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Review

Phytochemical constituents of *Cassia fistula*

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Since the advent of modern drug treatments, traditional medicine has greatly receded in occidental societies. Moreover, only a limited number of medicinal plants have received detailed scientific scrutiny thereby prompting the World Health Organisation to recommend that this area be comprehensively investigated. *Cassia fistula* Linn is used extensively in various parts of the world against a wide range of ailments, the synergistic action of its metabolite production being most probably responsible for the plant's beneficial effects. This paper reviews the primary and secondary metabolite composition of vegetative and reproductive plant parts and cell cultures thereby derived, with emphasis on potent phenolic antioxidants such as anthraquinones, flavonoids and flavan-3-ol derivatives. This paper also appraises the antioxidant and free radical propensities of plant parts and cell culture extracts. The data so far generated clearly sets the basis for a clearer understanding of the phytochemistry of the plant and derived cultures and opens the possibility of the potential utilization of the phenolic rich extracts from medicinal plants in food system or as prophylactics in nutritional/food supplement programs. Thus traditional medicinal plant- derived antioxidants may protect against a number of diseases and reduce oxidation processes in food systems. In order to establish this, it is imperative to measure the markers of baseline oxidative stress particularly in human health and disease and examine how they are affected by supplementation with pure compounds or complex plant extracts from the traditional medicinal plants.

Key words: *Cassia fistula,* medicinal plant, callus cultures, anthraquinones, flavonoids, flavan-3-ols, antioxidant, free radical scavenging, oil-in-water emulsion system.

INTRODUCTION

Native to India, the Amazon and Sri Lanka*, Cassia fistula* Linn., a semi-wild Indian Labernum also known as the Golden Shower, has become extensively diffused in various countries including Mauritius, India, South Africa, Mexico, China, West Indies, East Africa and Brazil as an `ornamental tree for its beautiful bunches of yellow flowers. Recognized by the British Pharmacopoeia (Mukhopadhyay et al., 1998), *C. fistula*, a member of the Leguminosae family, is widely used for its medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin and a tonic (Satyavati and Sharma, 1989) and has been reported to treat many other intestinal disorders like healing ulcers (Biswas et al., 1973; Kirtikar and Basu, 1975). The plant has a high therapeutic value and it exerts an antipyretic and analgesic effect (Patel et al., 1965). Besides, it has been found to exhibit antinflammatory and hypoglycaemic activity (Datta and Kumar, 1985). In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in the treatment of haematemesis, pruritus, leucoderm and diabetes has been suggested (Alam et al., 1990; Asolkar et al., 1992). *C. fistula* extract is used as an anti-periodic agent and in the treatment of rheumatism (Biswas et al., 1973; Kirtikar and Basu, 1975) and the leaf extract is also indicated for its anti-tussive and wound healing properties

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Abbreviations: 2,4-D, 2,4 dichlorophenoxyacetic acid; FRAP, ferric reducing antioxidant power; TEAC, Trolox equivalent antioxidant capacity; DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHT, Butylated hydroxytoluene, NF-κB, Nuclear factor- κB; AP-1, Activator protein-1; MS , Murashige and Skoog.

(Bhakta et al., 1998a,b). It has been concluded that plant parts could be used as a therapeutic agent in the treatment of hypercholesterolaemia partially due to their fibre and mucilage content (El-Saadany et al., 1991). There are reports indicating its antibacterial activity against a wide spectrum of bacteria namely *Escherichia Coli, Bacillus mycides, Bacillus subtilis, Mycobacterium smegmatis*, *Klebsiella aerogenes, Pseudomonas aerogenes* and *Proteus vulgaris* (Perumal et al., 1998). Antitumor (Gupta et al., 2000), hepatoprotective (Bhakta et al., 1999), antifertility (Yadav and Jain, 1999), antioxidant (Chaminda et al., 2001; Siddhuraju et al., 2002; Luximon-Ramma et al., 2002) properties of *C. fistula* as well as its actions on the central nervous systems (Mazumdar et al., 1998) and inhibitory effect on leukotriene biosynthesis (Sunil Kumar and Müller, 1998) have been suggested. Besides its pharmacological uses, the plant extract is also recommended as a pest and disease control agents in India (Jaipal et al., 1983; Sharma and Basandrai, 1999; Raja et al., 2000). Thus *C. fistula* is well anchored in its traditional uses and has now found widespread acceptance across the world.

