Pleione 7(2): 406 - 412. 2013. **ISSN: 0973-9467**

Cassia javanica Linnaeus [Caesalpiniaceae]: Phytochemical analysis and antimicrobial activity against multi-drug resistant hospital isolates of *Staphylococcus aureus*

Parinita Neog[1, 2], Shuvasish Choudhury[2], Amitabh Bhattacharjee[3], Pankaj Chetia[4], Manjur Ahmed Laskar[1] and **Manabendra Dutta Choudhury[1,5]**

[1]Ethnobotany & Medicinal Plant Research Laboratory, Department of Life science and Bioinformatics, Assam University, Silchar-788011, Assam, India
[2]Natural Product Process Laboratory, Central Instrumentation Laboratory, Assam University, Silchar-788011, Assam, India
[3]Department of Microbiology, Assam University, Silchar-788011, Assam, India
[4]Bioinformatics Center (DBT-BIF), Assam University, Silchar-788011, Assam, India
[5]*Corresponding author: e-mail*: drmdc@bioinfoaus.ac.in
[Received 06.09.2013; accepted 24.11.2013]

Abstract

Cassia javanica Linnaeus of Caesalpiniaceae is an ethnomedicinal plant of northeast India used as a home remedy in diarrhoea and skin infections. Ethyl acetate and methanol extracts of its bark, leaf and flower were studied and antimicrobial activity was verified against multi-drug resistant Gram positive bacteria. Antibacterial activity was found against *Staphylococcus aureus* ATCC 25923 and multi-drug resistant five other different strains of *Staphylococcus aureus*. Presence of overgrowth and bacterial resistance was not observed. Ethyl acetate extract of flower had the highest relative zone diameter 87.5 % in comparison with Vancomycin. Phytochemical screening of four different extracts of *Cassia javanica* (bark, leaves and flower) were tested and detected the presence of alkaloid, steroid, reducing sugar, saponin, flavonoid and anthraquinone.

Key words*: Cassia javanica*, natural product, multi-drug resistance, antimicrobial

INTRODUCTION

Plants are the richest source for bioactive herbal secondary metabolites those have been existing from thousands of years ago and always play a vital role in curing diseases. Recent antibacterial drug discovery research is based on finding out plant derived active secondary metabolites and their semi synthetic forms those can be used as drugs to treat human infectious diseases (Newman *et al* 2000). For these reasons, in search of a cure of diseases traditionally used medicinal plants are verified for their study and with their pharmacological study it was found that they are the database of natural composite sources which are less toxic with less side effect and can be future substitutes of synthetic drugs (Pandian *et al* 2006) and can act as most promising new anti-infectious agents (Ushimaru *et al* 2007). Plant derived bioactive natural products are potent herbal drugs to treat recent multi-drug resistant bacterial infections.

In these days, bacterial drug resistance is a common cause of treatment failure leading to death (Ahmad & Beg 2001). Moreover, susceptible bacteria can easily develop resistance towards a drug for their survival by an evolutionary process. It was reported that from 1959 to 1973 25% plant products dispensed from pharmacies in the United States (plants) which increased in 1996, and sales of botanical medicines increased 37 %. It was reported that 91 plant-derived compounds were taken for clinical trials in between September 2007 (Saklani & Kutty 2008).

Our present research is based on phytochemical screening of ethnomedicinaly important plant *Cassia javanica* Linnaeus and testing bioactivity of its phytoconstituents against multi-drug resistant bacterial isolates. *Cassia javanica* Linnaeus is a flowering plant from family Fabaceae (Chittam & Deore 2013). It is very difficult to state about its place of origin because of its widespread cultivation in all over America, Malaysia and tropical Asia. *Cassia javanica* is widely used in the traditional formulation for the treatment of nosocominal infections within indigenous community (Chittam & Deore 2013). Young leaves have already proved as a hypoglycemic agent and having anti-oxidant activity (Kumavat *et al* 2012; Kaur & Arora 2008). Ripe pods are used as laxative (Ganesan 2008). Moreover, in China and tropical Asian regions it is known as an antimicrobial to decrease pathogenic infections, fever, measles and intestinal problems. Cheng *et al* (2006), in his research stated that *Cassia javanica* has HSV type-2 antiviral activity and was very useful in chickenpox, constipation, gastric pain and cold. The bark and seeds are common home remedies in Thailand for pyretic infections. Phytochemical screening of ethyl acetate and methanol extracts of different parts of *C. javanica* testifies the presence of its active phytoconstituents and their sensitivity tests proved it to be a potent bactericidal antimicrobial agent (Pandith 2012; Bauer *et al* 1966).

