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Secondary metabolites from ornamental flowers: A study of common avenue trees of the family Caesalpiniaceae

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ABSTRACT

To explore the phytochemical constituents of the aqueous, petroleum ether, chloroform, ethanol and acetone extracts of petals of Cassia siamea Lam., Cassia fistula Linn., Delonix regia Linn. and Peltophorum pterocarpum (DC.) Baker ex. Heyne. Fresh fallen petals (50 g) were collected and 200 ml of water, petroleum ether, chloroform, ethanol and acetone were added in separate conical flasks, which were kept in a shaker at room temperature for 24 hours. After incubation, the extracts were filtered through Whatman No.1 filter paper, and collected and stored at 4°C. The extracts were concentrated using vacuum evaporator and dried at 60°C. Preliminary phytochemical screening was performed by the Harborne method. Screening of the petals of C. fistula and D. regia proved the presence of phytochemicals such as alkaloids, phenolic compounds, flavonoids, saponins, glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, proteins and carbohydrates. Alkaloids were found to be absent in petals of D. regia, alkaloids, saponins and glycosides were absent in the petals of P. pterocarpum, and petals of C. siamea were devoid of glycoside and phenolic compounds. The petal extracts studied here can be used as broad-spectrum antimicrobial agents after extensive investigation.

Keywords: Caesalpiniaceae, *Cassia siamea* Lam., *Cassia fistula* Linn., *Delonix regia* Linn., *Peltophorum pterocarpum* (DC.) Baker ex. Heyne

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INTRODUCTION

Ornamental flowers of avenue trees are highly promising and unutilized resources having tremendous and proven economic importance. In recent years, there is a growing interest concerning floral chemistry and extraction and purification of active principles, which are the bases of several valuable phyto-drugs. Plants have evolved with various coloured pigments on their petals based on their need, to increase their survival and reproductive potential. Colour of petals varies based on the time and season of flowering. In general, flowers which bloom in the evening are dark or white in colour to attract nocturnal pollinators, whilst the flowers which bloom during early morning or daytime are pretty with bright-coloured petals to attract nectarivores. In tropical and sub-tropical regions yellow-coloured flowers are common throughout the year while colours related to red can be found only during the late winter and summer seasons. Different colours among flowers are due to the presence of various kinds of pigments dissolved uniformly in the vacuolar solution of epidermal cells. Such pigmented bodies have been a source of secondary metabolites, which possess a wide array of bioactive compounds, used to treat various kinds of health problems in living organisms.

Cassia fistula Linn. commonly called ‘Amaltas’, ‘Golden shower tree’ and ‘Indian laburnum’, is an ornamental, medium-sized avenue tree growing up to a height of 10-20m with beautiful branches of yellow flowers. The 30-feet-tall graceful tree produces yellow 3 in. flowers that hang in clusters 12 to 18 in. in length. Typically, bloom occurs just after leaf drop in May, so that for several weeks the naked tree is covered with long yellow chains, each with a dozen or more flowers. Pollination is achieved through bees which cover the tree during bloom [1]. The extracts derived from different parts of this plant have antibacterial, antipyretic, analgesic, antioxidant, anti-inflammatory and hypoglycaemic properties and are used in the treatment of various disorders such as haematemesis, pruritus, leucoderma, rheumatism, skin diseases, tuberculosis, and eye and liver ailments [2-5]. Various parts of this plant are known to be important sources of secondary metabolites, mainly phenolic compounds such as fistucacidin (3,4,7,8,4-pentahydroxyflavan), (-)epiafzelechin, (-)epiafzelechin-3-*O*-glucoside, (-) epicatechin, procyanidin B2, biflavonoids, triflavonoids, rhein glucosides, sennoside A and B, chrysophenol and physcion isolated from the leaves [6,7]; kaempferol, α-leucopelargonidin tetramer having a free glycol unit, bianthraquinone glycoside, rhein, fistulin, alkaloids, triterpenes from the flowers [8]; and oxyanthraquinone and dihydroxyanthraquinone from the bark [9].

Cassia siamea Lam., also known as *C. javanica* and *Senna siamea*, a tree species indigenous to India, Burma, Sri Lanka, Indonesia and Malaysia, and introduced to semi-arid and sub-humid tropics, has been referred to in ayurvedic literature for the treatment of various ailments; nowadays it is being cultivated as an ornamental and avenue tree. Parts of this plant are widely used for the treatment of insomnia and various other conditions such as diabetes, hypertension, asthma, constipation and diuresis [10]. Tender leaves of this species are used as vegetable during famine in Gujarat drylands [11]. It is also used as a green leafy vegetable by the Thai [12].

