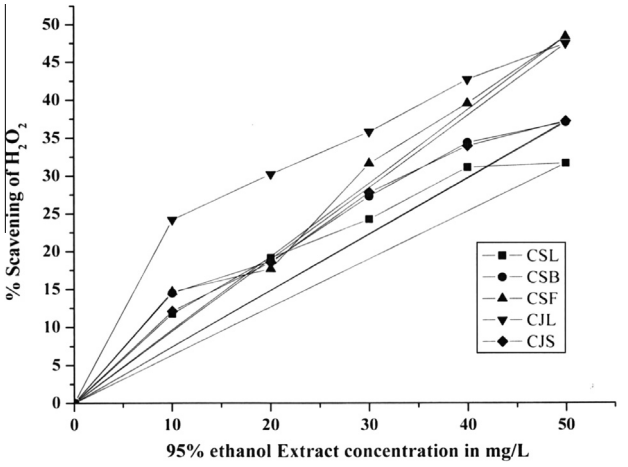

Figure 4 Percentage scavenging of H_2O_2 in different dosages of aerial parts of *C. siamea* and *C. javanica* in ethanolic extract. (i) –■– CSL (ii) –●– CSB (iii) –▲– CSF (iv) –▼– CJL (v) –♦– CJS.

ison. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene using Eq. (4).

$$\%\,\text{Inhibition} = [1 - (A_{S(0)} - A_{S(60)}/ - (Ac_{(0)} - Ac_{(60)})] \times 100 \quad (4)$$

where $A_{S(0)}$ the initial absorbance of the sample at $A_{S(60)}$ the absorbance of the sample at 60 min, $Ac_{(0)}$ the absorbance of the negative control at 60 min. The extract concentration providing 50% antioxidant activity (IC_{50}) was calculated from the graph of antioxidant activity percentage against extract concentration.

2.9. Assay of H_2O_2 scavenging activity

The ability of all seed extracts to scavenge hydrogen peroxide was determined according to the method described previously (Ruch et al., 1989). A solution of hydrogen peroxide (2 mM)

Table 1 IC_{50} for DPPH scavenging, β-carotene scavenging and H_2O_2 scavenging of different extracts of *C. siamea* and *C. javanica* plants.

Species	Solvent	DPPH scavenging activity (IC_{50} in μg/mL)	β-Carotene bleaching effect (IC_{50} in μg/mL)	H_2O_2 (IC_{50} in μg/mL)
CSL[a]	95% Ethanol	244.94 ± 7.94	**140.52 ± 2.69***	179.3 ± 14.47
CSB[b]		152.30 ± 12.68	239.31 ± 5.58	**69.2 ± 1.40**
CSF[c]		239.63 ± 19.21	409.31 ± 32.05	52.08 ± 0.66
CJL[d]		309.55 ± 10.92	283.92 ± 3.66	53.74 ± 0.83
CJS[e]		150.79 ± 20.00	369.54 ± 1.95	66.93 ± 0.27
CSL	Methanol	238.03 ± 7.81	**143.99 ± 1.93***	104.77 ± 6.08
CSB		159.84 ± 7.93	236.55 ± 2.71	**52.03 ± 2.12****
CSF		144.12 ± 12.71	206.63 ± 4.19	**51.64 ± 0.35****
CJL		325.07 ± 7.26	210.66 ± 2.27	**49.73 ± 0.29*****
CJS		**87.98 ± 2.95****	379.11 ± 4.95	140.59 ± 1.49
CSL	Water	242.22 ± 7.20	**130.74 ± 4.78***	108.07 ± 4.65
CSB		128.87 ± 7.43	255.11 ± 3.67	130.66 ± 8.82
CSF		155.31 ± 9.60	228.86 ± 5.30	**55.5 ± 0.18****
CJL		254.62 ± 13.89	394.84 ± 8.51	**52.02 ± 0.41****
CJS		**83.51 ± 7.07****	445.11 ± 4.95	**63.48 ± 0.55****

Each value is expressed as mean ± standard deviation ($n = 3$) and $p < 0.003$, which is extremely significant.
Bold letters show extremely significant relation for statistical analysis.
[a] *C. siamea* leaf.
[b] *C. siamea* bark.
[c] *C. siamea* flower.
[d] *C. javanica* leaf.
[e] *C. javanica* seeds.
* $p < 0.05$ Moderately significant.
** $p < 0.01$ Significant.
*** $p < 0.001$ Extremely significant.

