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Phytochemical and Biological Potential of *Cassia tora* Linn.

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Authors' contributions

This article has been prepared in collaboration between all authors. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Cassia tora Linn. (Caesalpinaceae) is a semi-wild annual herb grown widely in different places of south-east Asia including India, Northern Australia and Americas. This plant species is well known for having potential in traditional medicine practices for the treatment of a variety of disorders and ailments ranging from simple cough, hypertension to diabetes. Recent scientific investigation reveals its phytochemical as well as biological potential. *C. tora* has been proven to be medicinally effective for having antimicrobial, antiantioxidant, antihypertensive, antidiabetic and antimutagenic activities, just to name a few. This paper encompasses a comprehensive review on phytochemical and biological aspects of *Cassia tora* L.

Keywords: Cassia tora; anthraquinone; naphthopyrone; antioxidant; antimutagenic; hepatoprotective; antidiabetic; antiplasmodial.

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1. INTRODUCTION

Plant-based traditional remedies are well known for their potential health benefits in a variety of human disorders or ailments since time immemorial. According to the World Health Organisation (WHO), it has been estimated that 80% of the world's population is still dependant on traditional medicines for maintaining their health and combating various diseases [1-3]. Besides, 56% of world's populations in the rural areas rely chiefly on herbal medicine and supplementation for their primary health care needs [4]. Today, microbial infections, hypertension, diabetes, malaria and cancer are the common health problems in rural communities throughout the world. A huge number of traditionally important medicinal plants have been known to be biologically effective against these diseases [5]. One such potential plant is *Cassia tora* Linn*.,* which is a small semi-wild annual herb Fig. 1 belonging to the family Caesalpinaceae. The plant is native to south-east Asia, Northern Australia, Africa and Latin America. *C. tora* is commonly found in waste grounds and secondary forest, and grows wild along roadsides throughout the tropical and subtropical plains of India, Korea, China, Japan, Philippines, Vietnam, Indonesia and North America [6]. Many *Cassia* species having beautiful yellow flowers are grown as ornamental plants throughout the Thailand and India [7]. *C. tora* is commonly known as *Foetida cassia*, *Sickle senna*, *Sickle*, *Coffee pod*, *Chakvad*, *Ktanta*, *Charota* and *Chakramarda* in India. *C. tora* is a stout, erect, smooth, half-woody annual herb which is one meter or less in height. Leaves are alternate, even-pinnate, 1-2 cm long; leaflets in 2-4 pairs, oblong-ovate to obovate, 3-4 cm long, obtuse, attenuate at base; stipules linear lanceolate, falling early. The leaves are furnished with glands on the main rachis between the leaflets. The flowers are grouped 1-2 in leaf axils, showy, nearly regular, 5-merous, yellow; 10 stamens with 7 fertile and 3 abortive anthers in pairs, in the axils of the upper leaves about 1.5 cm across. Fruits are linear pods, 4-angled, up to 10 cm long with thick (3-4 mm) margins [8]. The seeds are flattened in the same direction as the pods are composed of hull (27%), endosperm (32%) and germ (41%) [9-10].

Fig. 1. *Cassia tora* L. plant

In ancient system of medicine, *C. tora* was used to treat a variety of medical complications like bronchitis, constipation, conjunctivitis, ulcer, hypertension, hypercholestrolemic, liver damage [11-12], fungal infection, diabetes [13-14] edema, glaucoma, nyctalopia, ringworm, skin diseases [15] plaque and caries [16]. According to Ayurveda, the leaves and seeds are acrid, anthelmintic, antiperiodic, cardiotonic, laxative and liver tonic [17-18]. Decoctions of different parts of *C. tora* showed analgesic, anticonvulsant, antipyretic, antibacterial,

antifungal, antihelmintic, diuretic, expectorant, laxative, purgative and are also useful in the treatment of glaucoma, hypertension, skin disease, ringworm, leprosy, flatulence, colic, dyspepsia, constipation, cough, itch [19]. The herb has been reported for its usefulness in the form of infusions and tinctures for treating skin diseases like psoriasis, leprosy etc. [20-22]. The use of *C. tora* seeds in beverages such as coffee, tea etc. is very popular in some parts of the world [23-24]. In anticipation of its ethnomedicinal claims documented from different regions of the world, scientific investigation have been carried out in order to explore the potential of this plant species as herbal medicine in a diverse range of therapeutic areas.

In the present study, a review of the published literature on *C. tora* has been carried out with an aim to acquire comprehensive information on phytochemical and biological potential of this medicinally useful plant species. A good number of articles published in various peer-reviewed journals (cited in PubMed, BioMed etc.) relevant to the topic were obtained from their respective web-based sources, and arranged thereafter in a systematic manner for the purpose of their inclusion/rejection in the article to be prepared. Any article relevant to the selected topic was referred and included in list, unless otherwise rejected. However, the present review article comprises a comprehensive enumeration of phytopharmacological importance of *Cassia tora* L.