Delonix regia Linn. is an ornamental, medium-sized tree planted in gardens in all the warmer and damper parts of India. It is commonly known as 'Gulmohar' in Marathi [13]. Traditionally, the parts of this plant have been used as anthelmintic, antimicrobial, anticancer, emetic and central nervous system depressant agents, and in the treatment of anaemia and fever [14]. Adje *et al.* characterized the anthocyanin pigment of *D. regia* [15]. Various parts of the plant have antioxidant [16, 17], larvicidal [18], antibacterial, antifungal [19], anti-inflammatory, analgesic [20], nutritional [21], antimalarial [22], hepatoprotective [23], anti-diarrhoel [24], gastroprotective [25], antiarthritic [17] and antirheumatic [13] properties. Its aqueous and alcoholic extracts have been found to be active against roundworm. The bark contains leucocyanidin, lupeol, tannin, β-sitosterol and free OH-proline as the major amino acid. Flower anthers are a rich source of zeaxanthin. Leaves contain tannins, lupeol and β-sitosterol [13], and seeds contain lectins [26].

Peltophorum pterocarpum (DC.) Baker ex. Heyne, is a species native to Sri Lanka, the Andamans, the Malay Peninsula and North Australia. It is commonly called 'Copper pod' or 'Yellow flame tree'. It is a very attractive avenue tree with its spreading crown of many branches consisting of feathery mimosa-like leaves, abundant bright yellow blooms and profuse copper red seedpods. In terms of biodiversity, it serves as a good nectar source for hymenopteran insects including honey bees, bumble bees and several economically important wasps [27, 28]. Apart from these it has potent medicinal value. Traditionally, the bark of the tree is used to treat wounds by the Paliyar tribe [29]. The bark and leaves of the plant have antimicrobial [30-35], antioxidant [36], antifungal [37-39], apoptotic [40] and haematological [41] properties. This tree blooms twice a year. The medicinal value of flowers, especially petals, still remains unexplored. Hence the present study was aimed to explore the biologically active constituents of the petals of *C. fistula, C. siamea*, *D. regia* and *P. pterocarpum* known in Tamil as 'Konrai', 'Mangalkonrai', 'Malai Varachi' and 'Perungondrai', respectively.

EXPERIMENTAL SECTION

The petals of *C. fistula*, *C. siamea*, *D. regia* and *P. pterocarpum* were collected from Scott Christian College Campus, Nagercoil, Tamilnadu, India and authenticated by a plant taxonomist of the Department of Botany, Scott Christian College, Nagercoil by using the *Flora of Scott Christian College Campus* [42]. Extracts were prepared from fresh petals. Fifty grams of petals of the flowers was collected and kept in separate conical flasks and 200 ml of water, petroleum ether, chloroform, ethanol and acetone were added, and the contents were mixed in a shaker at room temperature for 24 h. After incubation, the extracts were filtered through Whatman No.1 filter paper and collected and stored at 4°C. The extracts were concentrated using vacuum evaporator and dried at 60°C. Preliminary phytochemical screening was performed by using standard methods [43].

RESULTS

Preliminary phytochemical test were performed on the aqueous and solvent extracts of the petals of *C. fistula*, *C. siamea*, *D. regia* and *P. pterocarpum* and the results showed that various phytoconstituents are present in the petals of the selected flowers (Table 1). In the present investigation, alkaloids were found only in the aqueous and acetone extracts of *C. fistula* and in the aqueous extract of *C. siamea*, whereas extracts prepared from the petals of *D. regia* and *P. pterocarpum* were devoid of alkaloids. Phenolic compounds were noticed in all the extracts of *D. regia* and in the ethanol and acetone extracts of *C. fistula*. Phenolic compounds were also noticed in the aqueous, petroleum ether, ethanol and acetone petal extracts of *P. pterocarpum*, whereas they were not detected in the petal extracts of

C. siamea. Aqueous, ethanol and acetone extracts of *C. fistula* showed the presence of flavonoids, whereas in *D. regia*, flavonoids were noticed in petroleum ether, chloroform and acetone extracts. Aqueous, ethanol and acetone petal extracts of *P. pterocarpum* also showed the presence of flavonoids. In *C. siamea* all the extracts showed the presence of flavonoids. Saponins were found in the aqueous and petroleum ether extract of *C. fistula*, aqueous, ethanol and acetone extracts of *C. siamea* and in the aqueous, petroleum ether, ethanol and acetone extracts of *D. regia*. Saponins and glycosides were entirely absent in the petal extracts of *P. pterocarpum* and *C. siamea*. Glycosides were noticed in the ethanol extract of *C. fistula* and in the aqueous and ethanol extracts of *D. regia*.