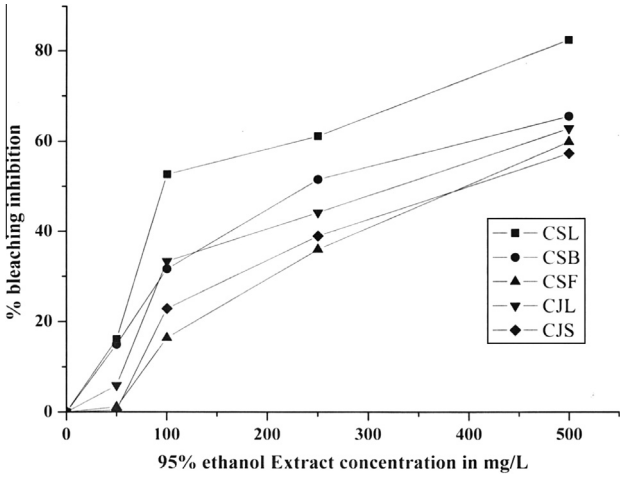

Figure 5 Percentage bleaching of β-carotene in different dosages of aerial parts of *C. siamea* and *C. javanica* in ethanolic extract. (i) –■– CSL (ii) –●– CSB (iii) –▲– CSF (iv) –▼– CJL (v) –♦– CJS.

was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically from absorption at 230 nm. Extracts samples (10–50 μg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentages of scavenging of hydrogen peroxide of extracts of aerial parts and standard compounds are calculated by using the following Eq. (5).

$$\%\text{Scavenged}\, H_2O_2 = ([A_0 - A_1]A_1) \times 100 \quad (5)$$

where A_0 was the absorbance of the control, and A_1 was the absorbance in the presence of the extracts and standards.

2.10. Acute toxicity test studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method; Ecobichon, 1997). Female *Swiss albino* mice ($n = 6$) selected by random sampling technique and were used for acute toxicity study. The animals were kept fasting overnight providing only water. Subsequent to administration of the extracts, the animals were observed closely for the first 3 h for any toxic manifestations such as increased locomotors activity, salivation, clonic convulsion, coma and death. Subsequent observations were made at regular intervals for 24 h. The animals were observed for a further week. The ethanolic extracts were safe up to a dose of 2000 mg/kg body weight, so 200 mg/kg (p.o.) and 400 mg/kg

Table 2 Anti inflammatory activity of different parts of *C. siamea* and *C. javanica* in 95% ethanol system.

Drug	Dose (mg/kg)	Change in mean paw volume (in ml)				
		0 min (Initial)	After 60 min	After 120 min	After 180 min.	After 240 min
Control		0.25 ± 0.03	0.34 ± 0.05	0.46 ± 0.10	0.52 ± 0.09	0.70 ± 0.05
Standard	5	0.27 ± 0.03	**0.38 ± 0.03****	0.36 ± 0.03*	0.33 ± 0.02	0.3 ± 0.03
CJL[a]	200	0.27 ± 0.04	0.36 ± 0.05*	0.33 ± 0.05	0.31 ± 0.05	0.29 ± 0.04
CJL[a]	400	0.27 ± 0.04	0.37 ± 0.05*	0.34 ± 0.05	0.32 ± 0.05	0.3 ± 0.04
CJF[b]	200	0.39 ± 0.06	**0.54 ± 0.06****	0.5 ± 0.06*	0.48 ± 0.06	0.50833 ± 0.06
CJF[b]	400	0.29 ± 0.03	**0.46 ± 0.03*****	**0.45 ± 0.03*****	**0.44 ± 0.03*****	**0.41 ± 0.06****
CSL[c]	200	0.25 ± 0.07	0.39 ± 0.06*	0.36 ± 0.06	0.33 ± 0.06	0.31 ± 0.06
CSL[c]	400	0.25 ± 0.02	**0.35 ± 0.02*****	**0.33 ± 0.02*****	**0.3 ± 0.02****	0.27 ± 0.02
CSF[d]	200	0.32 ± 0.07	**0.48 ± 0.06****	**0.45 ± 0.06****	**0.43 ± 0.06****	0.42 ± 0.07
CSF[d]	400	0.33 ± 0.01	**0.49 ± 0.02*****	**0.47 ± 0.03****	**0.44 ± 0.032****	**0.42 ± 0.03****
CSS[e]	200	0.32 ± 0.01	**0.51 ± 0.02*****	**0.49 ± 0.02****	0.46 ± 0.01*	0.44 ± 0.02
CSS[e]	400	0.34 ± 0.04	**0.48 ± 0.04*****	**0.45 ± 0.04*****	0.41 ± 0.04***	**0.38 ± 0.04*****
CJS[f]	200	0.41 ± 0.05	0.53 ± 0.05*	0.49 ± 0.05	0.46 ± 0.05	0.43 ± 0.05
CJS[f]	400	0.38 ± 0.04	**0.56 ± 0.04*****	**0.54 ± 0.03*****	**0.51 ± 0.04*****	**0.49 ± 0.04****
CJB[g]	200	0.31 ± 0.03	**0.5 ± 0.03*****	**0.49 ± 0.02*****	**0.47 ± 0.03*****	**0.45 ± 0.03*****
CJB[g]	400	0.3 ± 0.04	**0.44 ± 0.02*****	**0.4 ± 0.03****	**0.38 ± 0.03****	0.35 ± 0.03*
CSB[h]	200	0.33 ± 0.03	**0.46 ± 0.03*****	**0.44 ± 0.04****	0.4 ± 0.04*	0.38 ± 0.04
CSB[h]	400	0.28 ± 0.04	**0.4 ± 0.04****	0.35 ± 0.04	0.32 ± 0.03	0.29 ± 0.04