PHYTOCHEMICALS OF *C. FISTULA IN VIVO*

A majority of the ascribed biological effects of *C. fistula* extracts have been attributed to their primary and secondary metabolite composition. Primary metabolite analysis has essentially been focussed on the seed, pollen, fruit, leaf and pod. The seeds are rich in glycerides with linoleic, oleic, stearic and palmitic acids as major fatty acids together with traces of caprylic and myristic acids (Abu Sayeed et al., 1999). It has been reported that the stembark of *C. fistula* is also a potential source of lupeol, ß-sitosterol and hexacosanol (Sen and Shukia, 1968). With the view to exploring alternative and effective sources of protein, Niranjan and Katiyar (1979) noted a percentage of 31% of crude proteins comprising mainly globulin and albumin in the wild seeds. The same study emphasized that the seeds were rich sources of cephalin and lecithin phospholipids and contained 11.8% carbohydrates. In an earlier study it was reported that one of the major carbohydrates in the seeds was galactomannan consisting of 8 different types of sugar moieties (Lal and Gupta, 1976). A detailed biochemical analysis of the flower's pollen, suspected to play a significant allergenic role, showed a protein composition of 12% with appreciable amounts of free amino acids such as phenylalanine, methionine, glutamic acid and proline. Carbohydrate, lipid and free amino acid contents were of the order of 11.75, 12 and 1.42%, respectively (Mondal et al., 1998). The edible fruit tissue of the Indian laburnum fruit was reported to be a rich source of potassium, calcium, iron and manganese than fruits like apple, apricot, peach, pear and orange (Barthakur et al., 1995). The same authors reported 15.3, 13 and 7.8% of aspartic acid, glutamic acid and lysine of the total amino acids, respectively, in the pulp. The protein (19.94%) and carbohydrate (26.30%) contents (Vasi and Kalintha, 1980) are indicative of the potential of the fruit to be an important source of nutrients and energy. Apolar compounds including 5-nonatetracontanone, 2-hentriacontanone, triacontane, 16-hentriacontanol and β-sitosterol along with an oil (probably an isoprenoid compound) showing antibacterial activity have also been isolated in *C. fistula* pods (Misra et al. 1996).

C. fistula plant organs are known to be an important source of secondary metabolites, notably phenolic compounds (Table 1). Fistucacidin, an optically inactive leucoanthocyanidin (3,4,7,8,4'-pentahydroxyflavan) was first extracted from the heartwood (Padmanabha Rao and Venkateswarlu, 1965). The presence of kaempferol (Figure 1) and a proanthocyanidin whose structure has been established as a leucopelargonidin tetramer having a free glycol in the acetone extract of the flower has been documented (Narayanan and Seshadri, 1972). Morimoto et al. (1988) isolated (-)-epiafzelechin 3-O-B-D-glucopyranoside, 7 biflavonoids and two triflavonoids together with (-)-epiafzelechin, (-)-epicatechin and procyanidin B-2 (Figure 1) from the leaves. Proanthocyanidins containing flavan-3-ol (epiafzelechin and epicatechin) units with an abnormal 2S-configuration have also been observed in pods together with the common flavan-3-ols and proanthocyanidins like catechin, epicatechin, procyanidin B-2 and epiafzelechin (Kashiwada et al., 1996). Vaishnav and Gupta (1996) showed the presence of rhamnetin 3-O-gentibioside (Figure 1) in *C. fistula* roots. An investigation that characterized the contents of total phenolics, proanthocyanidin and flavonoid in vegetative and reproductive organs of *C. fistula* found in Mauritius and harvested at different stages, showed that among the vegetative organs, the young and old leaves showed the highest total phenolic, flavonoid and proanthocyanidin contents (Luximon-Ramma et al.. 2002). The study indicated that the highest levels of phenolics were contained in the pod, which coincidentally is the harvest stage and organ recommended by the Pharmacopoeias (Luximon-Ramma et al., 2002) (Table 2).

Although Liptak and Szentgali (1937) indicated the presence of a yellow substance that became red on addition of alkali in the pulp of the fruit, it was not until 1952 that this substance named rhein (Figure 2), was identified as a major anthraquinone derivative in the pulp of *C. fistula* (Modi and Khorana, 1952). The compound 1,8-dihydroxy-3-anthraquinone carboxylic acid was isolated from the pods (Modi and Khorana, 1952). This was confirmed by Kapadia and Khorana (1966) when it was shown that free rhein are complexed with sennidin-like compounds in *C. fistula* pods. A bianthraquinone glycoside, fistulin, together with kaempferol and rhein have been isolated from ethanolic extracts of *C. fistula* flowers (Kumar et al., 1966). Free rhein, rhein glucoside

Table 1. Secondary metabolites in *C. fistula* plant parts.