2. METHODS

2.1 Phytochemical Importance

During the past few decades, extensive studies have been carried out on isolation and characterization of chemical constituents Fig. 2 of various parts of *Cassia tora* L. Wilkinson *et al.* (1969) [25] reported that leaves and stems of *C. tora* contain fatty acid esters like palmitate (20.8%), stearate (6.4%), oleate (5.7%), linoleate (13.1%), linolenate (26.0%) and other shorter or longer homologs of straight or branched chain compounds (C_{34}). Emodin, tricontan-1-ol, stigmasterol, *β*-sitosterol-*β*-D-glucoside, succinic acid, *d*-tartaric acid, uridine, quercitrin, isoquercitrin [26] and ononitol monohydrate have also been reported from the leaves of this plant [27]. A water soluble complex polysaccharide consisting of D-galactose, D-glucose, D-mannose and D-xylose in the molar ratio of 2:2:7:1 have been isolated from the defatted seeds. The polysaccharide has a highly branched structure with α-linked D-galactopyranose and D-xylopyranose as end residues into which 1, 4 and 1, 4, 6-linked-*β*-D-mannopyranose and glucopyranose units of the main chain are likely to be attached [28]. Besides, leaves and seeds contain anthraquinones like chrysophanol, physcion, emodin, rhein, euphol, basseol, obtusifolin, obtusin, chryso-obtusin, rubrofusarin, aurantio-obtusin, chrysophonic acid-9-anthrone including their glycosides and naphthopyrones like rubrofusarin, orrubrofusarin, naptho-alpha-pyrone-toralactune, cassiaside including their glycosides [8,26,29,30]. 9-[β-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl)oxy]-10-hydroxy-7-methoxy-3-methyl-1*H*-naphtho[2,3*c*]pyran-1-one, 6-[(*α*-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranosyl)oxy]-rubrofusarin and rubrofusarin-6-*β*-gentiobioside are the most predominant naphthopyrone glycosides reported so far [26,31]. Phenolic glycosides like rubrofusarin triglucoside, nor-rubrofusarin gentiobioside, demethylflavasperone gentiobioside, torachrysone gentiobioside, torachrysone tetraglucoside torachrysone apioglucoside [32,33] torachrysone (8-*O*-[*β*-D-glucopyranosyl(1→3)-*O*-*β*-D-glucopyranosyl(1→6)-*O*-*β*-D-glucopyranoside]), toralactone (9-*O*-[*β*-D-glucopyranosyl-(1→3)-*O*-*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranoside]) [34] and alaterinin (2-*O*-*β*-D-glycopyranoise) have been isolated from the seeds [35] From the butanol-soluble part of the

methanol extract of the seeds, 2-acetyl-3-*O*-*β*-D-apiofuranosyloxy-8-*O*-*β*-D-lucopyranosyloxy-1, 6-dimethoxynaphthalene (cassitoroside) [36] and from the roasted seeds, 10-[(*β*-D-glucopyranosyl-(1→6)-*O*-*β*-D-glucopyranosyl)oxy]-5-hydroxy-8-methoxy-2-methyl-4*H*-l-naphtho[1,2-*b*]pyran-4-one (isorubrofusarin gentiobioside) along with isorubrofusarin gentiobioside, alaternin and adenosine have also been isolated and identified [37] Two more anthraquinones such as 1-desmethylaurantio-obtusin and 1-desmethylchryso-obtusin have been isolated from the seeds [38]. Nine compounds were isolated from the roots of *C. tora*. Their structures were elucidated as α-amyrin octacosanoate, palmitic acid, chrysophanol, beta-sitosterol, physcion, chrysophanol-8-methyl ether, betulone, beta-sitosterol-beta-D-glucoside and ononitol [39,40]. *Cassia tora* gum obtained from the seeds is comprised of at least 75% high molecular weight (approximately 200,000-300,000) polysaccharide consisting primarily of a linear chain of 1,4-*β*-D-mannopyranose units with 1,6-linked *α*-D-galactopyranose units. The ratio of mannose to galactose is about 5:1. The composition of saccharides is mannose (77.2-78.9%), galactose (15.7-14.7%) and glucose (7.1-6.3%) [41,42]. Leaves of *cassia tora* were analyzed to determine proximate nutrient content, amino acid composition and some selected mineral elements. Data obtained for proximate analysis showed that crude protein and crude fibre contents were found to be 11.63% and 27.07% respectively. Seventeen amino acids were also found in varying proportions in the plant. Results of the elemental analysis showed that the leaves of *C. tora* contain following minerals (in g/100g) Ca 3.52, Fe 0.22, Na 0.10, Mg 0.86, Zn 0.04, Mn 0.10, Co 0.02, K 0.76 etc. [43].

It is now well understood that *C. tora* L. serves as a potential source of many bioactive phytoconstituents, as well as essential nutritional components and minerals. Because of the fact that phytochemicals that have been reported from different parts of *C. tora* are biologically active, the health benefits of different plant preparations and/or extracts are attributed to be due to the presence such active components. Moreover, the traditional claims of this plant as described in the introduction section could be validated on the ground of results obtained from bio-efficacy studies of different medicinal components isolated from *C. tora*. The details of their importance towards biological activities are summarized wherever necessary in the following section.