n = Six animals in each group; values are represented as mean ± Standard Error Mean (SEM) Change in mean paw volume (in ml) after administration of control, sample and standard drugs.
Bold letters show extremely significant relation for statistical analysis.
[a] *C. javanica* leaf.
[b] *C. javanica* flower.
[c] *C. siamea* leaf.
[d] *C. siamea* flower.
[e] *C. siamea* seeds.
[f] *C. javanica* seeds.
[g] *C. javanica* bark.
[h] *C. siamea* bark.
* $p < 0.05$ moderately significant.
** $p < 0.01$ significant.
*** $p < 0.001$ extremely significant.

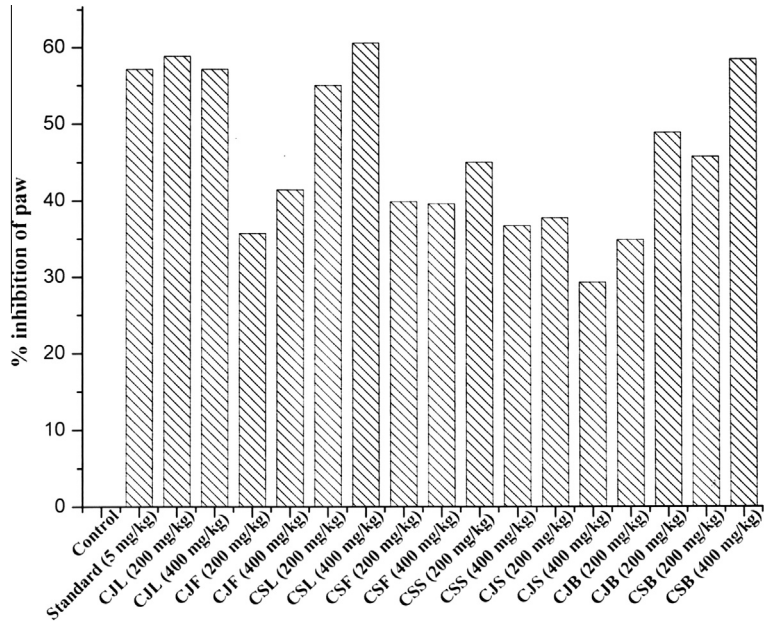

Figure 6 Reduction of paw edema of rats with percentage inhibition in two dosages of aerial parts of *C. siamea* and *C. javanica* in ethanolic extract.

(p.o.) were used as moderate dose for the evaluation of anti-inflammatory activities (Ghose, 2005).

2.11. Carrageenan-induced rat paw edema of ethanolic extract

Paw edema was induced by injecting 0.1 ml of 1% W/V λ-carrageenan in physiological saline into the subplantar tissues of the left hind paw of each rat (Winter et al., 1962). The extracts of aerial parts of *C. siamea* and *C. javanica* (200 mg/kg and 400 mg/kg p.o.) were administered orally 30 min prior to λ-carrageenan administration. The paw volume was measured at 60, 120, 180 and 240 min by the mercury displacement method using a plethysmograph. The percentage inhibition of paw volume in drug treated group was compared with the control group. Diclofenac sodium (5 mg/kg p.o.) was used as standard reference (Nsonde Ntandou et al., 2010).

2.12. Statistical analysis

All results were obtained with means ± standard deviation of three parallel measurements. The data were analyzed by a one-way analysis of variance (ANOVA) using Graph pad INST-3 to determine the effects of extracts. Significant differences between extract means were determined by two-way ANOVA tests.

3. Results and discussion

3.1. Total phenolic content

Natural polyphenols have chain-breaking antioxidant activities and are believed to present many degenerative diseases including cancer and atherosclerosis (Jayaprakasha et al.,