Secondary metabolite	Plant part/organ	References
Fistucacidin (3,4,7,8,4'-pentahydroxyflavan	Heartwood	Padmanabha Rao, 1965
Oxyanthraquinone, dihydroxyanthraquinone	Bark	Rani et al., 1998
(-) epiafzelechin, (-) epiafzelechin-3-O-glucoside, (-) epicatechin, procyanidin B2, biflavonoids, triflavonoids, rhein, rhein glucoside, sennoside A, sennoside B, chrysophanol, physcion,	Leaves	Kashiwada et al., 1996; Kaji et al., 1968; Kashiwada et al., 1996; Mahesh et al., 1984.
Kaempferol, leucopelargonidin tetramer (with free glycol unit), rhein, fistulin, alkaloids, triterpenes	Flowers	Narayanan and Seshadri, 1972; Kumar et al., 1966; Guri-Fakim et al., 1994
Rhein, volatile oil, waxy and resinous derivatives	Fruit pulp	Liptak and Szentagali, 1937
Fistulic acid, 3-formyl-1-hydroxy-8-methoxy anthaquinone, 3B-hydroxy-17-norpimar-8(9)-en-15-one	Pods	Misra et al., 1997
Chrysophanol	Seeds	Khana and Chandra, 1984
Rhamnetin-3-O-gentiobioside	Roots	Vaishnav and Gupta, 1996
Proanthocyanidins, flavonoids	Vegetative organs: young leaves, old leaves, twigs, bark Reproductive organs: flower bud, flower, pod	Luximon-Ramma et al., 2002

Table 2. Polyphenolic contents and antioxidant activities (FRAP and TEAC values) in in vivo vegetative and reproductive organs of the total extracts of C. fistula and in vitro callus extract harvested after 35 days of culture.

Plant Organ/Extract	Total phenolics[a]	Total flavonoids[b]	Total proanthocyanidins[c]	TEAC[d]	FRAP[e]
Young leaves	11	9	2	98	51
Old leaves	12	6	3	102	64
Twigs	9	3	2	93	64
Bark	13	4	2	157	95
Flower buds	44	8	20	893	380
Flowers	32	8	14	453	317
Pods	54	14	21	992	811
Callus (Day 35)	31.4	-	17.6	853	655

[a]mg gallic acid equivalent/ g dry weight.
[b]mg quercetin equivalent/ g dry weight.
[c]mg cyanidin chloride equivalent/ g dry weight.
[d]µmol/g dry weight.
[e]in units of µmol Fe(II)/g dry weight.

and sennosides A and B (Figure 2) occur in the leaves. While rhein and its glucoside could be isolated in pure state, others are obtained as mixtures (Kaji et al., 1968). In later studies the characterisation of sennosides and their contents in leaves and pods on seasonal basis have been substantiated (Lohar et al. 1975; Asseleih et al., 1990; Chowdhury et al., 1996; Dutta and De, 1998). The structure of a new colouring matter, fistulic acid (Figure 2), an anthraquinone acid, was elucidated from the pods (Agrawal et al., 1972). In a detailed study on the *Cassia* genus, the anthraquinones chrysophanol, rhein and physcion (Figure 2) were identified in the leaves (Mahesh et al., 1984; Khanna and Chandra, 1996). The first report on the isolation and characterisation of 3-formyl-1-hydroxy-8-methoxy anthraquinone was made in pods (Rani and Kalidhar, 1998). The presence of anthraquinones seems to be closely linked to the plant's physiological processes. A proposed picture (Fairbairn