MATERIALS AND METHODS

Plant material collection and preparation

Different parts of *Cassia javanica* plant, bark, leaves and flowers were collected separately from Assam University campus, Silchar, at spring time, as its flowering season is from April to June. Mounted herbarium specimen was prepared in Natural Product Process Laboratory under Central Instrumentation Laboratory, Assam University, Silchar, Assam. Collected plant materials were air-dried under shade at room temperature. Completely dried materials were ground to get fine powder and were dipped in different solvents according to their polarity gradient – hexane, ethyl acetate and methanol by the maceration technique at room temperature (Handa *et al* 2008). 160 g powder was dipped in 500 ml solvent for 3 days. The dipped bottles were shaken and stirred at an interval of 24 hour. Last day, Whatman filter papers were used to filter them and stored in the refrigerator at 4° C for further use (Adegoke *et al* 2010).

Phytochemical screening

Phytochemical screening of all crude extracts was performed under specific chemical test methods described by different authors (Rajesh *et al* 2010; Pandith 2012). Hexane extracts failed in the screening test but ethyl acetate and methanol extracts of different parts of *Cassia javanica* gives a positive indication about the presence of alkaloid, saponin, tannin, steroid, reducing sugar, flavonoid and anthraquinone.

Bacteria collection and culture

Pathogenic Gram positive cocci were collected from Silchar Medical College under the supervision of Dr. Atanu Chakraborty. Samples were directly collected from patients; cultured and isolated pure cultures were preserved in -20° C deep freezer by pure culture preservation process (Benson 2001). Test organisms include *Staphylococcus aureus ATCC* 25923 and 5

pathogenic strains of *Staphylococcus aureus*. All biochemical tests were performed for their confirmation. All chemicals were purchased from HiMedia. Nutrient agar, Macconkey agar, Simmon citrate agar, Cetrimide agar, Mannitol salt agar was used for their culture. Liquid culture media were peptone water and Muller hinton broth medium.

Antimicrobial disc preparation and activity test

Antimicrobial discs were prepared by punching Whatman filter paper no. 1 at 6 mm diameter, soaked in different volumes of concentrated plant extract at a potency of 1 µg and 5 µg, dried and sterilized in a UV chamber at 365 nm. Vancomycin 30µg paper discs were used as positive control and sterilized discs (without antimicrobial treatment) were used as a negative control. One freshly isolated bacterial colony was transferred to the sterilized Muller Hinton broth medium and incubated at 37° C for 24 hours for visible growth. Bacterial growth turbidity was standardized with 0.5 McFarland standards (1-2 x 10^8cfuml^{-1}) (Isenberg 2007). Muller hinton agar medium was used for bacterial sensitivity determination by Kirby-Bauer method. Bacteria were spread over the plate by lawn culture technique and after 3 – 4 minutes, concentrated antimicrobial disc, positive control and negative control were placed over it. Plates were incubated overnight at 37° C for 24 hours. Next day, results and relative inhibitory zone formation was determined by the formula (Chia & Yap 2011):

$$\% \text{ RIZD} = \frac{\text{IZD of sample} - \text{IZD negative control}}{\text{IZD of antibiotic agent}}$$

,IZD= inhibition zone diameter
RIZD= relative inhibition zone diameter

The antimicrobial disc potency was determined by the formula- $W = \frac{1000}{P} \times V \times C$. Here W= weigh of antibiotic (mg) dissolve in volume (ml), C= final concentration of the solutions. P= potency of the antimicrobial disc (Wiegand *et al* 2008). All extracts of *Cassia javanica* exhibits positive results only against *Staphylococcus aureus*.

RESULT AND DISCUSSION

Medicinal plants have always proved to be the source of bioactive therapeutic phytochemicals based on their traditional belief and medicinal properties. *Cassia javanica* is a traditionally believed herbal pant used against numerous infectious diseases. Phytochemical screening test of ethyl acetate and methanol extract of different parts of *Cassia javanica* showed the presence of alkaloid, reducing sugar, saponnin, tannin, flavonoid and anthraquinone as shown in Table 1.

