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Antimicrobial potential and structural elucidation of bioactive compounds from flower extract of *Cassia javanica* L.

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The present study was aimed to investigate the antimicrobial activity and characterization of bioactive constituents from flower extract of *Cassia javanica* L. Antimicrobial activity was tested against human pathogens in six different solvents using the well diffusion method. Of these, ethyl acetate and ethanolic crude extracts showed effective antimicrobial activity against all the tested human pathogens. The bioactive constituents of the flower extract revealed the presence of some biologically active substance (alkaloids, cardiac glycosides, flavonoids, glycosides, phenolic compounds, phlobatannins, triterpenoides, saponins, volatile oils and tannins). In the GC-MS analysis, 14 bioactive compounds were identified. Present study concludes that the different extracts of *C. javanica* flower contain a broad spectrum of bioactive compounds and also exhibit antimicrobial activity against all the tested human pathogens, therefore, *C. javanica* flowers can be tapped source to discover the natural bioactive products that may serve as leads in the developments of novel therapeutic drugs.

Keywords: *Cassia javanica*, Antimicrobial activity, Bioactive compounds, GC-MS.

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Introduction

Plants have been a valuable source of natural products for maintaining human health, though the identification of active compounds from this valuable source came to light in recent years[1]. Medicinal plants constitute an important natural wealth of a country and they play a significant role in providing primary health care services to rural people[2] as well as substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries[3]. Moreover, 80 % of individual from developed countries use traditional medicines, having various compounds with medicinal properties[4].

A dramatic increase in microbial antibiotic resistance has developed over the last forty years in both the agriculture and medical sectors, this forced the researchers to develop new antimicrobial drugs for controlling pathogenic species[5]. In general, plants have the capacity to produce a large number of organic phytochemicals with complex structural diversity such as secondary metabolites[6,7], which served as plant self defense mechanisms against pests and pathogens[8] with interesting biological activities, this secondary metabolites can be utilized for medicinal purposes. Therefore, such plants should be investigated for better understanding their properties, safety and efficiency[9]. Since plant products have no side effect[10] and they are comparatively cheaper. As a result, many researchers have paid attention to plants sources and biologically active compounds isolated from herbs[11].

The genus *Cassia* L. comprises 580 species of herbs, shrubs and trees, which are widely distributed throughout the world, of which only 20 species are indigenous to India[12]. Many of the *Cassia* spp. possess a good amount of medicinal properties[13] and they revealed potential in Ayurvedic medicine[14]. Especially, recent literature provides information about therapeutic uses of the plant *C. javanica*. Bark of *C. javanica* is used as one of the ingredients in antidiabetic Ayurvedic formulation. Leaves are proved to be active against Herpes simplex infection. It is conventionally believed that this herb can reduce fever, decrease the virulence of pathogenic organisms, regulates the flow of chi and lubricate the intestine. In China it is applied to treat gastric pain, cold, malaria, measles, chickenpox and constipation[15].

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C. javanica is a small to medium-sized tree, 3-20 m tall with widely spreading horizontal branches and showy blossoms, trunk of young trees either smooth or armed with stump-remnants of branches. Leaves paripinnate with 5-15 pairs of leaflets, taproot, petioles 1.5-4.0 cm long, inflorescence a axillary racemose, many-flowered; flowers colour bright rose or pink, fading to white with red sepals (4-10 mm long), petals 15-35 mm long, whitish to reddish or buff, stamens 10, 3 longer ones with filaments 2 cm long, 4 shorter with filaments about 1 cm long and 3 reduced with filaments about 1 cm long and minute anthers and phenology period is July to November (Plate 1). Since, very far of literature is currently available to substantiate antimicrobial prospective of the *C. javanica* L. flower. The present attempt was made to examine the antimicrobial properties and bioactive compounds characterization of the *C. javanica* L. flower.

