Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 7 (3): 150-157, 2013
ISSN 1819-3455 / DOI: 10.3923/rjmp.2013.150.157

Phytochemical Screening and Antimicrobial Activity of Anthraquinones Isolated from Different Parts of *Cassia nodosa*

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ABSTRACT

The study aims in investigating the phytochemical screening and antimicrobial activity of isolated anthraquinones from different parts of *Cassia nodosa* against various bacterial and fungal strains to confirm their effective use in different traditional medicines against these pathogenic strains. Fresh plant parts of *C. nodosa* were collected, dried at room temperature, powdered and extraction of anthraquinones were done using standard protocol. Extracted compounds were screened by UV, IR, ^{1}HNMR, ^{13}CNMR techniques. Agar well diffusion method was used to test the antimicrobial activity of isolated anthraquinones against pathogenic microbial strains. The phytochemical screening revealed the presence of Emodin and Rhein in the plant. Maximum level of Emodin (0.66 mg gdw^{-1}) and Rhein (0.30 mg gdw^{-1}) was observed in leaves. The crude compounds exhibited a significant antimicrobial activity. The emodin demonstrated maximum activity against *F. moniliforme* (IZ = 26.00 mm) while rhein was more active against *A. flavus* (IZ = 21.00 mm). The inhibitory effects of Emodin and Rhein extracted from various parts of *C. nodosa* against various pathogenic micro-organisms had clearly demonstrated the usefulness of *C. nodosa* in the treatment of various diseases caused by these pathogenic strains.

Key words: *Cassia nodosa*, anthraquinones, antimicrobial activity, microdilution method

INTRODUCTION

Ayurveda recommends the application of different parts of plant species in the forms of paste, powder, juice and decoction for treatment of various infections. Despite of tremendous progress in human medicines, infectious diseases caused by the bacteria, fungi, viruses and parasites are still a major threat to the public health. *S. aureus* and *E. coli* which causes frequent urinary tract infections, are the most common organisms used by other workers (Roia and Smith, 1977) to check bactericidal activity; also while, *P. aeruginosa* (Urine infection) and *S. typhi* (Intestinal infection) chosen as the test organisms because of their high resistance to the antibacterial agents. Thus any plant which could exhibit pronounced activity against either of these organisms might yield an important antibiotic (Noriaki *et al*., 2005).

Anthraquinone glycosides are important cathartic compounds. They are used as purgatives and are widely employed in gastriatic and pediatric medicines for their unique pharmacological effects (Sinha and Verma, 1994). Anthraquinone derivatives have also been widely used in the analytical chemistry, mainly as strong chelating agents and chromophores (Hatano *et al*., 1999; Uddin *et al*., 2003; Jiang *et al*., 2005).

Cassia nodosa commonly called pink *Cassia* is a common ornamental tree belonging to the family Leguminosae. It is a perennial tree 3-5 m height and scattered in the India, Pakistan, Bangladesh and Burma. Traditionally it is useful in the indigenous medicine, as its pods and leaves showed purgative activities and cytotoxic activity (Rizk and Heiba, 1990). Bark of *C. nodosa* is used as one of the ingredients in antidiabetic ayurvedic formulation. Leaves are proved to be active against Herpes simplex infection (Cheng *et al*., 2006) and was moderately active against *P. aeruginosa* and *S. epidermidis* (Mohtar and Shaari, 2000). The presence of these antidiabetic phytochemicals of *C. nodosa* leaves may give desired pharmacological action. It shows hyperglycemic effect on normal and streptozotocin induced diabetic rats (Kumavat *et al*., 2012). *C. occidentalis* showed antimicrobial activity against various human pathogenic strains (Arya and Yadav, 2010; Yadav *et al*., 2010). Different plant parts of *Cassia* also showed varying degree of antioxidant activity (Arya *et al*., 2010, 2011).

So far, anthraquinones have been isolated and characterized from *C. Senna* (Rai, 1978; Abo *et al*., 2000), *C. laevigata* (Singh *et al*., 1980), *C. angustifolia* (Lemli *et al*., 1983; Fernandes *et al*., 1982; Kitanaka *et al*., 1985), *C. multijuga* (Singh, 1981), *C. tora* (Takahashi *et al*., 1981), *C. javanica* (Tiwari and Sharma, 1981; Singh and Singh, 1988), *C. alata* (Rai and Shok, 1982; Yadava *et al*., 1998), *C. sophera* (Malhotra and Misra, 1982), *C. obtusifolia* (kitanaka and Tokedo, 1985), *C. marginata* (Singh and Singh, 1987), *C. fistula* (Ahuja *et al*., 1988), *C. grandis* (Verma and Sinha, 1996; Verma *et al*., 1997) and *C. nigricans* (Ayo *et al*., 2007), both *in vivo* and *in vitro* but any systematic study has been lacking, both qualitatively and quantitatively, in the selected *Cassia* species.