2.2 Biological Potential

2.2.1 Antioxidant activity

Herbal antioxidants are capable of neutralizing free radicals and thus play a major role in the prevention of certain diseases such as cancer, cataracts, cerebral pathologies and rheumatoid arthritis [44]. The antioxidant property of different extracts of *C. tora* is described herein. Ethanol extract showed 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and its IC_{50} was found to be 8.0 µg/mL as reported by Anh et al. [45]. Ethyl acetate fraction of methanol extract exhibited more antioxidant potency and was found to be more effective in protecting low dense lipoprotein against oxidation in a concentration-dependent manner [46]. Alaternin, cassiaside and rubrofusarin gentiobioside isolated from the seeds of *C. tora* showed DPPH radical scavenging activity, among them alaternin was more potent than the others [47]. Methanol and aqueous extracts of seeds produced strong antioxidant effect on peroxidation of linoleic acid. Antioxidant activity of methanol extract was stronger than *α*-tocopherol but weaker than that of butylated hydroxyanisole. Methanol extract, on further fractionation, one fraction possessed significant antioxidant activity which was equal to that of butylated hydroxyanisole and much greater than *α*-tocopherol and showed 85.8% inhibition on peroxidation of linoleic acid. Subfraction of this obtained from methanol-water

eluent solvent exhibited much stronger antioxidant activity and was identified as 1,3,8-trihydroxy-6-methyl-9,10-anthracenedion on the basis of UV, HPLC, IR, MS and NMR analysis [48]. The antioxidant properties of aqueous extract of seeds has been significantly affected by different degree of roasting. The aqueous extract of unroasted seeds showed 94% inhibition of peroxidation of linoleic acid at a dose of 0.2 mg/mL, which was higher than that of *α*-tocopherol (82%). Aqueous extracts were prepared from roasted seeds at 175°C for 5 min and at 200°C for 5 min exhibited 83% and 82% inhibition of linoleic acid peroxidation, respectively. The decreased activities have been observed with longer roasting time or higher roasting temperature. The IC_{50} of aqueous extract in liposomal oxidation (induced by the Fenton reaction) was 0.41 mg/mL, which was higher than that of *α*-tocopherol [(IC_{50}) 0.55 mg/mL]. Aqueous extract of unroasted seeds also exhibited good antioxidant activity in enzymatic and non-enzymatic microsomal oxidative systems (Yen and Chuang, 2000). Aqueous extracts from seeds treated with different degree of roasting also produced effects on the oxidative damage to deoxyribose, DNA and DNA base *In vitro.* It was found that extract alone induced a slight strand breaking of DNA. In the presence of Fe^{3+}/H_2O_2, the strand breaking of DNA was accelerated at a concentration of 2μg/mL. However, at still higher concentrations (>5 μg/mL) decreased activity was observed. Extract also accelerated the oxidation of deoxyribose induced by Fe^{3+}-EDTA/H_2O_2 at a concentration of 0.2 mg/mL but inhibited the oxidation of deoxyribose induced by Fe^{3+}-EDTA/H_2O_2/ascorbic acid. Furthermore, the oxidation of 2'-deoxyguanosine (2'-dG) was accelerated to form 8-OH-2'-dG induced by Fe^{3+}-EDTA/H_2O_2. The pro-oxidant action on the oxidation of 2'-dG was in the order of unroasted > roasted at 150°C > roasted at 200°C > roasted at 250°C [49]. Yen et al. [50] showed that aqueous extract also modulated the oxidative DNA damage in human lymphocytes induced by hydrogen peroxide. Seed extract showed positive results for folin-ciocalteu, ferric-reducing power, lipid peroxidation of linoleate with fentrons reagents and DPPH radical scavenging assays. Phenolic compounds of seeds showed antioxidant activities of 47.41±2.17 mg rutin equivalents per gram [51]. In another study, both seed extract and its major components norrubrofusarin-6-*β*-D-glycoside showed a positive action against hydrogen peroxide induced cytotoxicity in Chinese Hamster Lung Cells. They showed a strong anticlastrogenicity against micromycin C-induced micronuclai reticulocytes formation in mouse peripheral blood [52]. *C. tora* plant extract also exhibited a potent peroxynitrite scavenging activity; further analysis identified that phenolic active components, alaternin and nor-rubrofusarin glucoside were considered to be potent peroxynitrite scavengers [53]. Gluco-aurantioobtusin isolated from methanol seeds extract showed marked inhibitory and scavenging activities against trolox and peroxynitril with an IC_{50} value of 49.64±0.37 μM (positive control; trolox: 26.07±1.05 μM) for total reactive oxygen species generation and 4.60±1.12 μM (positive control; penicillamine: 0.24±0.04 μM) for peroxynitril [54]. In a study with methanol extract of leaves by Rejiya et al. [55], it has been observed that a correlation existed between concentration of extract and percentage inhibition of free radical reducing power and inhibition of lipid peroxidation with IC_{50} value 180 μg/mL for nitrogen oxide scavenging and 82.6, 192.3 μg/mL for inhibition of lipid peroxidation in rat's liver and brain, respectively.