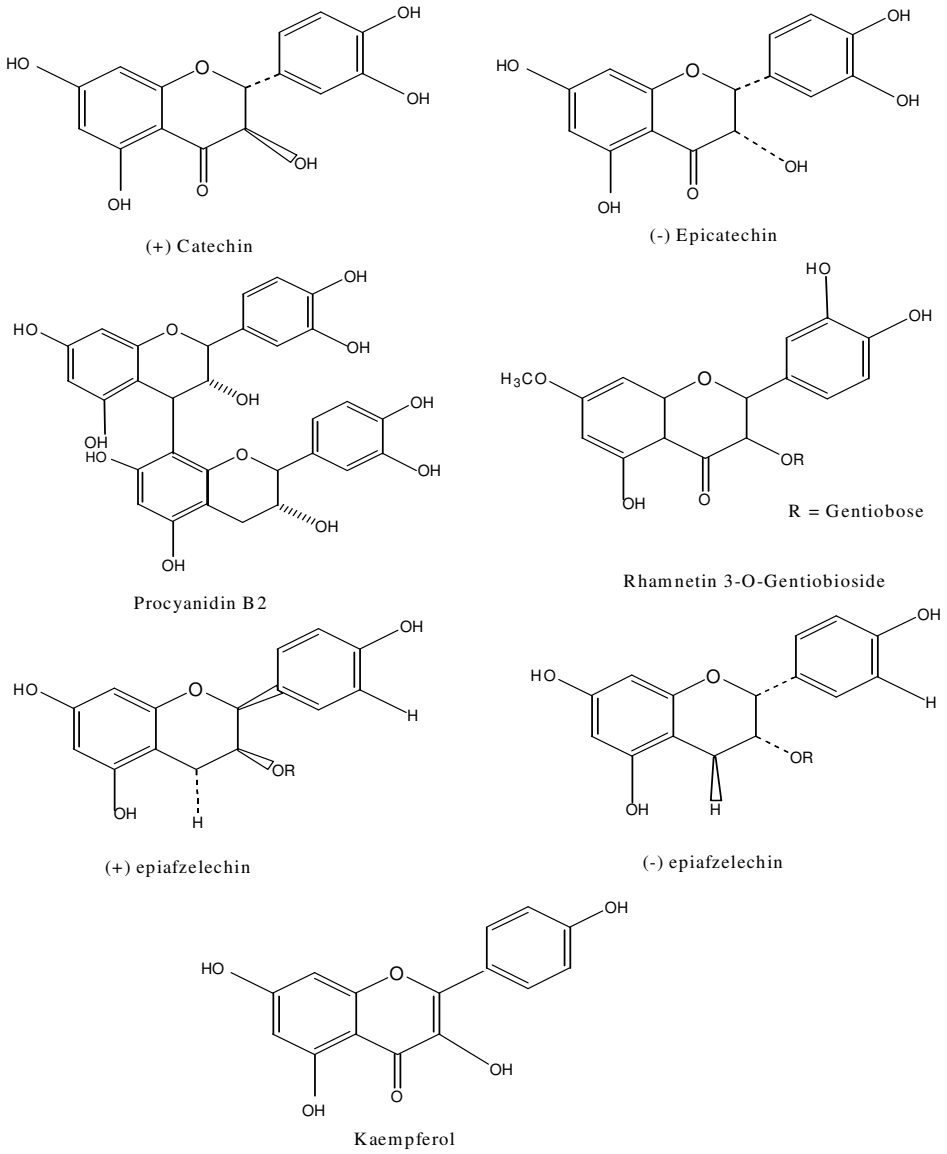
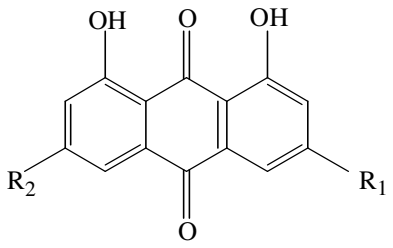


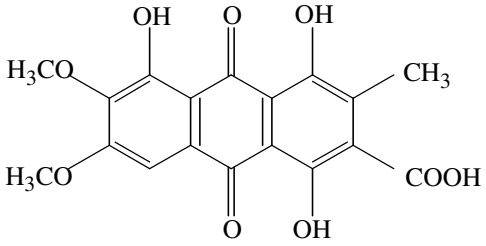
Figure 1. Chemical structures of main flavanol and flavonol derivatives in *C. fistula.*

Table 3. Metabolite production in *C. fistula* tissue cultures.

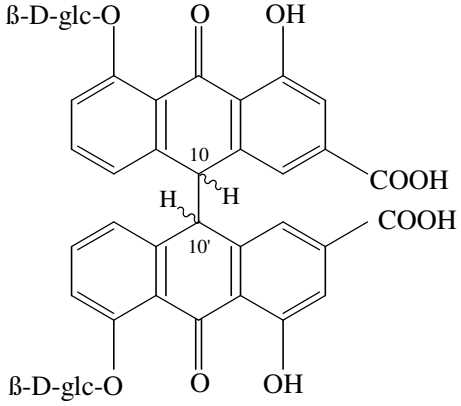
Medium/culture	Observations	Reference
MS/callus	Polyphenol synthesis influenced by sucrose, 2-4-D, kinetin and giberillic acid in presence and absence of light	Subbaiah et al., 1974
MS/callus	Polyphenol production determined by auxin concentration of medium, accumulation of phenolics restricted to most rapid growth phase, highest peroxidase activity in phenolic rich tissues	Shah et al., 1976
MS/callus	Infection of callus by Ranikhet disease virus (RDV) induced the production of an interferon-like antiviral factor	Babbar and Madan, 1981
MS/callus	Production of Chrysophanol and physcion	Ahuja et al., 1988
MS/callus	Incorporation of L-phenylalanine in culture media induced polyphenolic enhancement correlated with an increased growth, increase in proanthocyanidin synthesis	Neergheen et al., 2002
MS/callus	Effect of magnetic field induced an increase in polyphenolic production and biomass production, increase in proanthocyanidins synthesis	Chisolm and Steinberg, 2000