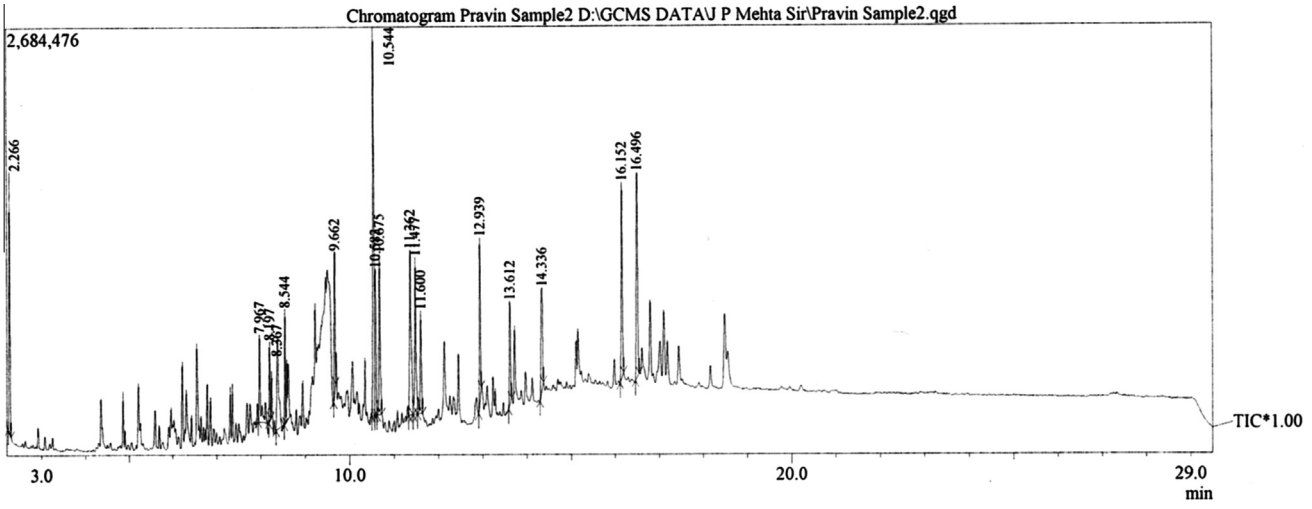

Figure 7 Gas chromatograph of 14 separated components from crude extract of *C. siamea* using mass spectral universal detector.

2001). Phenolics are aromatic secondary plant metabolites and are called high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlets, superoxide free radicals and hydroxyl radicals (Roginsky, 2003). Many studies have reported the antioxidant activities of phenolics from plant sources in different systems (Hagerman et al., 1998; Sekretar et al., 2004; Tachakittirungrod et al., 2007; Kobus et al., 2009).The contents of total phenolics in crude extracts of aerial parts of *C. siamea* and *C. javanica* are depicted in Fig. 1. The crude extracts obtained from aerial parts of *C. siamea* and *C. javanica* were characterized by their contents of total phenolics expressed as gallic acid equivalent (GAE) for *C. siamea* leaf (CSL), *C. siamea* bark (CSB), *C. siamea* flower (CSF), *C. javanica* leaf (CJL) and *C. javanica* seeds (CJS) in 95% ethanol, methanol and water. Lowest total phenolics were obtained from the water extract of CSF: 69.37 mg/g (GAE) and highest total phenolics were obtained from the 95% ethanolic extract of CJL: 910.85 mg/g (GAE). This is due to fact that solubility of phenolics is less in water. The calculations of total phenolics were carried out by using standard gallic acid curve with least square regression ($y = 0.016x + 0.029$; $R^2 = 0.9968$; standard deviation of residuals from line = 0.01855). Similarly, total flavonoid content equivalent to quercetin (QU. E) for *C. siamea* and *C. javanica* was calculated from the standard curve of QU with least square regression ($y = 0.0001x + 0.011$; $R^2 = 0.9941$; standard deviation of residuals from line = 0.001514) and is depicted in Fig. 2.

Aerial parts of both plants and different solvents used for extraction were responsible for the variation in total phenolic contents. The order of phenolic content obtained from three solvents was 95% ethanol > methanol > water for aerial parts. Variations in phenolic contents of various extracts are attributed to polarities of different compounds present in various aerial parts, and such differences have been reported elsewhere (Duh et al., 1990). Similarly, the order of flavonoid content obtained from three solvents was 95% ethanol > methanol > water for both plants. The results of the extracted material showed that leaves of *C. javanica* had highest phenolic and flavonoid contents.

3.2. DPPH radical scavenging assay

DPPH is a stable free radical in aqueous or methanol and ethanol solution and accept an electron or hydrogen radical to become a stable diamagnetic molecule. It is used as a substrate to evaluate the antioxidative activity of antioxidants (Baumann et al., 2002). The reduction capability of DPPH radicals was determined by a decrease in its absorbance at 517 nm. The decrease in absorbance of DPPH radical was caused by antioxidant, and was due to the scavenging of hydrogen donation. DPPH assay showed that extracts of CSB in water had the highest scavenging with 39.47% ($R^2 = 0.9624$; standard deviation of residuals from line = 3.869) scavenging at 50 μg/mL concentration. Results of DPPH assay also showed that extracts of CSL, CSB, CSF, CJL and CJS were in the order of methanol > 95% ethanol > water. The scavenging effect of extracts on the DPPH radical increased with an increasing concentration of each extract and is shown in Fig. 3. The degree of discoloration indicates the scavenging capacity of the extract. The effect of antioxidants on the DPPH radical scavenging was due to the result from their hydrogen donating ability (Hallivel and Guttreridge, 1989). IC_{50} value for water extract of CJS was moderate (83.5 μg/mL ± 7.07; $p < 0.001$; $R^2 = 0.8233$) in comparison to the standard BHT (Table 1). Similarly, many researchers reported that some plant extracts showed a lower activity against the DPPH, if compared with BHT (Ramarathnam et al., 1995; Halliwell, 1997). In addition, there was a statistically significant correlation between the amount of phenolic compounds and DPPH scavenging activity in all the extracts.