2.2.2 Antifungal, antibacterial and antishigellosis activity

Several studies reported that *C. tora* possess antifungal activities. Besides chrysophanic acid and other hydroxyanthraquinone derivatives, the major antifungal compound identified was chrysophanic acid-9-anthrone. The compound inhibited *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum* and *Geotrichum candidum* in broth in the presence of L-ascorbic acid at 100 μg/mL as antioxidant [56]. In a study conducted by Ahmad *et al*. [57], ethanol extract of seeds showed positive results for *C. albicans* with clear

inhibition zones of 8.8 mm diameter at 25 mg/mL and 11.1 mm diameter at 30 mg/mL. Ethanol extract of leaves and aqueous extracts of seeds and leaves showed no distinct inhibition zone. Ethanol extract of leaves inhibited the *M. canis* growth but ethanol extract of seeds showed no effects on *M. canis* growth. The ethanol and aqueous extracts of seeds and leaves were found not effective against the growth of *A. fumigates*. Phongpaichit et al. [58] observed that methanol extracts of leaves possesses antifungal activities on pathogenic fungi *M. gypseum, T. rubrum* and *Penicillium marneffei* with the 50% inhibition concentration (IC_{50}) of hyphal growth at 1.2, 1.8 and 1.8 mg/mL, respectively. Leaves extract also affected *M. gypseum* conidial germination. In another study, methanol leaves extract retarded the growth of four pathogenic fungi in order of *T. rubrum>T. mentagraphytes> A. fumigatus> M. Canis* [59]. The fungicidal activities of *C. tora* seeds extract and their active principles were compared with three commercially available anthraquinones. The response varied with the pathogen tested at concentration of 1 mg/mL, the chloroform fraction of seeds extract showed a strong fungicidal activity against *Botrytis cinerea*, *B. graminis, Phytophthora infestans* and *Rhizoctonia solani*. Emodin, physcion and rhein, isolated from the chloroform fraction using chromatographic techniques, showed strong and moderate fungicidal activities against *B. cinerea, E. graminis, P. infestans* and *R. solani*. Furthermore, aloe-emodin showed strong and moderate fungicidal activities against *B. cinerea* and *R. solani*, respectively but did not inhibit the growth of *E. graminis, P. infestans, P. recondita* and *Pyricularia grisea*. A little or no activity was observed for anthraquinone and anthraquinone-2-carboxylic acid [60].

Aqueous extract of seeds inhibited *Staphyllococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* at concentration of 100 µg/mL, 200 µg/mL and 250 µg/mL, respectively but did not inhibit the growth of *Bacillus subtilis*. Ethanol extract inhibited *B. subtilis* at 64 µg/mL, where as it was not effective against the *S. aureus*, *P. aeruginosa* and *E. coli*. Methanol extract was effective against both the *S. aureus* and *E. coli* at the concentration of 64 mg/mL but was ineffective against *P. areuginosa* and *E. coli.* Aqueous extracts of seeds exhibited better antibacterial activity compared to their petroleum ether, methanol and ethanol extracts in terms of zone of inhibition against all the four organisms [61]. In another study, ethanol extracts of crude and fermented leaves showed equal or nearly equal antibacterial activity against *S. aureus, B. subtilis*, *E. coli* and *P. aeruginosa* [62]. The effects of phenolic glycosides of seeds were tested on growth of *E. coli* K12, *P. aeruginosa* PAO1 and some strains of *S. aureus*. Among them, torachrysone, toralactone, aloe-emodin, rhein and emodin showed noticeable antibacterial effects on four strains of methicillin-resistant *S. aureus* with a minimum inhibitory concentration of 2-64 mg/mL. On the other hand, the phenolic compounds did not show strong antibacterial effects on *E. coli* and *P. aeruginosa* [34].

Menghani and Soni [63] observed that petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water extracts of *C. tora* exhibited antibacterial potency against *Proteus vulgaris, Pseudomonas aeruginosa, Trichophyton rubrum, Streptococcus aureus, Escherichia coli, Klebsiella pneumoniae, Shigella sonnei* etc. and antifungal activity against *Candida albicans, Aspergillus niger* and *Aspergillus flavus* etc. The antibacterial activity of leaf extract was also studied by Dave et al. [64]. Panda and Ray [65] tested the water, ethanol, petroleum ether, benzene and chloroform extracts of *C. tora* against *Candida albicans* MTCC 854 and *Cryptococcus neoformans* by disc diffusion method. Study results revealed that the ethanol extract of *C. tora* showed best antifungal activity with zone of inhibition of 16 mm against *Candida albicans*, while benzene extract showed better zone of inhibition i.e., 13 mm against *Cryptococcus neoformans*. Ethanolic and aqueous extracts obtained from the leaves of *C. tora* were investigated for their antibacterial activity in zone of

inhibition at 0.15 mg and 0.31 mg doses respectively. Both the extracts exhibited significant antibacterial activity when compared with the standard reference drug, ciprofloxacin [66].

Ethyl acetate fraction of the root extract showed antishigellosis activity. The ethyl acetate fraction of the petroleum ether extract of dry powdered roots showed maximum zone of inhibition ranging from 23-25 mm at the concentration of 200µg/disc. The minimum inhibitory concentration of ethyl acetate, ethanol and chloroform extracts was found from 32-64 µg/mL, whereas, methanol and petroleum extract showed values from 128-512 µg/mL [67].