	R_1	R_2
Chrysophanol	CH_3	H
Rhein	COOH	H
Emodin	CH_3	OH
Physcion	CH_3	OCH_3



Fistulic Acid



	C-10	C-10’
Sennoside A	R	R
Sennoside B	R	S

Figure 2. Chemical structures of main anthraquinone derivatives in *C. fistula*.

and Shrestha, 1967) is that shortly after photosynthetic activity begins in the seedling, anthraquinones and their glycosides are formed. These accumulate rapidly in the developing leaf and are then possibly translocated to the developing ovaries and fruits. There, they accumulate in comparatively high concentrations but become depleted as the seeds develop, more particularly at later stages.

Besides phenolics and their derivatives, a certain amount of alkaloids have also been reported in the flowers (Asseleih et al., 1990), while traces of triterpenes have been observed in both flowers and fruits (Gurib-Fakim et al., 1994). Misra et al. (1997) isolated a new diterpene, 3B-hydroxy-17-norpimar-8(9)-en-15-one from the pods of *C. fistula*.

PHYTOCHEMICALS IN *C. FISTULA* CELL CULTURES

Over the past years there has been increasing interest in the use of plant tissue cultures as industrial biosynthetic sources of useful secondary products. The production of secondary metabolites by plant cell cultures is extensively documented (Zenk et al., 1977; Banthorpe, 1994; Dornenburg and Knorr, 1997; Bourgaud et al., 2001; Bahorun et al., 2002; Ramachandra Rao and Ravishankar, 2002). However, data are more limited on the production of secondary compounds in *C. fistula* cultures (Table 3). One of the first studies was on the influence of sucrose, 2-4-D, kinetin and giberillic acid under different light conditions on the production of polyphenolics in *C. fistula* callus cultures (Subbaiah et al., 1974). It was observed that sucrose at higher levels enhanced polyphenol synthesis and 2,4-D and kinetin nhibited the production when applied at supraoptimal levels. While the initiation of polyphenol production was strongly influenced by 2,4-D and kinetin, its accumulation was mainly dependent on the carbohydrate level in the medium. An investigation of the hormonal effect on polyphenol accumulation in *C. fistula* callus cultures (Shah et al., 1976) have indicated that the initiation of polyphenol synthesis was largely due to the auxin concentration in the medium and that the accumulation of phenolics was essentially restricted to the exponential growth phase. The tissue peroxidase activity was higher in phenolic rich calli at an optimal 2,4-D concentration of 0.2 mg/L. Ahuja et al. (1988) reported the presence of chrysophanol and physcion as the main anthraquinone constituents in undifferentiated *C. fistula* callus cultures. These two compounds also occur in *C. angustifolia*, *C. obtusifolia, C. tora, C. torosa* and *C. senna* tissue cultures (Ahuja et al., 1988) and *in vivo* (Mahesh et al., 1984). Interestingly, use of a colorimetric method based on Borntrager's reaction has enabled us to estimate that *C. fistula* callus cultures contain 170 mg/100 g fresh weight of anthraquinones consisting of 8 main anthraquinone derivatives including fistulic acid.