Terpenoids were found in almost all the extracts of *C. fistula*, *D. regia* and *P. pterocarpum* except the chloroform extract of *C. fistula* and ethanol extract of *P. pterocarpum*. Petals of *C. siamea* showed the presence of terpenoids only in the acetone extract. The presence of steroids and coumarins were noticed in all the five extracts of *C. fistula*. In *D. regia*, aqueous, chloroform and ethanol extracts showed the presence of steroids, and petroleum ether, chloroform and acetone extracts showed the presence of coumarins. In *P. pterocarpum* presence of steroids were noticed in petroleum ether and chloroform extract, and coumarins were detected in all the extracts. In *C. siamea* steroids were noticed in the aqueous, petroleum ether and ethanol extract, and coumarins were noticed in aqueous, ethanol and acetone extracts.

Quinones were noticed in the chloroform and acetone extracts of *C. fistula*, aqueous, petroleum ether and ethanol extracts of *D. regia*, in the petroleum ether extract of *P. pterocarpum* and in the acetone extract of *C. siamea*. Phytosterols were not detected in the petal extracts of *C. fistula* whereas, the petroleum ether and chloroform extracts of *D. regia* petals showed the presence of phytosterols. All the extracts of *P. pterocarpum* showed the presence of phytosterols. Phytosterols were noticed in the petroleum ether, ethanol and acetone extracts of *C. siamea*. The petals of *C. fistula* showed the presence of protein in aqueous, ethanol and acetone extract but the petals of *D. regia* showed the presence of protein in petroleum ether, chloroform and ethanol extracts. In the petals of *P. pterocarpum* proteins were detected in aqueous, petroleum ether, chloroform and ethanol extracts. Proteins were detected in the aqueous and acetone extracts of *C. siamea*. Carbohydrates were noticed in all the extracts of *C. fistula* and *D. regia* except ethanol extract of *C. fistula*. Carbohydrates were noticed in the aqueous and chloroform petal extracts of *P. pterocarpum* and in the aqueous, chloroform, ethanol and acetone petal extracts of *C. siamea*.

DISCUSSION

Plant compounds that are perceived by humans to have colour are generally referred to as 'pigments'. Their varied structures and colours have long fascinated chemists and biologists, who have examined their chemical and physical properties, their mode of synthesis, and their physiological and ecological roles. Plant pigments also have a long history of use by humans. In the present study both yellow- and red-coloured petals, which are very common among the avenue trees of the study area, were used for the analysis of phytoconstituents. *Delonix regia* is the only plant having red petals, the other three species bearing yellow petals. The presence of yellow and red pigments in petals of flowers of the family Caesalpiniaceae may be due to anthocyanins and carotenoids.

Anthocyanins, a class of flavonoids derived ultimately from phenylalanine, are water-soluble, synthesized in the cytosol, and localized in vacuoles. They are the basis for a wide range of colours, from orange/red to violet/blue. In addition to various modifications to the structure of anthocyanin, a specific colour also depends on co-pigments, metal ions and pH. They are widely distributed in the plant kingdom [44]. These pigments normally occur in plants as glycosides attached to sugars. They are derived from phenylalanine, are water-soluble, synthesized in the cytosol and localized in vacuoles. Moreover, anthocyanins present in flowers attract bird and insect pollinators, as well as animals and birds that disperse their fruits and seeds. They also protect plants from ultraviolet irradiation.

The carotenoid class of compounds includes yellow, orange and red pigments. These pigments are soluble in hydrocarbon solvents (carotenes) or ethyl alcohol (xanthophylls) and occur in specialized protoplasmic bodies (plastids) in higher plants. Carotenoids are present along with chlorophyll, in the chloroplasts of green leaves and immature fruits. As leaves approach senescence and fruits mature, chloroplasts 'degenerate' into chromoplasts. With the breakdown of the photosynthetic apparatus, the production of carotenoids increases [45]. Carotenoids in fruits and seeds serve to attract animals that disperse them.