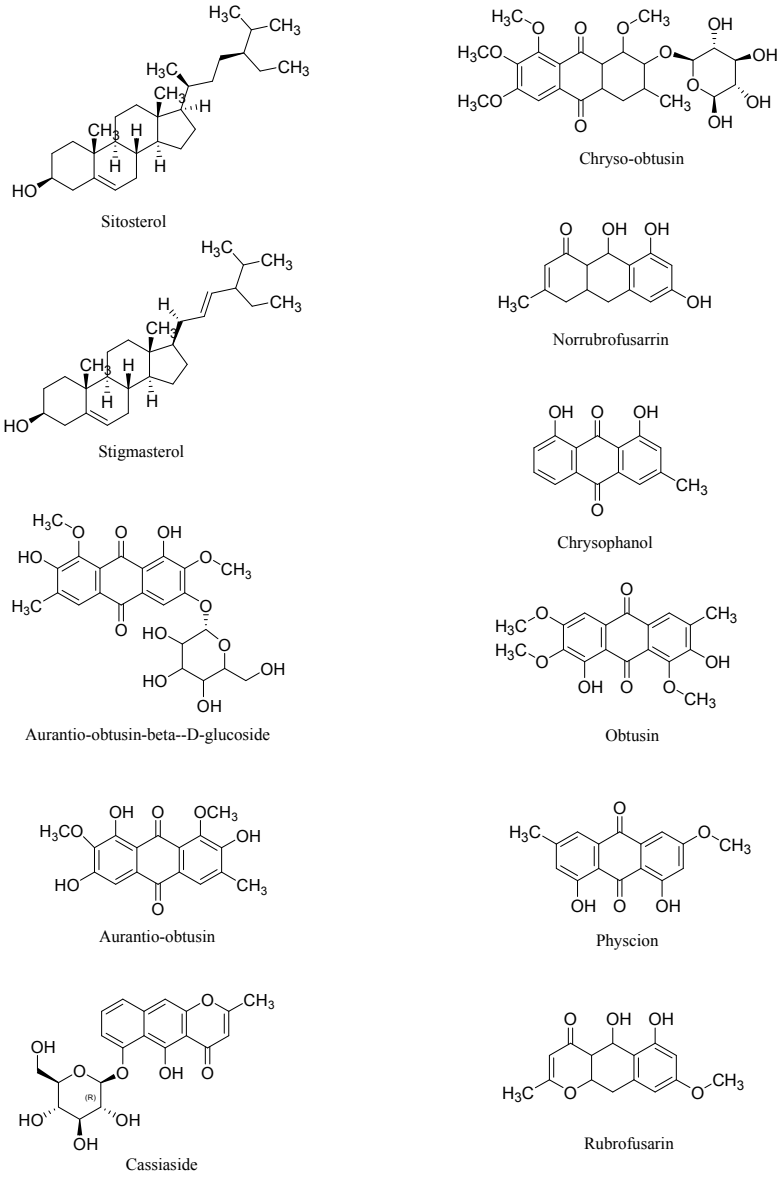

Fig. 2. Structures of some biologically important chemical constituents of *Cassia tora* L.

2.2.3 Antihyperlipidemic, hepatoprotective and hypotensive activity

Ethanol extract of seeds and its fractions were investigated by Patil et al. [68] on triton induced hyperlipidemic profile in albino rats. Extract and its ether soluble and aqueous soluble fraction reduced serum level of total cholesterol by 42.07, 40.77 and 71.25%, respectively. On the other hand, it increased the serum high dense lipoprotein cholesterol level by 6.72, 17.20 and 19.18%, respectively, decreased triglyceride level by 26.84, 35.74 and 38.46%, respectively and reduced low dense lipoprotein cholesterol level by 69.25, 72.06 and 76.12%, respectively.

The effects of soluble fiber on lipid metabolism were also investigated in male Sprague Dawley rats, fed with one of three experimental diets, a normal diet, a high cholesterol diet or a high cholesterol diet with 5% soluble fiber, for 5 weeks. The serum concentration of total cholesterol in rats fed soluble fiber was decreased by 27% but the serum high density lipoprotein cholesterol level was increased by 37%. Liver total cholesterol and triglyceride levels were reduced 50% in rats fed with soluble fiber diet [69]. Fiber supplement also reduced serum lipid level in Korean type II diabetic patients. *C. tora* fiber supplement was given to seven men and eight women, 57.1±2.9 years old type II diabetic subjects with instructions to take two packs per day for 2 months. Fiber supplement showed significant changes in the level of serum total cholesterol, serum triglycerides and low density lipoprotein-cholesterol. Serum total cholesterol was moderately decreased in the treated group compared with the age and gender matched placebo group, as the ratio of apolipoprotein B to apolipoprotein A1. Levels of serum triglycerides and low-density lipoprotein-cholesterol tended to decrease more in the fiber supplemented group than in the placebo group getting maltodextrin [70]. Tablets composed of *C. tora, Radix Polygori Multiflori*, *Polygonatum sibicum*, *Lycium barbarum* and *Crataegus pinnatifida* was used in the treatment of 130 cases of hyperlipemia and achieved an effective rate of 87.0% in lowering serum cholesterol and 80.8% in lowering triglyceride [71].