Addition to the culture media of appropriate precursors or related compounds sometimes stimulates secondary metabolite production. As far as *C. fistula* cell cultures are concerned, a study conducted by Babbar and Maddan (1981) on callus cultures infected by an animal virus (Ranikhet Disease Virus, RDV) showed a surprising production of interferon-like antiviral factors. The incorporation of L-phenylalanine in the culture media of 3-year-old calli initiated from young leaves of *C. fistula* induced a polyphenolic production enhancement correlated with an increased growth (Neergheen and Bahorun, 2002). Maximum increase in growth and optimum total phenol (5,044 g/100 g dry weight gallic acid equivalent) and proanthocyanidins synthesis (3,417 g/100 g dry weight cyanidin chloride equivalent) were obtained using 100 mg/L L-phenylalanine. Under that condition, within a subculture period, an overall 23% biomass increase, 21% total phenolic rise and a 61% total proanthocyanidins production enhancement were observed compared to control calli. Furthermore polyphenolic production in both phenylalanine treated and control cultures were higher than that of young leaves of *C. fistula* from which the cultures were derived. The appreciable amount of total phenols synthesized by these cultures were very close to that present in *C. fistula* pods, which had previously been shown to be the richest organs with 54 mg/g dry weight of total phenols (Table 2) (Luximon-Ramma et al., 2002). An almost 2 fold increase in proanthocyanidin level was observed in phenylalanine treated calli in comparison with the pods which contained an average amount of 21 mg/g dry weight (Table 2). The precursor feeding experiment used in this study has proved to be a low cost and an efficient means leading to the optimization of callus growth, total phenolics and more particularly flavan-3-ol derivatives which are known to be potential antioxidant prophylactic agents (Neergheen and Bahorun, 2002; Rechner et al., 2002).

One other original strategy used to increase the production of polyphenolic compounds in *C. fistula* callus cultures was the application of magnetic field. This technique is encompassed in a new rapidly developing branch of biophysics, namely magnetobiophysics. Studies were conducted for the first time on magnetotropism (effect of an imposed magnetic field on plant life) by Savostin in 1930 who observed 100% increase in the rate of elongation of wheat seedlings under the influence of a magnetic field. There have been several reports showing increased seedling growth, seed vigour and crop yield when the dormant seeds of corn, beans, barley and wheat were exposed to a magnetic field (Bhatnagar and Deb, 1977; Pittman, 1977; Gusta et al., 1978; Gubbels, 1982; Kavi, 1983; Pietruszeweskis, 1993). In a more recent work, the effects of imposed continuous North and South magnetic fields (1 milli Tesla) on the growth of callus cultures of *C. fistula* and their polyphenolics contents were investigated (Aukhez et al., 2001). It was shown that north pole magnetic field exposed callus cultures showed a more or less similar growth pattern as control cultures but with an average percentage decrease of 24 and 23% for total phenolic and proanthocyanidins production, respectively. Despite a lower biomass production, south pole treated calli produced 24% and 31% more total phenolics and proanthocyanidins, respectively, than non treated cultures.

ANTI-TUMOUR ACTIVITY, ANTIOXIDANT AND FREE RADICAL SCAVENGING POTENTIALS OF *C. FISTULA* EXTRACTS

Over the past years, there has been an exponential growth in the number of reports indicating that excessive free radical production and lipid peroxidation are actively

involved in the pathogenesis of a wide number of diseases including atherosclerosis (Chisolm and Steinberg, 2000) cardiac and cerebral ischemia (Lopez and Casado, 2001), carcinogenesis (Galati and O'Brian, 2004), neurodegenerative disorders (Gerlach et al., 2003), diabetic pregnancy (Viana et al., 2000), rheumatic disorders (Hänninen et al., 2000), and play a major role in the ageing process (Golden et al., 2002) and DNA damages (Termini, 2000). There is overwhelming evidence showing that natural antioxidants play a role in wellness, health maintenance, and the prevention of the above chronic and degenerative diseases. Furthermore, autooxidation of fats and oils in processed foods may also be prevented by the use of natural oxidation inhibitors or antioxidants (Adegoke et al., 1998; Reddy et al., 2005). Consequently, there has been a growing interest in the potential health-promoting properties of phytochemicals of plant origin. Special attention has been given to vitamin E, vitamin C and more particularly to phenolic derivatives including anthraquinones, xanthones, phenolic acids, phenolic diterpenes, flavonoids, catechins, proanthocyanidins and anthocyanins. These substances have also been reported to exhibit biological effects including antibacterial, anti-viral, anti-inflammatory, antithrombotic, antimutagenic, anticarcinogenic, antiageing and vasodilatory actions (Middleton and Kandaswami, 1994; Bravo, 1998; Caroll et al., 1998; De Bruyne et al., 1999; Di Carlo et al., 1999; Duthie et al., 2000; Middleton et al., 2000; Ferguson, 2001). The bioactive actions ascribed to polyphenols are almost certainly mediated partly by their free radical scavenging, antioxidant and metal complexing actions (Rice-Evans et al., 1996; Bahorun et al., 2004), their ability to decrease localized oxygen concentration and to decompose peroxides (Aruoma, 1996), their interaction with several enzymes (Laughton et al., 1991; Chang et al., 1993; Di Carlo et al., 1999) and to synergistic effects with other antioxidants (Filipe et al., 2001). However, other possible mechanisms of actions include their binding to carcinogens, their ability to inhibit phase I and induce phase II carcinogen metabolising enzymes and their potential to modulate signal transduction pathways (Ferguson, 2001). They may prevent tumour development by inducing tumor cell apoptosis by inhibiting DNA topoisomerase II and p53 downregulation or by causing mitochondrial toxicity, which initiates mitochondrial apoptosis (Galati et al., 2000; Birt et al., 2001; Ren et al., 2003; Galati and O'Brian, 2004). 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 tumour volume (Gupta et al., 2000). Further research need to be undertaken to ascertain the mechanisms of DNA protection, hence delineating the antimutagenic and anti-carcinogenic effects of *C. fistula* extracts.