Materials and Methods

Plant material

Fresh flowers of the species *C. javanica* L. (Family- Caesalpiniaceae) were collected during January to April 2011 from various regions of the Pudukkottai district in Tamil Nadu, India. Plant was identified using the facility of Rapinat Herbarium, St, Joseph's college, Tiruchirappalli, Tamil Nadu, India and identified voucher specimen was deposited to the Research and PG Department of Botany, H.H The Rajah's College, Pudukkottai. The flowers were separated from the plant and thoroughly washed with fresh water and kept for shade dry at room temperature to get rid off moisture, until further analysis (Plate 1).

Preparation of extract

Dried flower materials were powdered with electric blender, at room temperature and 2g of the powdered sample was soaked in 20 mL of different solvents (ethanol, ethyl acetate, chloroform, benzene and water) overnight. Later, the samples were filtered under vacuum using Whatman No. 1 filter paper and stored in airtight screw-capped bottles at 5ºC for further analysis.

Preparation of inoculum

Seven clinical pathogenic organisms were obtained from the Microbial Clinical Laboratory, KMC Hospital, Tiruchirappalli, India. Out of the seven organisms, four were bacteria (*Escherichia coli, Salmonella typhi, Bacillus subtilis* and *Streptococcus pyogenes*) and three were fungi (*Candida albicans, Fusarium solani* and *Trichophyton rubrum*). Stock culture was maintained at 5ºC on slopes of nutrient agar for bacteria and potato dextrose agar (PDA) for fungi. Active cultures for further experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of respective media. For antibacterial and antifungal activity, tubes were incubated for 24 h at 37 ± 2ºC and 25 ± 2ºC for 48 h, respectively. Muller-Hinton Broth (for bacteria) and PD Broth (for fungi) were prepared for streaking and fresh slant cultures were prepared and stored in refrigerator at 5ºC for future requirements.

In vitro antimicrobial tests

Spectrum of antibacterial activity was studied by using the techniques described by Bauer *et al*[16]. Gentamicin sensitivity disc (30 mg; Hi-Media) was used as a positive control and respective solvents

Plate 1—Photographs of a twig and flowers of *C. javanica* L.

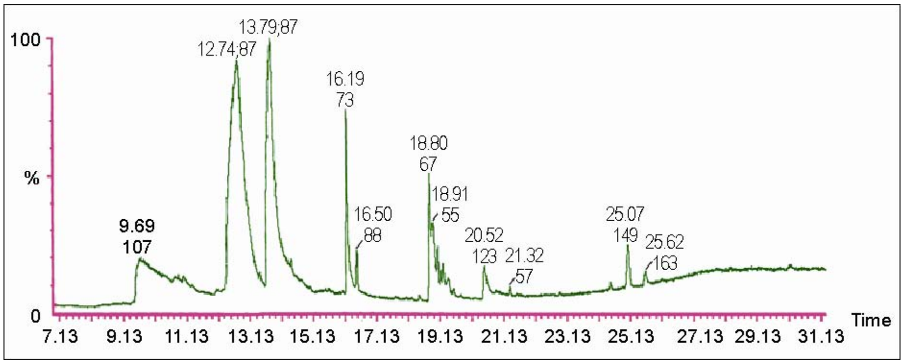

Fig. 1—GC-MS chromatogram of the *C. javanica* flower crude extracts

Table 1—Antimicrobial activity of *C. javanica* flower extracts in different solvents

S.No	Strains	Inhibition zone of diameter (mm)[a] after 24 - 48 h					
	Bacteria	A	B	C	ET	EA	Gentamicin
1	*E. coli*	-	-	-	8.4±0.6	24.1±0.7	23.5
2	*S. typhi*	-	-	-	7.7±0.2	19.8±0.2	28.3
3	*B. subtilis*	11.5±0.4	11±0.25	-	10.2±0.4	21.8±0.4	25.8
4	*S. pyogenes*	-	-	-	8.6±0.05	13.7±0.8	31.7
	Fungi						
5	*C. albicans*	18.6±0.02	11.6±0.07	15.8±0.6	18.2±0.2	21.3±0.2	35.6
6	*F. solani*	-	-	13.1±0.2	15.7±0.1	24.4±0.2	28
7	*T. rubrum*	-	-	-	-	19.7±0.4	26.2

Aqueous (A), Benzene (B), Chloroform (C), Ethanol (ET), Ethyl acetate (EA), No activity (-).
[a] -Values are mean ± standard deviation of three determination.

were taken as negative controls. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Biochemical screening

Biochemical tests in the flower of *C. javanica* were done using standard methods: carotenoids[17], total starch[18], total sugars[19], total proteins[20], total free amino acids[21], total phenols[22] and lipids[23]. Secondary metabolites were qualitatively tested by the standard methods of Harborne[24] and Odebiyi and Sofowara[25].