Reports of several studies indicated the hepatoprotective activity of *C. tora* leaves. In an experimental model, carbon tetrachloride induced liver injury in albino rats was reduced by oral administration of extract at the dose of 100-600 mg/kg body weight. It increased the level of liver marker enzymes SGPT, SGOT, ALP and the production of malanoaldehyde, an end product of peroxidation of lipid without affecting the levels of Vitamin E and glutathione [72]. Dhanasekaran et al. [73] reported that ononitol monohydrate isolated from leaves possess similar hepatoprotective activity on carbon tetrachloride induced hepatotoxicity in wister rats at a oral dose of 20 mg/kg body weight. Serum and histopathological studies revealed that ononitol monohydrate decreased the levels of serum transaminase, lipid peroxidation and TNF-α, but increased the levels of antioxidant and hepatic glutathione enzyme activities without any adverse effect on liver tissues.

Hepatoprotective activity of petroleum ether, methanol and aqueous extracts of *C. tora* seeds was studied in paracetamol induced hepatotoxic animal model. Aqueous and methanol extract significantly decreased the levels of serum bilirubin, SGOT, SGPT and SALP as compared to the hepatotoxic group. Histopathological study of aqueous and methanol extracts treated groups showed minimal inflammation with moderate portal triaditis and their normal lobular architecture. Whilst, hepatotoxic group showed serious hepatocytic necrosis and inflammation along with portal triaditis in the centrilobular region. Moreover, the effects of these extracts were comparable with that of standard drug, silymarin [74].

Aqueous and methanol seed extracts elicited hypotensive effects on anesthetized rats, as investigated by several workers. The ability of the extract to reduce blood pressure was significantly observed in vagotomized rats and induced effects were greatly antagonized in rats whose sympathetic nervous systems were interrupted by transection of the spinal cord [75,76] In another experiment with pentobarbital anesthetized rats reported by Chan *et al*. (1976), [77] the medial portion of the medullary reticular formation was attributed to be responsible for the hypotensive effects of the extract. Methanol extract from both raw and roasted seeds inhibited angiotensin converting enzyme to more than 50% in experimental model at a concentration of 163.93 µg/mL, and further marker compound was identified as gluco-aurantioobtusin anthraquinon with an IC_{50} value of 30.24±0.20 µM. Conversely, aurantioobtusin obtained from the acid hydrolysis of gluco-aurantioobtusin, showed no activity [55].

2.2.4 Antidiabetic activity

In a study with streptozotocin-induced diabetic rats [78], it was observed that *C. tora* seeds lowered plasma glucose level and this lowering effect was as acute as seen even at the first week of feeding. In another investigation, butanol fraction of methanol extract of seeds was studied on postprandial glucose control and insulin secretion from the pancreas in the normal and streptozotocin induced diabetic male *Sprague-Dawley* rats. The postprandial glucose control was monitored during a 240 min period using a maltose loading test. Extract lowered postprandial glucose level from 30 min to 180 min in normal rats (20 mg per 100 g body weight per day) as compared to the control rats without extract (P<0.05). In diabetic rats, those levels in the treated group seemed to be decreased during the 30 to 180 min, but only the glucose level at 30 min showed significant difference compared to that of the control group. Moreover, the extract delayed the peak time of the glucose rise in both normal and diabetic rats. Oral administration of fraction to the diabetic rats for 5 days, 12 hr fasting serum glucose level was decreased in the diabetic rats (P<0.05). Degree of a decrease in 12 hr fasting serum insulin levels was significantly less in the diabetic treated group as compared to diabetic control rats. On the last day of feeding, β cells of the pancreas were stimulated by 200 mg/dL glucose through a 40 min pancreas perfusion. Amounts of the insulin secreted from the pancreas during the first phase (11 to 20 min) and the second phase (21 to 40 min) in the extract fed diabetic rats were significantly greater than those of the diabetic control group (P<0.05). These findings indicated that constituents of *C. tora* seeds possess beneficial effect on postprandial blood glucose control which might be partially mediated by stimulated insulin secretion from the pancreas of the diabetic rats [79].

Chaurasia et al. [80] investigated the antidiabetic screening of methanol extract and its ethyl acetate, *n*-butanol and dichloromethane fraction of seeds of *C. tora* employing single dose and prolonged treatment in normal and alloxan induced diabetic albino rats. Methanol extract was given orally at a dose of 50, 100, and 200 mg/kg body wt. and its sub-fractions were given at a dose of 100 mg/kg body wt. Methanol extract at a dose of 200 mg/kg body wt. showed significant antidiabetic activity in normal, acute as well as prolonged treatment groups. The *n*-butanol fraction of methanol extract was found to be more significant in reducing the blood glucose level after single dose as well as prolonged treatment and the observed effect was equivalent to that of standard drug, Glibenclamide.

Jain et al. [81] studied the effect of *n*-butanol fraction of ethanolic extract of *C. tora* seeds on biochemical parameters and on *β*-cells regeneration in alloxan induced diabetic rats. The *n*-butanol extract was administered orally in different doses (0.1, 0.25, and 0.5 g/kg body wt.)

to different groups of normal or alloxan induced diabetic rats either as single dose daily or multiple dose spanning over two weeks. Oral administration of the *n*-butanol extract significantly decreased blood glucose level in normal and alloxan induced diabetic rats both in single dose as well as multiple dose experiment when compared with the control group. A significant ($p<0.05$) reduction was also observed in total cholesterol, triglycerides, asparate amino transferase (AST) and alanine amino transferase (ALT) in serum to the control levels in diabetic animals. The increased serum urea, uric acid and creatinine levels in diabetic rats were decreased significantly following administration of the *n*-butanol extract.