The phenolic-rich *in vivo* plant organs and *in vitro* cell culture extracts of *C. fistula* makes the latter species a potential antioxidant prophylactic candidate warranting a detailed evaluation of its antioxidant and free radical scavenging properties. It is becoming clear that for *in vitro* and *in vivo* characterization of antioxidant propensities, no one method can give a comprehensive prediction of antioxidant efficacy. So use of more than one method is recommended and there should be greater caution in extrapolating the *in vitro* data (Frankel, 1993; Frankel and Meyer, 2000; Aruoma, 1996, 2003). FRAP, TEAC, antioxidant activities based on thiocyanate method, oil and water emulsion systems, lipid peroxidation, superoxide scavenging, DPPH radical scavenging assays have been used to assess the free radical scavenging capacities and the reducing potentials of the antioxidant constituents of *C. fistula* extracts (Chaminda et al., 2001; Siddhuraju et al., 2002; Luximon-Ramma et al., 2002). TEAC and FRAP assays show that in *C. fistula* vegetative organs, the bark had the highest antioxidant potential followed by the old leaves, the young leaves and the twigs (bark>old leaves>young leaves>twigs). The reproductive organs had the highest antioxidant propensities compared to the vegetative parts (Table 2). In the reproductive organs, the order of efficacy was pods>flower buds>flowers. The antioxidant capacities of the reproductive organs parallel their contents of high levels of catechins, oligomeric and polymeric proanthocyanidins (Luximon-Ramma et al. 2002). Further studies based on lipid peroxidation, DPPH radical and $O_2^{\cdot-}$ scavenging (Siddhuraju et al., 2002) also underline the antioxidant capacity of *C. fistula* extracts. Here too the antioxidant capacities were directly related to the total phenolic contents of the extracts where the presence of established antioxidants such as xanthones, flavans, flavonols and di-anthraquinones are potentially responsible for the activities (Yen et al., 2000). In a study highlighting the antiradical and antilipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardioprotection, *C. fistula* root extracts were shown to exhibit potent DPPH radical scavenging and lipid peroxidation actions, and to afford a 30% protection against deoxyribose damage (Chaminda et al., 2001).

Callus cultures of *C. fistula* initiated from young leaves show that maximum antioxidant and free radical scavenging actions (FRAP and TEAC) were obtained when phenolic production was optimum after 35 days of culture (Table 2). Here again, a substantive contribution of proanthocyanidins to the overall antioxidant efficacy is observed as indicated by the high correlation coefficients (TEAC: $r = 0.843$; FRAP: $r = 0.948$). The antioxidant efficacy of this callus extract was investigated in a stripped 30% oil-in-water emulsion system. The extract was effective in preventing hydroperoxide formation in

Table 4. The effect of a *C. fistula* plant extract and BHT on the oxidation of stripped sunflower oil emulsions during storage at 30°C.

Sample	Mean peroxide value (meq/kg)			
	Day 0	Day 2	Day 5	Day 7
Control	9.87 ± 2.063	45.76 ± 1.320	155.6 ± 8.585	219.47 ± 11.887
BHT (0.02%)	0.92 ± 0.016	37.4 ± 0.001	5.14 ± 0.660	5.6 ± 1.320
C. fistula callus extract (0.1%)	0.95 ± 0.003	3.27 ± 0.660	5.14 ± 0.660	5.79 ± 1.057

Control: without antioxidant or plant extract.
BHT: Butylated hydroxytoluene.

Table 5. The effect of a *C. fistula* plant extract and BHT on the oxidation of stripped sunflower oil emulsions during storage at 30°C.