GC-MS analysis

GC-MS analysis of the *C. javanica* flower extract was performed using Perkin Elmer GC Clarus 500 MS system for identification of different components present in the extract, under the following conditions: column – dimethyl polysiloxane DB-1fused silica capillary column (30m x 0.25 mm x 0.1µm of film thickness); carrier gas – helium (1mL/ min); injector temperature –250^o C; detector temperature - 200^o C; column temperature – 35-180^o C at 4^o C / min – then 180 – 250^o C at 10^o C / min; MS electron impact 70 $_e$V. Identification of the constituents was achieved based on comparison of mass spectra with the library ones (NIST Ver.2.1).

Results

Antimicrobial activities of *C. javanica* flower extracts were screened against human pathogens (bacteria and fungi) using different solvents, viz. aqueous, benzene, chloroform, ethanol, and ethyl acetate. Among them, ethyl acetate and ethanol extract showed effective antimicrobial activity. In ethyl acetate, maximum inhibition was in *F. solani* (24.4 ± 0.2 mm) and the minimum in *S. pyogenes* (13.7 ± 0.8 mm) while in ethanol extract, the maximum inhibition was in *C. albicans* (18.2 ± 0.2 mm) and the minimum in S. *typhi* (7.7 ± 0.2 mm). Chloroform, benzene and aqueous extracts showed moderate antimicrobial activity. The gentamicin (30 µg/disc) was used positive control for comparing the bioassay (Table 1).

Results of the biochemical screening in the fresh flower extract of *C. javanica* were presented: carotenoids (0.731 ± 0.03 mg/g), total soluble sugars (37.83 ± 6.64 mg/g), total soluble proteins (52.75± 1.73 mg/g), total free amino acids (6.130 ± 0.54 mg/g), total phenol (32.81± 6.19 mg/g), hydroxyl phenols (14.07± 4.69 mg/g) and lipids (13.33 ± 5.77 mg/g). Further, alkaloids, cardiac glycosides, flavonoids, phenolic compounds, phlobatannins, tannins, tritrepenoides, volatile oils and saponins were also present in the *C. javanica* flower.

Gas Chromatography and Mass Spectroscopy analyses were carried out on the flower extract of *C. javanica* and 14 compounds were identified. Active principles with their Retention time (RT), Molecular formula (MF), Molecular weight (MW), Concentration (%) and Nature of the compounds are presented in Table 2. The GC-MS chromatogram showed the peak identities of the identified compounds (Fig. 1). In the present investigation, a variety of compounds has been detected *viz*. phenol,4-propyl- (a), 3-O-methyl-d-glucose (b), myo-Inositol, 4-C-methyl- (c), n-hexadecanoic acid (d), hexadecanoic acid, ethyl ester (e), Phytol (f), 9-12-octadecadienoic acid (Z,Z)- (g), 9,12,15- octadecatrienoic acid, (Z,Z,Z)- (h), 9,12-octadecadienoic acid-methyl ester (E,E)- (i), octadecanoic acid (j), octadecanoic acid-ethyl ester, phenol, 4-amino-3-methyl-(syn:m-cersol,4-amino-), 1,2-benzenedicarboxylic acid-diisooctyl ester (k) and propanal,2-(4-ethoxyphenyl)-2-methyl.

Discussion

Due to the value of medicinal plants as potential source of new compounds of therapeutic value researchers have been investigating them for their antimicrobial usefulness and as an alternative source to existing drugs[26,27]. Plant extracts which are available for controlling microbial growth, non-pollutive, cost effective, non hazardous and do not disturb ecological balance are preferred[28].