Hexane extracts of flower and leaf failed to show any indication of the presence of active metabolites but hexane extract of bark showed the presence of anthraquinone. In this screening test, ethyl acetate extract of flower (EF) indicated the presence of steroid and flavonoid, ethyl acetate extract of bark (EB) showed the presence of reducing sugar, steroid, tannin, flavonoid and anthraquinone and lastly, ethyl acetate extract of leaf (EL) showed the presence of single steroid metabolite. Methanol extract of flower (MF) and methanol extract of bark (MB) both indicate the presence of alkaloid, steroid, saponin, tannin and antroquinones. Methanol extract of leaf (ML) results in the presence of all phytoconstituents like alkaloid, reducing sugar, steroid, saponnin, tannin, falvenoid and anthraquinone as shown in Table 1.

Nine different broad spectrum and narrow spectrum antibiotics were purchased from HiMedia and were tested against *Staphylococcus aureus* ATCC 25923 and 5 different strains of *S. aureus* to find out the recent multi-drug resistant strains. Four Penicillin resistant *S. aureus* (PRSA) and three Oxacillin resistant *S. aureus* (ORSA) strains were identified which is listed below in Table 2 along with their inhibition zone diameter. Vancomycin with inhibition zone diameter 15 mm against *S. aureus* is reported as Vancomycin intermediate *Staphylococcus aureus* (VISA).

Table 1: Phytochemical screening results of hexane, ethyl acetate and methanol extracts of flower, leaf and bark extracts of *Cassia javanica* Linnaeus

Plant material (160gm)	Solvent (500ml)	Polarity	Alkaloid	Reducing sugar	Steroid	Saponin	Tannin	Flavonoid	Anthra-quinone
Flower	Hexane	0.01	-	-	-	-	-	-	-
	Ethyl acetate	4.4	-	-	+	-	-	+	-
	Methanol	5.1	+	-	+	+	+	-	+
Bark	Hexane	0.01	-	-	-	-	-	-	+
	Ethyl acetate	4.4	-	+	+	-	+	+	+
	Methanol	5.1	+	-	+	+	+	-	+
Leaf	Hexane	0.01	-	-	-	-	-	-	-
	Ethyl acetate	4.4	-	-	+	-	-	-	-
	Methanol	5.1	+	+	+	+	+	+	+

Table 2: Sensitivity test results of *Staphylococcus aureus* ATCC 25923 and 5 pathogenic *S. aureus* strains against 9 different marketed antibiotics. Inhibition zone diameter with interpretation R: resistant, I: intermediate and S: sensitive.

Product code	Antimicrobial agent	Disc content (µg)	*S. aureus ATCC* 25923	*S. aureus* 1 (mm)	*S. aureus* 2 (mm)	*S.aureus* 3 (mm)	*S. aureus* 4 (mm)	*S. aureus* 5 (mm)
SD060	Ciprofloxacin	5	24	14 (R)	18 (I)	17 (I)	30 (S)	29 (S)
SD073	Imipenem	10	48	37 (S)	38 (S)	40 (S)	40 (S)	40 (S)
SD010	Co-trimoxazole	25	24	17 (S)	19 (S)	19 (S)	26 (S)	24 (S)
SD037	Tetracycline	30	26	26 (S)	27 (S)	25 (S)	27 (S)	30 (S)
SD045	Vancomycin	30	20	15 (I)	17 (S)	16 (S)	16 (S)	17 (S)
SD215	Linezolid	30	30	26 (S)	27 (S)	25 (S)	27 (S)	31 (S)
SD013	Erythromycin	15	26	13 (R)	14 (R)	(R)	24 (S)	27 (S)
SD028	Penicillin G	10	35	(R)	10 (R)	(R)	31 (S)	10 (R)
SD088	Oxacillin	1	16	(R)	9 (R)	9 (R)	11 (S)	12 (S)

In sensitivity test of disc agar diffusion method all ethyl acetate and methanol extracts of *Cassia javanica* other than ethyl acetate extract of leaf shows a very good potential bacterial inhibition zone diameter at a disc potency of 1 µg and 5 µg. Noticeable increase in the zone diameter was observed as the disc potency increases. Relative inhibition zone diameter (RIZD) was calculated in comparison to Vancomycin (30 µg).

The ethyl acetate extract of bark and flower has the highest relative inhibition zone diameter 87.5 against *S. aureus* 4 at disc potency 5 µg. Methanol extract of leaf (ML) has the lowest activity 37.5 against *S. aureus* 3. The inhibition zone diameter and relative inhibition zone diameter of MB, EB, Mf, EF and ML against all Gram positive *Staphylococcus aureus* are listed in Table 3. Ethyl acetate extract of leaf did not show any sign of antibacterial activity when performed.