3.3. H_2O_2 scavenging activity

The results showed excellent H_2O_2 scavenging activities for all aerial parts at a concentration range from 10 to 50 μg/mL and is depicted in representative Fig. 4.

IC_{50} for standard ascorbic acid was 49.47 ± 1.22 μg/mL ($R^2 = 0.9891$), while IC_{50} for methanolic extract of CJL was 49.74 ± 0.29 μg/mL ($R^2 = 0.9553$; standard deviation of residuals from line = 1.848). Similarly, water extract of CJL had IC_{50} value 52.07 ± 0.41 μg/mL with linear regression (R^2) = 0.9792, while 95% ethanolic extract of CJL had IC_{50} value 53.75 ± 0.84 μg/mL with linear regression (R^2) = 0.9941(Table 1). These results suggest that bioactive compound(s) present in the different extracts of CJL have very good impact on the scavenging activity of H_2O_2 system. Hydrogen peroxide itself is not very reactive but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cell (Gordon, 1990). Thus, the removing of H_2O_2 is very important for antioxidant defense in cell or food systems. Scavenging of H_2O_2 by extracts is attributed to their phenolics, which could donate electrons to H_2O_2. The extracts might be used to provide a good H_2O_2 scavenger for humans and foods especially, CJL extracts in all three solvents showed excellent ability to scavenge H_2O_2.

3.4. β-Carotene bleaching effect

The bleaching effect was measured by the peroxidation of β-carotene. Antioxidants can reduce the extent of β-carotene destruction by neutralizing the linoleate-free radical and other free radicals formed in the system (Vongadow et al., 1997). Accordingly, the absorbance decreased rapidly in reaction mixtures without extracts, whereas in the presence of extracts, the reaction mixtures retained their color and thus, absorbance for a longer time (Fig. 5).

The IC_{50} of different extracts in inhibiting the bleaching of β-carotene suggest that bleaching is moderately inhibited by extracts of aerial parts of both plants in three different solvents with linear regression (R^2) ranging from 0.8471 to 0.9991 with $p < 0.003$, which is extremely significant if compared with standard propyl galate solution (Table 1). This is because the presence of different antioxidant molecules in extracts might be responsible for the inhibition of β-carotene destruction by neutralizing the effect of linoleate-free radical and other free radicals formed in the system (Cheung et al., 2003).

3.5. In vivo anti-inflammatory assay

The acute toxicity results showed that etahnolic extracts of *C. siamea* and *C. javanica* was safe up to a dose of 2000 mg/kg

Please cite this article in press as: Mehta, J.P. et al., *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of *Cassia* speciesCassia species –>. Arabian Journal of Chemistry (2013), http://dx.doi.org/10.1016/j.arabjc.2013.06.010

body weight. Based on acute toxicity data, two different dosages 200 and 400 mg/kg (p.o.) were selected for *in vivo* anti-inflammatory study and results are depicted in Table 2. The results of aerial parts were compared with positive control of diclofenac sodium (NSAID) (5 mg/kg p.o.) and were authenticated by statistical analysis.

The ethanolic extracts of aerial parts of *C. siamea* and *C. javanica* (200 and 400 mg/kg p.o.) reduced the swelling induced in the rats paw by carrageenan on each of the five occasions. It was measured and it was observed to be dose dependent. Bark extracts of *C. javanica* in ethanol showed a significant impact on the reduction of edema rate at a lower dosage that is 200 mg/kg (p.o.) on each of the four occasions with $p < 0.001$, which is considered as extremely significant. The anti-inflammatory activity is demonstrated here for the first time by using aerial parts. However, the *Cassia* genus presents in other anti-inflammatory species such as *Cassia italica* (Jain et al., 1997). It was observed from results of percentage inhibition of paw edema that leaf extracts of *C. siamea* and *C. javanica* in ethanol have a very significant impact on the reduction of paw edema if compared with standard positive control and it is shown in Fig. 6. This study confirmed the anti-inflammatory properties of ethanolic extracts of aerial parts of *C. siamea* and *C. javanaica*. The traditional methods of extraction are promising since the yield extracts are devoid of cytotoxicity or acute toxicity on rats and therefore, in the present study we have given focus to traditional methods for the extraction of active ingredients from aerial parts of both plants.