2.2.5 Antimutagenic and immunostimulatory activity

The antimutagenic activity of the methanol extract of seeds was demonstrated against aflatoxin B_1 (AFB_1) using the *Salmonella typhimurium* assay and direct-acting mutagen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine assay methods. The numbers of revertants per plate were decreased significantly with methanol extract using *S. typhimurium* TA100 and TA98 assay methods. Pure compounds obtained from ethylene dichloride and *n*-butanol fractions of methanol seed extract were reported to possess significant antimutagenic activity but the water fraction was found to be inactive. Neither the methanol extract nor its fractions were capable of inhibiting the direct-acting mutagen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine [82]. Aqueous extract of unroasted seeds markedly suppressed the mutagenicity of 2-amino-6-methyldipyrido(1,2-*a*:3′:2′-*d*)imidazole (Glu-P-1) and 3-amino-1,4-dimethyl-5*H*-pyrido(4,3-*b*)indole (Trp-P-1) when tested with *S. typhimurium* TA98 and TA100. Extract, especially unroasted sample, showed much lower value of IC_{50} than the other roasted samples. For strain TA98, the IC_{50} of aqueous extract of unroasted seeds toward Trp-P-1 and Glu-P-1 were 0.15 and 0.57 mg/mL; whereas for strain TA100, the IC_{50} were 0.20 and 0.17 mg/mL, respectively. The antimutagenicity was decreased with increase in roasting temperature in the following order of unroasted > roasted at 150°C > roasted at 250°C [83].

Benzo (*a*) pyrenes are well known carcinogens and widely distributed in the environment [84,85]. The elimination of these polycyclic aromatic hydrocarbons from the body requires their deactivation to aqueous soluble metabolites. Some of the physiological enzymes involved in benzo[*a*]pyrenes metabolism in the body include cytochrome P-450, epoxide hydratase and arylhydrocarbonhydroxylase etc. which are induced by various substances found in edible plants. There are certain evidences that consumption of certain dietary vegetables like spouts, cabbages, broccoli, alfalfa and fibers may reduce the incidence of stomach and colon cancer [86].

Aqueous seed extract of *C. tora* reduced benzo[*a*]pyrene induced DNA damage in human hepatoma cell line HepG2 via the comet assay without exogenous activation mixtures. The inhibitory effects of aqueous extract on DNA damage were 72%, 60% and 23% with unroasted, roasted at 150ºC and roasted at 250ºC, respectively at a concentration of 1 mg/mL. Ethoxyresorufin-*O*-dealkylase activity of HepG2 cells was effectively inhibited by aqueous extracts of seeds and a similar trend of inhibition was observed in the order of unroasted > roasted at 150ºC > roasted at 250°C with inhibition of 64%, 42% and 18%, respectively. The activity of NADPH cytochrome P-450 reductase was also decreased up to 50% and 38% by unroasted and roasted at 150ºC samples, respectively. Furthermore, glutathione S-transferase activity was increased up to 1.26 and 1.35 fold by treatment with unroasted and roasted at 150ºC samples at 1 mg/mL. Aqueous extract alone, at concentration of 0.1-2 mg/mL, showed neither cytotoxic nor genotoxic effect towards HepG2 cells. The inhibitory effects of chrysophanol, emodin and rhein on benzo[a]pyrene-mediated DNA damage in HepG2 cells were 78, 86 and 71%, respectively, at 100 µM [87].

In the Comet assay performed on human lymphocytes, aqueous extracts of seeds exhibited significant protective effect on Trp-P-1-mediated DNA damage followed the order of unroasted > roasted at 150ºC > roasted at 250°C with 55%, 42% and 29% protection, respectively at a concentration of 0.5 mg/mL. Pre-treatment of the lymphocytes with aqueous extract of unroasted seeds resulted in 30% repression of DNA damage at concentration up to 0.5 mg/mL. However, no significant effect on excision-repair system was found during DNA damage expression time in post-treatment scheme (P>0.05). Aqueous extract showed no significant protective effect on direct-acting mutagen N-methyl-NT-nitrosoguanidine (MNNG) in the Comet assay. Aqueous extract of unroasted seeds exhibited the greatest potency on scavenging the reactive intermediates of Trp-P-1 and exhibited 38.7% of protective effect on DNA damage at a concentration of 0.5 mg/mL. Aqueous extract of unroasted seeds showed 84% scavenging effect on oxygen free radicals generated in the activation process of mutagen detected by electron paramagentic resonance system [83].

Rejiya et al. [56] studied the *in vitro* antiproliferative activity of methanol extract of leaves against human cervical cancer cells (HeLa). Extract exhibited a marked concentration dependent inhibition on proliferation measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide (MTT) assay, reduced DNA content up to 39.92 µg/1 × 10^6 per cell compare to 62.0 µg/1 × 10^6 for control group determined by modified diphenylamine method and apoptosis by caspase 3 activity. The IC_{50} of the extract was 191µg/mL which was considerably active when compared with IC_{50} value of anticancer drug cisplatin at the dose of 174µg/mL.