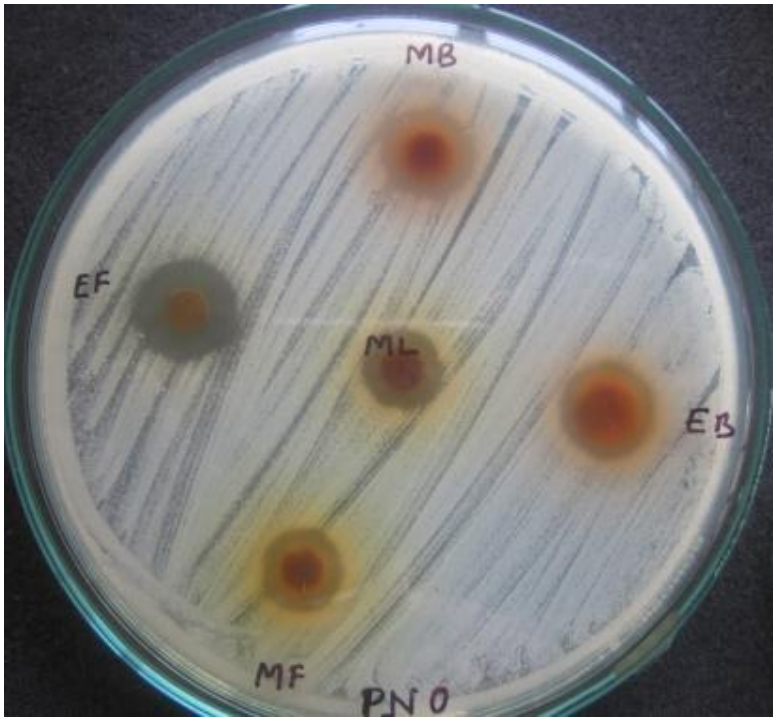

Figure 1: Inhibition zone diameter of all extracts

Table 3: Inhibition zone diameter (IZD) and Relative inhibition zone (RIZD%) of MB: methanol extract of bark, EB: ethyl acetate extract of bark, MF: methanol extract of flower, EF: ethyl acetate extract of flower and ML: methanol extract of leaf against *Staphylococcus aureus* ATCC 25923 and 5 pathogenic *S. aureus* strains. Vancomycin: positive control, sterilized paper disc: negative control

Relative Inhibition Zone Diameter (RIZD%) of different extracts of *Cassia Javanica* L. in comparison with Vancomycin												**Controls**	
Organisms	**Disc potency (µg)**	**MB**		**EB**		**MF**		**EF**		**ML**		**Positive**	**Negative**
		IZD	**RIZD%**	**IZD**	**RIZD%**	**IZD**	**RIZD%**	**IZD**	**RIZD%**	**IZD**	**RIZD%**	**Vancomycin (30µg)**	**Paper disc**
***S. aureus* ATCC 25923**	1	14	70	14	70	11	55	11	55	9	45	20	0
	5	16	80	15	75	14	70	14	70	11	55		
***S. aureus* 1**	1	10	66.7	11	73.3	10	66.6	12	80	7	46.6	15	0
	5	12	80	12	80	12	80	12	80	8	53.3		
***S. aureus* 2**	1	11	64.7	11	64.7	10	58.8	11	64.7	7	41.1	17	0
	5	12	70	13	76.4	13	76.4	12	70.5	9	52.9		
***S. aureus* 3**	1	11	68.7	11	68.7	11	68.7	12	75	0	0	16	0
	5	13	81.2	13	81.2	12	75	13	81.2	6	37.5		
***S. aureus* 4**	1	11	68.7	13	81.2	12	75	13	81.2	7	43.7	16	0
	5	13	81.2	14	87.5	12	75	14	87.5	8	50		
***S. aureus* 5**	1	11	64.7	12	70	10	58.8	12	70.5	0	0	17	0
	5	11	0.64	14	82.3	12	70.5	14	82.3	0	0		

The reported Phytochemical screening test of *Cassia javanica* and its antibacterial activity in comparison with recent antibiotic drug Vancomycin proved that *Cassia javanica*,

a tradition herbal medicinal plant has the most potent bactericidal activity against multi-drug resistant *Staphylococcus aureus* nosocominal strains. This species could be a source of most promising future antibacterial drug in antibacterial drug discovery research as plant derived natural products already have been proved as novel antibacterial drugs. Scientific investigation of this research is based on the traditional knowledge of the indigenous people about the medicinal plants of north east India.