4. Conclusion

In conclusion, four major families of compounds were present in the aerial parts of both plants and may play an important role in the anti-inflammatory and antioxidant properties. These families are triterpenes flavonoids, anthraquinones and phytosterols. A representative gas chromatograph is depicted in Fig. 7 clearly suggest that as many as 14 known and lesser known components were separated from the aerial parts of *C. siamea* and *C. javanica* using universal mass detector. Out of 14 components 5-Amino-1H-[1,2,4] triazole-3-carboxylic acid butyl ester, 9,19-Cyclolanost-23-ene-3,25-diol, (3.beta.,23E) and 7-hydroxy-6-methoxy-2H-1-benzopyran-2-one are not normally reported to be present in the aerial parts of *C. siamea* and *C. javanica* plants.

Our results are encouraging as the sources for NSAID drug are natural and hence, it may not have side effects like synthetic molecules.

The traditional methods of extraction are promising since the yield extracts are devoid of acute toxicity on rats. In order to go toward a valuation of these traditional extracts in the form of phyto-drugs, follow-up studies must be carried out in particular on their stability and the evolution of the haematological and biochemical parameters.

Acknowledgements

Authors are thankful to the Department of Biochemistry, Junagadh Agricultural University, Junagadh-362001, India for their kind support in carrying out some basic analysis of our crude extracts and also thankful to Department of Life Sciences, Maharaja Krishankumarsinhji, Bhavnagar University, Bhavnagar-364022-India.

References

Abatan, M.O., 1990. A note on the anti-inflammatory action of plants of some *Cassia* species. Fitoterapia 61, 336–338.

Ahn, B.Z., Degen, U., Lienjayetz, C., Pachaly, P., Zymalkowski, F., 1978. Constituents of *Cassia siamea*. Arch. Pharmacol. 311, 569–578.

Baumann, J., Wurn, G., Bruchlausen, V., 2002. Prostaglandin synthetase inhibiting O_2^- radical scavenging properties of some flavonoids and related phenolic compounds. Naunyn Schmiedebergs Arch. Pharmacol. 308, R27.

Boakye-Gyasi, E., Woode, E., Ainooson, G.K., Obiri, D.D., Ansah, C., Duwejua, M., Donkoh, A., 2008. Anti-Inflammatoryandanti-pyreticeffectsofanethanolic extract of Palisota hirsuta K. Schum-roots. Afr. J. Pharm. Pharmacol. 2, 191–199.

Chen, P.N., Chu, S.C., Chiou, H.L., Kuo, W.H., Chiang, C.L., Hsieh, Y.S., 2006. Mulberry anthocyanins, cyaniding 3-rutinoside and cyaniding-3-glucoside, exhibited and inhibitory effect on the migration and invasion of a human lung cancer cell line. Cancer Lett. 28, 248–259.

Cheung, L.M., Cheung, P.C.K., Ooi, V.E.C., 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 81, 249–255.

De las Heras, B., Slowing, K., Benedi, J., Carretero, E., Ortega, T., Toledo, C., Bermejo, P., Iglesias, I., Abad, M.J., Ǵomez-Serranillos, P., Liso, P.A., Villar, A., Chiriboga, X., 1998. Antiinflammatory and antioxidant activity of plants used in traditional medicine in Ecuador. J. Ethnopharmacol. 61, 161–166.

Desmarchelier, C., Ciccia, G., Coussio, J., 2000. Recent advances in the search for antioxidant activity in South American plants. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry. Elsevier, Amsterdam, pp. 343–367 (vol. 22).

Duh, P.D., Tu, Y.Y., Yen, G.C., 1990. Antioxidant activity of water extract of harn jyur (Chrysanthemum morifolium Ramat). Leben-Wissen Technol. 32, 269–277.

Ecobichon, D.J., 1997. The Basis of Toxicology Testing. CRC Press, New York.

Elujoba, A.A., Ajulo, O.O., Iweibo, G.O., 1989. Chemical and biological analyses of Nigerian *Cassia* species for laxative activity. J. Pharm. Biomed. Anal. 7, 1453–1457.

Gbeassor, M., Kossou, Y., Amegbo, K., De Souza, C., Koumaglo, K., Denke, A., 1989. Antimalarial effects of eight African medicinal plants. J. Ethnopharmacol. 25, 115–118.

Geronikaki, A.A., Gavalas, A.M., 2006. Antioxidants and anti-inflammatory diseases: synthetic and natural antioxidants with anti-inflammatory activity. Combin. Chem. High Throughput Screen 9, 425–442.

Ghose, M.N., 2005. Fundamental of Experimental Pharmacology, thrid ed. Hilton and Company, Kolkata.