Different solvents have various degrees of solubility for different phytochemicals[29]. In present study, ethyl acetate and ethanol extracts had more inhibitory effects followed by chloroform, benzene and aqueous extracts and this study clearly revealed that organic solvents have more powerful antibacterial activity compared to water extracts[30-31]. Previously, many researchers reported antimicrobial activity in *Cassia* species, viz. *C. nigricans* Vahl[32], *C. angustifolia* Vahl[33] and *C. fistula* L.[34,35]. The present study also revealed a significant antimicrobial activity in *C. javanica* flower extract.

Plant biochemicals are the vital source of innumerable number of antimicrobial compounds and several phytochemicals such as tannins, saponins, terpenoides, flavonoids, phenolics, polyphenos and alkaloids[36]. These compounds are potentially significant therapeutic application against human pathogens, including bacteria, fungi[37]. Pervez ***et al***[38] reported three flavonoids (5, 3 Dihydroxy-2 phenyl-4-chrome-7, 4-digulucoside, Naringenin-7–glucoside and 3-glucoside of pelargonidin) from *C. javanica* flower, Panda ***et al***[35] and Pandya ***et al***[39] reported alkaloids, flavonoids, carbohydrates, glycosides, triterpenoides, phenolic compounds and saponins from *C. fistula* which had interference with molecular targets in their pathogens. The major targets include biomembrane, proteins and nucleic acid and these are still regarded as a valuable pool for discovering novel mode of action[40].

To fulfill the needs of novel chemical substance, we require an in-depth knowledge of chemical constituents of plants[41] for application in pharmaceutical industry[42]. Therefore biological activities of 14 various compounds identified by GC-MS study of flower extract of *C. javanica* were listed based on Dr. Duke's Phytochemical and Ethnobotanical Databases [Online] by Dr. Jim Duke of the Agriculture Research service/ USDA. Other species of genus *Cassia* reported as remedies to human diseases are: *C. auriculata* L. for the treatment of antihyperlipidaemic and antidiabetic[43]; *C. nigricans* can act as antifeedant, insecticide, antiulcer, antiplasmodial, anti-inflammatory, analgesic and antinociceptive[44]; *C. tora* L.[45] and *C. fistulaL.*[34] possess wound healing, ovicidal, antitumor, anti-inflammatory, antifungal, antibacterial and antioxidant activities and *C. italica* (Mill.) Lam. ex F.W. Ander. could be used as antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive[46].

Conclusion

In conclusion, terrestrial plants have opened up a new perspective for pharmaceutical research and development of novel antimicrobial agents for the treatment of microbial diseases. In this study flower extracts of *C. javanica* demonstrated a broad spectrum of antimicrobial activity against human pathogens. Further, the present results supported its use as a traditional and folklore medicine. The bioactive compounds, present in *C. javanica* flowers may lead to investigation of novel drugs against human diseases.

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References

1 Alam A, Vinay Sharma, Sharma S C and Kumari P, Antibacterial activity of the alcoholic extracts of *Entodon nepalensis* Mizush. against some pathogenic bacteria, *Report and Opinion*, 2012, **4**(10), 44-47.

2 Rukia A K, Use of medicinal plants for human health in Udzungwa Mountains Forests: a case study of New Dabaga Ulongambi Forest Reserve, Tanzania, *J Ethnobiol Ethnomed*, 2007, **3**(7), 1-4.

3 Phani Kumar G, Raj Kumar OP, Chaurasia and Shashi Bala Singh, Current status and potential prospects of medicinal plant sector in trans-Himalayan Ladakh, *J Med Plants Res*, 2011, **5**(14), 2929-2940.

4 Dahikar SB, Bhutada SA, Tambekar D H, Vibhute S K and Kasture S B, *In-vitro* antibacterial efficacy of solvent extracts of leaves of *Bauhinia racemosa* Lam. (Caesalpiniaceae) against enteric bacterial pathogens, *Int J Pharma Sci Drug Res*, 2011, **3**(1), 32-34.

5 Daboor S M and Haroon A M, *In vitro* Antimicrobial potential and phytochemical screening of some Egyptian aquatic plants, *Egypt J Aqua Res*, 2012, **38** (4) 233-239.