CONCLUSION

Now, it may be concluded that *Cassia javanica* Linnaeus is a medicinal plant of northeast India with a very high antibacterial activity and isolation identification and characterization secondary metabolites from from this species is taken under investigation for further research.

Acknowledgements

Authors acknowledge the services received from the Bioinformatics Center and DBT-Biotech Hub of Assam University, Silchar.

LITERATURE CITED

Adegoke, A.A.; Iberi, P.A.; Akinpelu, D.A.; Aiyegoro, O.A. & Mboto, C.I. 2010. Studies on phytochemical screening and antimicrobial potentials of Phyllanthus amarus against multipl antibiotic resistant bacteria. *Inter. J. Appl. Res. Nat. Prod.* 3(3): 6 – 12.

Ahmad, I. & Beg, A. Z. 2001. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. *J. Ethnopharm.* 74: 113 – 123

Bauer, R.W.; Kirby, M.D.K.; Sherris, J.C. & Turck, M. 1966. Antibiotic susceptibility testing by standard single disc diffusion method. *Amer. J. Clin. Pathol.* 45: 493 – 496.

Benson, 2001. Microbiological Applications Lab Manual, 8th Edition. The McGraw"Hill Companies.

Cheng, H.Y.; Yang, C.M.; Lin, T.C.; Shieh, D.E. & Lin, C.C. 2006. ent-Epiafzelechin-(4aR8)-epiafzelechin extracted from Cassia javanica inhibits herpes simplex virus type 2 replication. *J. Med. Micro.* 55: 201 – 206.

Chia, Y.Y. & Yap, W.S. 2011. *In vitro* Antimicrobial Activity of Hexane: Petroleum Ether Extracts from Fruits of *Momordica charantia* L. *Inter. J. of Phar. & Biol. Arch.* 2(3): 868 – 873.

Chittam, K.P. & Deore, S.L. 2013. *Cassia Javanica Linn*: A Review on Its Phytochemical and Pharmacological Profile. *J. Biomed. Phar. Res.* 2(1): 33 – 35.

Ganesan, S. 2008. Traditional oral care medicinal plants survey of Tamil Nadu. *Nat. prod. Radi.* 7(2): 166 – 172.

Handa, S.S.; Khanuja, S.P.S.; Longo, G. & Rakesh, D.D. 2008. *Extraction Technologies for Medicinal and Aromatic Plants*. International centre for science and high technology, Trieste.

Isenberg, H.D. 2007. *Clinical microbiology procedures handbook*. Washington, DC.

Kaur, P. & Arora, S. 2008. Comparison of antioxidant activity of different methanol extracts of *Cassia* and *Bauhinia* sp. *J. Chin. Clin. Med.* 5 (8): 457 – 462.

Kumavat, U.C.; Shimpi, S.N. & Jagdale, S.P. 2012. Hypoglycemic activity of *Cassia javanica* Linn. in normal and streptozotocin-induced diabetic rats. *J. Adv. Pharm. Tech. Rese*. 3(1): 47 – 51

Newman, D.J.; Cragga, G.M. & Snaderb, K.M. 2000. The influence of natural products upon drug discovery. *Nat. Prod. Rep*. 17: 215 – 234.

Pandian, M.R.; Banu, G.S. & Kumar, G. 2006. A study of the antimicrobial activity Alangium salviifolium. *Indian J. Pharm.* 38: 203 – 204.

Pandith, J.I. 2012. Phytochemical screening of certain plant species of Agra city. *J. Drug Deli. Ther.* 2(4): 135 – 138.

Rajesh, P.; Latha, S.; Selvamani, P.V. & Kannan, R. 2010. Phytochemical Screening and Toxicity Studies on the Leaves of Capparis sepiaria Linn. (Capparidaceae). *J. Basic Clin. Phar.* 1(1): 41 – 46.

Saklani, A. & Kutty, S. K. 2008. Plant-derived compounds in clinical trials. *Elsevier, Drug Disc. Tod.* 13: 161 – 171.

Ushimaru, P.I.; da Silva, M.T.N.; Stasi, L.C.D.; Barbosa, L. & Junior, A. F. 2007. Antibacterial activity of medicinal plant extracts. *Braz. J. Micro.* 38: 717 – 719.

Wiegand, I.; Hilpert, K.. & Hancock, R.E.W. 2008. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat. prot.* 3: 163 – 175.