Gordon, M.H., 1990. The mechanisms of the antioxidant action *in-vitro*. In: Hudson, B.J.F. (Ed.), Food Antioxidants. Elsevier, London/New York, pp. 1–18.

Gutteridge, J.M., 1995. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin. Chem. 41, 1819–1828.

Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeldt, P.W., 1998. High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46, 1887–1892.

Hallivel, B., Guttreridge, B.J., 1989. Free Radicals in Biology and Medicine, second ed. Oxford University Press, Oxford, UK.

Halliwell, B., 1997. Antioxidants and human disease: a general introduction. Nutr. Rev. 55, 44–52.

Huang, B., Ke, H.B., He, J.S., Ban, X.Q., Zeng, H., Wang, Y.W., 2011. Extracts of Halenia elliptica exhibit antioxidant properties *in vitro* and *in vivo*. Food Chem. Toxicol. 49, 185–190.

Jain, S.C., Jain, R., Sharma, R.A., Capasso, F., 1997. Pharmacological investigation of *Cassia* italica. J. Ethnopharmacol. 58, 135–142.

Jayaprakasha, G.K., Singh, R.P., Sakariah, K.K., 2001. Antioxidant activity of grape seeds (Vitis vinifera) extracts on peroxidation models *in vitro*. Food Chem. 73, 285–290.

Kaur, G., Alam, M., Jabbar, Z., Javed, K., Athar, M., 2006. Evaluation of antioxidant activity of *Cassia siamea* flowers. J. Ethnopharmacol. 108, 340–348.

Kaur, P., Arora, S., 2011. Superoxide anion radical scavenging activity of *Cassia siamea* and *Cassia javanica*. Med. Chem. Res. 20, 9–15.

Kobus, J., Flaczyk, E., Siger, A., Nogala-Kalucka, M., Korczak, J., Pegg, R., 2009. Phenolic compounds and antioxidant activity of extracts of Ginkgo leaves. Eur. J. Lipid Sci. Technol. 111, 1150–1160.

Lee, H.J., Hyun, E.A., Yoon, W.J., Kim, B.H., Rhee, M.H., Kang, H.K., Cho, J.Y., Yoo, E.S., 2006. *In vitro* anti-inflammatory and anti-oxidative effects of *Cinnamomum camphora extracts*. J. Ethnopharmacol. 103, 208–216.

Li, R.W., Myers, S.P., Leach, D.N., Lin, G.D., Leach, G., 2003. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. J. Ethnopharmacol. 85, 25–32.

Lin, C.C., Huang, P.C., 2002. Antioxidant and hepatoprotective effects of *Acathopanax senticosus*. Phytother. Res. 14, 489–494.

Mbatchi, S.F., Mbatchi, B., Banzouzi, J.T., Bansimba, T., Nsonde-Ntandou, G.F., Ouamba, J.-M., Berry, A., Benoit-Vica, L.F., 2006. *In vitro* antiplasmodial activity of 18 plants used in Congo Brazzaville traditional medicine. J. Ethnopharmacol. 104, 168–174.

Menichini, F., Tundis, R., Bonesi, M., Loizzo, M.R., Conforti, F., Statti, G., De Cindio, B., Houghton, P.J., Menichini, F., 2009. The influence of fruit ripening on the phytochemical content and biological activity of Capsicum chinense Jacq. cv Habanero. Food Chem. 114, 553–560.

Moreno, M.I.N., Isla, M.I., Sampietro, A.R., 2000. Comparison of the free radical scavenging activity of propolis from several regions of Argentina. J. Ethnopharmacol. 71, 109–114.

Mueller, M., Hobiger, S., Jungbauer, A., 2010. Anti-inflammatory activity of extracts from fruits, herbs and spices. Food Chem. 122, 987–996.

Nsonde Ntandou, G.F., Banzouzi, J.T., Mbatchia, B., Elion-Itou, R.D.G., Etou-Ossibi, A.W., Ramos, S., Benoit-Vicale, F., Abena, A.A., Ouamba, J.M., 2010. Analgesic and anti -inflammatory effects of *Cassia siamea* Lam. Stem bark extracts. J. Ethnopharmacol. 127, 108–111.

Nsonde-Ntandou, G.F., Ndounga, M., Ouamba, J.M., Gbeassor, M., Etou-Ossibi, A., Ntoumi, F., Abena, A.A., 2005. Ethnobotanical survey, chemical screening and effective treatment of certain plants used in traditional medicine to treat malaria in Brazzaville. Phytothérapie 1, 13–18.

Perry, E.K., Pickering, A.T., Wang, W.W., Houghton, P.J., Perru, N.S., 1999. Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy. J. Pharm. Pharmacol. 51, 527–534.

Qian, J.Y., Liu, D., Huang, A.G., 2004. The efficiency of flavonoids in polar extracts of Lycium chinese Mill fruits as free radical scavenger. Food Chem. 87, 283–288.