6 Kannan P, Ramadevi S R and Waheeta H, Antibacterial activity of *Terminalia chebula* fruit extract, *Afr J Microbiol Res*, 2009, **3**(4), 180-184.

7 Gobalakrishnan R, Kulandaivelu M, Bhuvaneswari R, Kandavel D and Kannan L, Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrata* L., *J Pharm Anal*, 2013, **3**(6), 460-465 , http://dx.doi.org/10.1016/j.jpha.2013.07.001.

8 Mazid M, Khan TA and Mohammad F, Role of secondary metabolites in defense mechanisms of plants, *Biol Med*, 2011, **3**(2), 232-249.

9 Nascimento G G F, Lacatelli J, Freitas P C and Silva G L, Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria, *Braz J Microbiol*, 2000, **31**(4), 886-891.

10 Kavishankar G B, Lakshmidevi N, Mahadeva Murthy S, Prakash H S and Niranjana S R, Diabetes and medicinal plants-a review, *Int J Pharm Biomed Sci*, 2011, **2**(3), 65-80.

11 Essawi T and Srour M, Screening of some Palestinian medicinal plants for antibacterial activity, *J Ethanopharmacol*, 2000, **70**(3), 343-349.

12 Anonymous,The Wealth of India- A Dictionary of Indian Raw Materials, Vol. II, Publications and Information Directorate, CSIR, Delhi, 1950, 93-98.

13 Ganapaty S, Thomas PS, Ramana KV, Vidyadhar K and Chakradhar V, A review of phytochemical studies of *Cassia* species, *J Nat Rem*, 2002, **2**(2), 102 -120.

14 Ledwani L and Oberoi S, A comparative study of fatty acids and sterols profiles in seed oil of five *Cassia* species, *Arch Appl Sci Res*, 2010, **2**(1), 295-301.

15 Chittam K P and Deore S L, *Cassia javanica* Linn.: a review on its phytochemical and pharmacological profile, *J Biomed Pharma Res*, 2013, **2**(1), 33-35.

16 Bauer A W, Kirby E, Sherris E M and Turk M, Antibiotic by standardized single disk method, *Am J Clin Pathol*, 1966, **45**(4), 493- 496.

17 Goodwin TW and Britton G, Distribution and analysis of carotenoids, *In*: Goodwin TW (ed), Plant pigments. Academic Press, London, 1988, 61-132.

18 McCready R M, Guggolz J, Silviera V, Owens, H S. Determination of starch and amylose in vegetables, *Anal Chem*, 1950, **22**(9), 1156-1158.

19 Dubois M, Gills K A, Hamilton J K, Reberts P A and Smith F C, Colorimetric method for the determination of sugars and related substances, *Anal Chem*, 1956, **28**(3), 350-356.

20 Lowry OH, Rosenberg NJ, Rarr A L and Randall R J, Protein measurement with the dolin-phenol reagent, *J Biol Chem*, 1951, **193**, 265-275.

21 Troll W and Canon K, A modified photometric ninhydrin method for the analysis of amino-imino acids, *J Biol Chem*, 1953, **200**(3), 803-811.

22 Swain T and Hillis W E, The phenolic constituents of *Prunus domestica* L. the quantitative analysis of phenolic compounds, *J Sci Food Agri*, 1959, **10**, 63-68.

23 Jayaraman J, Laboratory manual in biochemistry, Wiley Eastern Limited, New Delhi, India, 1981.

24 Harborne J B, Phytochemical Methods: A guide to modern technique of plant analysis, Chapman and Hall Ltd., London, 1973, 49-188.

25 Odebiyi O O and Sofowora E A, Phytochemical screening of Nigerian medical plants, *Llyodia*, 1978, **41**(3), 234-236.

26 Gowthami M, Tamil Selvi S, Senthil Kumar G and Panneerselvam A, Phytochemical analysis and antibacterial properties of leaf extract of *Azima tetracantha* (Lam.), *Asian J Plant Sci Res*, 2012, **2**(2), 110-114.

27 Kamble G S, Rasika C T, Pranav C C, Nirmala R D and Jyoti P S, Antimicrobial potential of medicinal plant-*Embelia basal*, *Int J Pharma Bio Sci*, 2011, **2**(2), 197-201.