Selection of appropriate solvents is crucial for effective phytoextraction of secondary metabolites for clinical studies. Due to their varied polarity and boiling point the extractable phytoconstituents and their bioactivity also vary. Alcohol has been found to be the most effective solvent for extracting phytochemicals from flowers than other solvents [46-50]. Methanol was found to be most effective solvent for extracting antioxidants from *C. fistula* flowers [51]. In the present study, acetone, chloroform, ethanol, petroleum ether and aqueous extracts were used for qualitative analysis of phytochemicals from the selected flowers. The petals of *C. fistula* and *D. regia* give up maximum compounds in acetone and petroleum ether extracts respectively. The petals of *P. pterocarpum* yielded maximum compounds in aqueous as well as in petroleum ether solvents. Preliminary findings of the present study contradict the reports of earlier workers; however, more trials are needed to confirm these findings.

Table 1. Preliminary phytochemical studies of petal extracts of *Cassia siamea* Lam., *Cassia fistula* Linn., *Delonix regia* Linn. and *Peltophorum pterocarpum* (DC.) Baker ex. Heyne

Phytoconstituents	Solvents																			
	Aqueous				Petroleum ether				Chloroform				Ethanol				Acetone			
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Alkaloids	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
Phenolic compounds	-	-	+++	+++	-	-	+	+++	-	-	+	-	+	-	+++	+++	+	-	+++	+++
Flavonoids	+++	++	-	+++	-	+	++	-	-	+	+	-	++	+	-	+++	+++	+	+	+++
Saponins	+	++	+	-	++	-	+++	-	-	-	-	-	-	+	++	-	-	+	+++	-
Glycosides	-	-	+++	-	-	-	-	-	-	-	-	-	+++	-	++	-	-	-	-	-
Terpenoids	++	-	+++	+++	++	-	+++	++	-	-	+	+	++	-	+++	-	+++	+	+++	++
Steroids	++	++	++	-	++	+	-	+	+	-	+++	+++	++	+	+	-	+	-	-	-
Coumarins	++	+	-	+++	+++	-	++	+	+++	-	+++	+++	++	+++	-	+++	++	++	+++	++
Quinones	-	-	++	-	-	-	+	++	++	-	-	-	-	-	++	-	+	+	-	-
Phytosterols	-	-	-	++	-	++	++	+++	-	-	++	+++	-	+	-	+++	-	+	-	+++
Proteins	+	+	-	++	-	-	++	++	-	-	+++	++	+	-	+	+++	+	++	-	-
Carbohydrates	++	+++	++	+++	++	-	++	-	+++	+++	+++	+++	-	++	+	-	+	+++	+	-

(A) Cassia siamea; (B) Cassia fistula; (C) Delonix regia; (D) Peltophorum pterocarpum; (-) absent; (+) low; (++) average; (+++) high.

The various phytochemical constituents detected in the flowers are known to be beneficial in the treatment of infectious diseases [52, 53]. Recently, a number of studies have been carried out on the phytochemistry of plants across the world [54-67]. In the present investigation petals of *C. siamea*, *C. fistula*, *D. regia* and *P. pterocarpum* showed the presence of alkaloids, phenolic compounds, flavonoids, saponins, glycosides, terpenoids, steroids, coumarins, quinones, phytosterols, protein and carbohydrates in one or more extracts. Alkaloids are nitrogen-containing heterocyclic organic bases with complicated structures usually with specific physiological functions. They are biosynthesized and derived from amino acids through mevalonic acid pathways. Most alkaloids are toxic to man and animals, especially in large doses. But in lower doses they have been reported to have great medicinal uses, e.g. as pain relievers, stimulants and antimalarials. Phenols, mainly the type of flavonoids from some medicinal plants are safe and bioactive, and have antioxidant, anticarcinogenic, antimutagenic, antitumoral, antibacterial, antiviral and anti-inflammatory properties. Therefore, nowadays substantial attention has been directed towards exploring these plants with bioactive principles. In addition, other phytochemical constituents such as tannins and several other aromatic compounds are secondary metabolites of plants that serve as defense mechanisms against microorganisms, insects and other herbivores. The whole plant or organism serves as an active laboratory for the production of natural products from primary metabolites. These are basic biological molecules also called ‘biochemist molecules’, found virtually in all plants and organisms.