Ramarathnam, N., Osawa, T., Ochi, H., Kawakishi, S., 1995. The contribution of plant food antioxidants to human health. Trends Food Sci. Technol. 6, 75–82.

Repetto, M.G., Llesuy, S.F., 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Braz. J. Med. Biol. Res. 35, 523–534.

Roginsky, V., 2003. Chain breaking antioxidant activity of natural polyphenols as determined during the chain oxidation of methyl linoleate in Triton X-100 micelles. Arch. Biochem. Biophys. 414, 261–270.

Ruch, R.J., Cheng, S.J., Klaunig, J.E., 1989. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. J. Carcinog. 10, 1003–1008.

Sabir, S.M., Rocha, J.B.T., 2008. Antioxidant and hepatoprotective activity of aqueous extract of Solanum fastigiatum (false Jurubeba) against paracetamol-induced liver damage in mice. J. Ethnopharmacol. 120, 226–232.

Saleem, A., Husheem, M., Harkonen, P., Pihlaja, K., 2002. Inhibition of cancer cell growth by crude extract and the phenolics of Terminalia chebula retz. Fruit. J. Ethnopharmacol. 81, 327–336.

Schinella, G.R., Tournier, H.A., Prieto, J.M., Mordujovich de Buschiazzo, P., Rios, J.L., 2002. Antioxidant activity of anti-inflammatory plant extracts. Life Sci. 70, 1023–1033.

Sekretar, S., Schmidt, S., Vajdak, M., Zahradnikova, L., Annus, J., 2004. Anti-oxidative and antimicrobial effects of some natural extracts in Lard. Czech. J. Food Sci. 22, 215–218.

Shon, M.Y., Kim, T.H., Sung, N.J., 2003. Antioxidants and free radical scavenging activity of Phellinus baumii (Phellinus of Hymenochaetaceae) extracts. Food Chem. 82, 593–597.

Sosa, S., Balic, M.J., Arvigo, R., Esposito, R.G., Pizza, C., Altinier, G., Tubaro, A., 2002. Screening of the topical anti-inflammatory activity of some Central American plants. J. Ethnopharmacol. 81, 211–215.

Sukma, M., Chaichantipyuth, C., Murakami, Y., Tohda, M., Matsumoto, K., Watanabe, H., 2002. CNS inhibitory effects of barakol, a constituent of *Cassia siamea* Lamk. J. Ethnopharmacol. 83, 87–94.

Tachakittirungrod, S., Okonogi, S., Chowwanapoonpohn, S., 2007. Study on antioxidant activity of certain plants in Thailand: mechanism of antioxidant action of guava leaf extract. Food Chem. 103, 381–388.

Thongsaard, W., Chainakul, S., Bennett, G.W., Marsden, C.A., 2001. Determination of barakol extracted from *Cassia siamea* by HPLC with electrochemical detection. J. Pharm. Biomed. Anal. 25, 853–859.

VanderJagt, T.J., Ghattas, R., Vanderjagt, D.J., Crossey, M., Glew, R.H., 2002. Comparison of the total antioxidant content of 30 widely used medicinal plants of New Mexico. Life Sci. 70, 1035–1040.

Vongadow, A., Joubert, E., Hansmann, C.F., 1997. Comparison of antioxidant activity of aspalathin with that of other plant phenols of Rooibosd tea (Aspalathon linearis), a-tocopherol, BHT, and BHA. J. Agric. Food Chem. 45, 632–638.

Winrow, V.R., Winyard, P.G., Morris, C.J., Blake, D.R., 1993. Free radicals in inflammation: second messengers and mediators of tissue destruction. Br. Med. Bull. 49, 506–522.

Winter, C.A., Risley, E.A., Nuss, G.W., 1962. Carrageenan-induced edema in the hind paw of rat as an assay for anti-inflammatory activity. Proc. Soc. Exp. Biol. Med. 111, 544–547.

Yonathan, M., Asres, K., Assefa, A., Bucar, F., 2006. *In vivo* anti-inflammatory and anti-nociceptive activities of Cheilanthes farinose. J. Ethnopharmacol. 108, 462–470.

Yoon, J., Baek, S.J., 2005. Molecular targets of dietary polyphenols with anti-inflammatory properties. Yonsei Med. J. 46, 585–596.

You-dim, K.A., McDonald, J., Kalt, W., Joseph, J.A., 2002. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults. J. Nutr. Biochem. 13, 282–288.

Please cite this article in press as: Mehta, J.P. et al., *In-vitro* antioxidant and *in-vivo* anti-inflammatory activities of aerial parts of *Cassia* speciesCassia species –>. Arabian Journal of Chemistry (2013), http://dx.doi.org/10.1016/j.arabjc.2013.06.010