The immunostimulatory activities of aloe-emodin, emodin, chrysophanol and rhein anthraquinones of seeds were studied on human peripheral blood mononuclear cells (PBMC). Studies were conducted on lymphocyte proliferation by bromodeoxyuridin (BrdU) immunoassay, secretion of interferon-gamma (IFN-γ) and interleukin 10 (IL-10) by an ELISA assay and elucidation of responding immune cells by flow cytometry. All four anthraquinones were found to be effective in stimulating the proliferation of resting human PBMC and/or secretion of IFN-γ at non-cytotoxic concentration. However, at the concentration of 10 µg/mL (35µM), rhein significantly stimulated proliferation of resting human PBMC (stimulation index =1.53) but inhibited IFN-γ secretion (74.5% of control). The augmentation of lymphocyte proliferation was correlated to the increase in number of $CD4^+$ T cells, while the elevated secretion of IFN-γ and IL-10 might have been due to the activated $CD4^+$ T cells [88].

Yang et al. [89] tested the fractions obtained from 17 clinically useful antitumour traditional Chinese herbs for their potential to restore the sensitivity of MCF-7/ADR and A549/Taxol cells to a known antineoplastic agent. The effects of these fractions were evaluated by MTT method and an assay of the cellular accumulation of doxorubicin. Fractions from the PB group (herbs with the ability to promote blood circulation and remove blood stasis) showed more significant effects than fractions from the CH group (herbs with the ability to clear away heat and toxic materials). Fractions from CH_2Cl_2 extracts were more effective than fractions from EtOAc extracts. *C. tora* L. is one among five herbs which could sensitise these resistant cancer cells at a non-toxic concentration ($10\,\mu g\,mL^{-1}$), and markedly increased doxorubicin accumulation in MCF-7/ADR cells. Park and Kim [90] studied that methanol extract of *C. tora* seeds was successively partitioned with diethyl ether, chloroform, ethyl acetate, and water, and the antitumor-promoting activity of the solvent fractions was determined by inhibition of Epstein-Barr virus early antigen (EBV-EA) activation induced by teleocidin B-4 in Raji cells. The diethyl ether (68.7%) and chloroform

(91.2%) fractions and the hydrolysate (94.3%) of the ethyl acetate fraction showed strong inhibitory activities.

2.2.6 Spasmogenic, antinociceptive, anti-inflammatory activity

The spasmogenic effect of methanol extract of leaves was evaluated on guinea pig ileum, rabbit jejunum and mice intestinal transit. The extract contracted smooth muscles of guinea pig ileum and rabbit jejunum in a concentration-dependent manner. The median effective concentration of the extract (EC_{50}) was found to be 1.33 mg/mL and 0.09 mg/mL on the isolated guinea pig ileum and rabbit jejunum, respectively. The extract showed increased intestinal transit in mice in a dose dependent manner; no effect was seen up to the dose of 200 mg/kg but at the dose of 400 mg/kg (P<0.05), intestinal transit was increased significantly. Extract of leaves (P<0.05) showed reduced acetic acid induced abdominal constrictions significantly in mice and the effect was comparable with that of standard drug, aspirin (150 mg/kg i.p.). The extract also showed reduced (P<0.05) nociceptive response in mice significantly. The LD_{50} values of the extract in mice were more than 2000 mg/kg for both oral and i.p. doses [91].

Methanol extract of leaves produced significant antiinflammatory activity on both acute and chronic models of inflammation in rats. The oral administration of extract significantly reduced carrageenan, dextran, histamine and serotonin-induced rat paw edema level up to 40.33%, 31.37%, 53.57% and 29.15%, respectively at the end of 3 hr at the dose of 400 mg/kg. Using glaucoma pouch chronic test in rat, extract exhibited 48.13% reduction in granuloma [92]. The ethanolic extract of the leaves of *C. tora* was studied for its effect on wound healing in rats excision wound model and observed that the wound contracting ability of the extract was significantly greater than that of control (ointment base) and comparable with the reference standard Nitrofurazone ointment. Thus, ethanolic extract of this plant have been shown to possess good anti-inflammatory potential [93].

2.2.7 Larvicidal and antiplasmodial activity

Larvicidal activity of methanol extract of seeds was confirmed against early 4^{th} stage larvae of *Aedes aegypti*, the mosquito that carries dengue fever and *Culex pipiens*, the mosquito that carries Japanese encephalitis in a dose dependent manner. Extract showed very good mortality rate against both the larvae of *A. aegypti* and *C. pipiens* [94].

Plant extract showed *in vitro* antiplasmodial activity against chloroquine-sensitive *Plasmodium falciparum* 3D7 and chloroquine resistant Dd2 (pyrimethamine sensitive) with 50% inhibitory concentration (IC_{50}) values less than 5 µg/mL on both tested strains [95].

3. CONCLUSION

In this review paper, phytopharmacological aspects of *Cassia tora* L. have been enumerated and described. The information was collected from recent literature sources and depicted in a comprehensive manner covering the details of scientific understanding of this valuable natural resource. This effort not only provides considerable evidence in support of traditional claims of *C. tora*, but also strengthens the basis of further exploration of novel bioactive compounds reported so far from this plant. However, future research would be directed to the discovery of new therapeutic strategy based on the novel phytomedicinal approach of *C. tora* L.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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