Bioactive compounds found in various plant parts possess multiple biological effects on human and non-human biota [68-72]. The reproductive organs of *C. fistula* had higher antioxidant levels compared to the vegetative parts [2], the order being pods>flower buds>flowers. The antioxidant capacity of the extracts of reproductive organs is directly proportional to the level of catechins, and oligomeric and polymeric proanthocyanidins [73]. The active compound rhein (1,8-dihydroxyanthraquinone-2-carboxylic acid) isolated from the flowers of *C. fistula* showed antifeedent activity against the lepidopteran pests *Helicoverpa armigera* and *Spodoptera litura*. It is also showed larvicidal activity against *H. armigera* and *S. litura* and the larvae that survived produced malformed adults [74]. The leaf extract of *C. fistula* possesses remarkable larvicidal, ovicidal and repellent activity against medically important vector mosquitoes [75]. The hydroalcoholic extract of *C. fistula* showed antioxidant activity by inhibiting DPPH (1, 1-diphenyl-2-picryl hydrazyl) and hydroxyl radical, and had higher total phenol content [51].

Cassia siamea has been known to contain pharmacologically active compounds. Two active anthraquinones, emodin and chrysophanol, and two bianthraquinones, cassiamin A and cassiamin B [76] extracted from the plant might be valuable as an anti-tumour-promoting and chemopreventive agent [77]. The pharmacologically active ‘barakol’ was also isolated from the leaves of the plant and its main effect is on the cardiovascular and central nervous system [78]. The plant also possesses antibacterial [12,79, 80] and antioxidant activity [81,82]. Morita *et al*. isolated two novel alkaloids with an unprecedented tricyclic skeleton, cassiarins A and B, from the leaves of *C. siamea*, which showed potent antiplasmodial activity [83]. The ethanolic extract of *C. siamea* was found to possess high toxicity in rats [84].

Higher plants are a rich source of novel bioactive substances that can be used to develop pharmaceutical drugs [85-89]. Our preliminary phytochemical evaluation of select extracts of *D. regia* flowers revealed the presence of phenolic compounds. In Ivory Coast, flowers of *D. regia* are traditionally used to prepare beverages with beneficial health properties mainly due to their polyphenolic contents [90]. Liquid Chromatography – Mass Spectroscopy and Nuclear Magnetic Resonance analyses have revealed the polyphenols to be rutin, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, quercetin trihexoside, quercetin 3-*O*-robinobioside, kaempferol rhamnosylhexoside and isorhamnetol rhamnosyl-hexoside [91]. Carotenoids and anthocyanins were extracted from petals of *D. regia*: astaxanthin, a ketocarotenoid and anthocyanins — peonidin-3-*O*-glucoside and petunidin-3-*O*-acetyl-glucoside. Ethanolic extract of *D. regia* flowers showed the presence of tannins and flavonoids, which may be responsible for the significant antidiarrhoeal and antiulcer activity of the flower [24]. The alcoholic extract of *D. regia* was found to have antiarthritic activity in adjuvant-induced arthritic rats, when treated for a period of 28 days [92]. In addition, *D. regia* synthesizes multiple kunitz-like α-amylase inhibitors, with different molecular masses and a wide biotechnological potential to control insect pests [93].

Phytochemical compounds have implicit therapeutic and pharmacological properties, and hence exert unique physiological effects [94-101]. The flower bud extracts of *P. pterocarpum* lowered fasting blood glucose level in alloxan-glucose-induced diabetic mice; this may be due to the presence of flavonoids and steroids in the buds [102]. In addition the petroleum ether and ethanolic extract of this species confirmed its cardiotonic effect due to the presence of a mixture of steroidal glycosides (campesterol-3-*O*-beta-D-glucopyranoside, stigmasterol-3-*O*-beta-D-glucopyranoside and β-sistosterol-3-*O*-beta-glucopyranoside) [103]. The methanol:ethyl acetate (1:9) extract of the flower buds of *P. pterocarpum* reduced blood glucose level in alloxan-induced diabetic mice, implying that new compounds for treating diabetes can be isolated.

Hence it can be concluded that petals of the members of the family Caesalpiniaceae are richly endowed with an unusual combination of biologically active carotenoids and anthocyanins, particularly cyanidin-3-*O*-rutinoside, holding a great promise for food and pharmaceutical applications. Since astaxanthin, which is rarely found in higher plants, is present in *D. regia*, the biochemical pathway of ketocarotenoid biosynthesis in higher plants can be elucidated [104].

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