Sample	Conjugated diene value (g/100 g)			
	Day 0	Day 2	Day 5	Day 7
Control	0.28± 0.032	0.42 ± 0.014	2.35 ± 0.1	3.39 (0.111
BHT (0.02%)	0.15 (0.006	0.24 (0.015	0.34 (0.032	0.43 (0.034
C. fistula callus extract (0.1%)	0.15 (0.003	0.21 (0.028	0.45 (0.030	0.47 (0.003

Control: without antioxidant or plant extract.
BHT: Butylated hydroxytoluene.

the emulsion prepared with stripped sunflower oil, during 7 days storage at 30ºC (Table 3). The antioxidant activity of 0.1% extract was comparable to that of 0.02% BHT. Conjugated diene determinations for the emulsions containing BHT and *C. fistula* extract remained stable during 7 days storage at 30ºC when compared to control emulsions (Table 4). These data clearly indicate that *C. fistula* extract was as effective as BHT in preventing formation of conjugated dienes (Table 5). These observations may be largely explained in part on the basis of interfacial phenomena having a pronounced effect on oxidative stability of lipid systems. The mechanism of this process might be related to the affinities of the antioxidants toward the water-oil interfaces in emulsions (Frankel et al., 1994). Accordingly, hydrophobic antioxidants located in the oil and the oil-water interface were proposed to be more active than hydrophilic antioxidants, which are partitioned into the aqueous phase and are not able to adequately protect lipids in the water-oil interface. Such behavior can also be explained by the “polar paradox” model of Porter (1993), which suggests that polar antioxidants are more active in non-polar lipids, whereas non-polar antioxidants with high hydrophilic-lipophilic balance (HLB) are more potent in polar lipid emulsions of high surface-to-volume ratio. Based on the foregoing, the antioxidant activity in this model food emulsion system can be ascribed to the additive effect of the relatively less polar phenolics due to increased concentration at the oil-water interface. Interestingly, a similar study on the stability of unstripped sunflower oil emulsions during storage at 30°C has revealed that antioxidant components in an aqueous *Crataegus monogyna* (Hawthorn) extract could be (-)-epicatechin, procyanidin B2, chlorogenic acid, flavonol glycosides and anthocyanins (Bahorun et al., 2003). It thus appears that the important criteria for high-antioxidant *C. fistula* extracts are high total phenolics with high proanthocyanidin content probably necessitating the oxidative potency of flavonoids and other phenolic derivatives to add up to the synergistic overall antioxidant effect of the extracts.

CONCLUSION

C. fistula is an important source of naturally occurring bioactive compounds. Polyphenolics abundantly present in both *in vivo* and *in vitro* extracts may prove to be very important, non-toxic chemopreventive agents against various oxidative stresses. This paper also highlights the importance of plant cell cultures as an alternative system for the production of biologically active metabolites, indicating that *C. fistula* callus cultures could represent an interesting supply of potential antioxidative and chemoprotective components like flavonoids and anthraquinones. The work so far achieved on *C. fistula* also sets the basis of future studies on the effects of its polyphenol containing extracts, which may have important practical implications for food quality, and their potential utilization in multicomponent biological/food systems.

It is becoming clear that traditional systems of medicine have become a topic of global importance. Current estimates from the World Health Organisation suggest that, in many developing countries, a large proportion of populations rely heavily on traditional practices. Herbal medicines or phytomedicines have often maintained popularity for historical and cultural reasons.

Concurrently, many people in developed countries are turning to alternative or complementary therapies, including medicinal herbs. Many of these plants are tropical plants emanating from less developed countries. *C. fistula* could be one of them particularly because of its low toxicity and its widespread use for its multiple medicinal effects. Although traditional medicines help to fill the gaps in modern health care, it is of utmost importance to evaluate the safety and bioefficacy of the extracts used. The phytochemical compositions and biological activities need to be well understood and the data gathered so far for *C. fistula* and its extracts aim at achieving that goal. Plant extract and traditional medicine-derived antioxidants may be important in protecting against a number of diseases and reducing oxidation processes in food systems. In order to establish this it is imperative to measure the markers of baseline oxidative stress particularly in human health and disease and examine how they are affected by use of the pure compounds or complex plant extracts from the traditional medicinal plants. It has become important to evaluate how the bioactive components in plant extracts affect cellular signaling processes and modulate oxidative stress mediated responses (Aruoma et al., 2003; Farombi, 2003). These are properties distinct from the classical antioxidant actions. Integrating traditional medicine into the health system require both demonstration of clinical and biochemical evidence of efficacy.

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