28 Joseph B, Muzafar A D and Vinod Kumar, Bioefficacy of plant extracts to control *Fusarium solani* f. sp. melongenae incitant of Brinjal Wilt, *Global J Biotech Biochem*, 2008, **3**(2), 56-59.

29 Marjorie MC, Plant products as antimicrobial agents, *Clin Microbiol Rev*, 1999, **12**(4), 564-582.

30 Maji S, Dandapat P, Ojha D, Maity C, Halder S K, Das Mohapatra P K, Pathak T K, Pati B R, Samanta A and Mondal K C, *In vitro* antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens, *J Phytol*, 2010, **2**(4), 57-64.

31 Vinoth B, Manivasagaperumal R and Balamurugan S, Phytochemical analysis and antibacterial activity of *Moringa oleifera* Lam., *Int J Res Biol Sci*, 2012, **2**(3), 98-102.

32 Ayo RG and Amupitan J O, Antimicrobial activity screening of crude extract from leaves of *Cassia nigricans* Vahl., *Chem Class J*, 2004, 24-26.

33 Makky E A, Mashitah M Y and Ibrahim M M, Impact of medicinal plants phytocomponents against antibiotic resistant bacteria, *J Chem Pharm Res*, 2012, **4**(1), 881-893.

34 Bhalerao SA and Kelkar TS, Traditional medicinal uses, phytochemical profile and pharmacological activities of *Cassia fistula* Linn., *Int Res J Biol Sci*, 2012, **1**(5), 79-84.

35 Panda S K, Padhi L P and Mohanty G, Antibacterial activities and phytochemical analysis of *Cassia fistula* Linn. leaf, *J Adv Pharm Tech Res*, 2011, **2**(1), 62-67.

36 Alagesaboopathi C and Sivakumar R, Antimicrobial properties of various extracts of *Andrographis neesiana* Wight. An endemic medicinal species from India, *Intern J Pharm Tech Res,* 2011, **3**(1), 27-31.

37 El Astal Z Y, Aera A and Aam A, Antimicrobial activity of some medicinal plant extracts in Palestine, *Pak J Med Sci*, 2005, **21**(2), 187-193.

38 Pervez M, Obgeride ON, Irshad K and Ashfag A, Flavonoids of *Cassia javanica* (Caesalpinaceae), *Pak J Sci*, 1995, **47**(1-2), 34-37.

39 Pandya D J, Vishal L Patel, Tusharbindu R Desai, Rutu R Lunagariya, Swati D Gajera and Amee J Mehta, Pharmacognostic and phytochemical evaluation of leaves of *Cassia fistula*, *IJPLS*, 2012, **3**(2), 1424-1429.

40 Yazdani D, Tan Y H, Zainal Abidin M A and Jaganath I B, A review on bioactive compounds isolated from plants against plant pathogenic fungi, *J Med Plant Res,* 2011, **5**(30), 6584-6589.

41 Yadav R N S and Munin Agarwala, Phytochemical analysis of some medicinal plants. *J Phytol,* 2011, **3**(12), 10-14.

42 Iqbal P J, Phytochemical screening of certain plant species of Agra city, *J Drug Delivery & Therap*, 2012, **2**(4), 135-138.

43 Yesu Raj J, Paul John Peter M and Joy V, Chemical compounds investigation of *Cassia auriculata* seeds: A potential folklore medicinal plant, *Asian J Plant Sci Res*, 2012, **2**(2), 187-192.

44 Ayo RG, Phytochemical constituents and bioactivities of the extracts of *Cassia nigricans* Vahl: A review, *J Med Plants Res*, 2010, **4**(14), 1339-1348.

45 Choudhary M, Yuvraj Gulia and Nitesh, *Cassia tora*: its chemistry, medicinal uses and pharmacology, *Pharmacology online*, 2011, **3**, 78-96.

46 Sermakkani M and Thangapandian V, GC-MS analysis of *Cassia italica* leaf methanol extract, *Asian J Pharm Clin Res*, 2012, **5**(2), 90-94.