MATERIALS AND METHODS

Collection and identification: *Cassia nodosa* Bunch is an ornamental tree belonging to family Leguminosae, popularly known as Pink Shower. Plant species were collected from Jaygragh fort of Amer at Jaipur. The plant was identified at Herbarium, Department of Botany, University of Rajasthan, Jaipur and given a specified voucher specimen No.

Processing and extraction: The all plant parts (root, stem, leaves, flowers and pods) of *Cassia nodosa* collected and were studied for antraquinones composition. Plant samples were first extracted in chloroform (50 mL g^{-1}) for 30 min (Rai *et al*., 1974) on water bath. Subsequently, the chloroform extract were filtered and the filtrate was evaporated to dryness, which was then takenup with 10 mL of 5% NaOH in 2% NH_3 solution (v/v). The reconstituted fraction was then Chromatographed on TLC (Silica gel; 1-propanol-ethyl acetate-water; along with standard markers (Harborne, 1973). The developed chromatograms were air-dried. Visualized under UV light alone and in presence of ammonia fumes and then sprayed with 5% (w/v) ethanolic KOH.

Two spots coinciding to the reference Emodin (R_f 0.96) and Rhein (R_f 0.78) were isolated by TLC, eluted and purified. Later, the isolated compounds were identified by using MP, UV, IR and NMR spectroscopy. Using spectrophotometric methods, the quantification of total anthraquinones (free and glycosidic) was made.

Sources of microorganisms: Standard strains of *Staphylococcus aureus* (ATCC 25923), *Salmonella typhi* (ATCC 13311), *Escherichia coli* (ATCC 5922) and *Pseudomonas aeruginosa* (ATCC 25922) were obtained from Microbiology Lab., SMS medical College, Jaipur and were grown on nutrient agar culture medium at 37°C for 24 h and fungi, *Aspergillus flavus*, *Aspergillus niger*,

Fusarium monilliformae and *Rhizoctonia betaticola* were obtained from seed pathology lab, Department of Botany, University of Rajasthan, Jaipur and grown on Potato Dextrose Agar (PDA) medium at 30°C for 48 h.

Antimicrobial activity: The test micro-organisms were grown in nutrient broth (Difco Co.) at 37°C for bacteria and Potato Dextrose Agar at 30°C for fungi, respectively. The Disc Diffusion method was used to determine the anti-microbial activities of the isolated anthraquinones. 6 mm discs were prepare from what man's filter paper No. 1. Solutions of varying concentrations ranging from 1.0×10^4 to 5.0×10^4 mg mL^{-1} $disc^{-1}$ were prepared. They were also prepared using the pure extruding solvent for each extract. The treated discs were air dried at room temperature to remove any residual solvent which might interfere with the determination. Culture supplemented with gentamycin and mycostatin were used as control. These were then placed over petriplates which were previously seeded with 100 µL suspension of each of microbial species. These plates were later incubated at 37°C for 24 h in case of bacteria and 48 h for fungi, after which the zone of inhibition could be easily observed and measured. Antimicrobial activity was expressed as the diameter of the zone of inhibition.

The Minimum Inhibitory Concentration (MIC) of the crude compounds was estimated by microdilution method (Jones and Barry, 1987). The dilutions of crude compounds was prepared ranging from 1×10^3 to 1×10^5 mg mL^{-1} and poured in presterlized molten nutrient agar and potato dextrose. The medium was then poured into sterile petridishes. The surface of the medium was allowed to dry before streaking with 24 h old microbial cultures. The plates were later incubated for 48 h and they were examined for the presence or absence of growth. The lowest concentration of the sample required to inhibit the growth of the test organism was recorded for each organism as the MIC. The All data are presented as mean values of five replicate of each microorganism.

RESULTS AND DISCUSSION

In the present investigation, anthraquinone profile has been studied *in vivo* of *C. nodosa*, where Emodin (R_f 0.96) and Rhein (R_f 0.78) from different plant parts of *C. nodosa* have been evaluated by chromatographic, spectroscopic and color reactions. The silica gel TLC of the fractions showed the presence of two anthraquinones after spray of 5% KOH in EtOH and in UV light and I_2 vapours (Table 1).

The compounds eluted from TLC were pooled together according to their TLC behaviour and isolated with the solvents and evaporated yielding two anthraquinones viz., Emodin (R_f 0.96) and Rhein (R_f 0.78). The spectral analyses of the active constituent, Spectra-1-Emodin and Spectra-2-Rhein from the different plant parts of selected *C. nodosa* plant parts are summersied in Table 2.

Table 1: Chromatographic data of the anthroquinones isolated from the selected *Cassia* species

	Rf ×100 in solvents			Colour			
Anthraquinones	PrEw$^+$	*BEA	BA^{++}	Day-light	UV light	I_2 vapours	Colour with chromatographic spray 5% KOH in ethanol
Emodin	96	97	96	YW	OR	YW-BN	Pink
Rhein	78	81	41	LT-OR	OR	OR-BN	Yellow

Abbreviations: $^+$PrEW: 1-Propanol, Ethyl acetate: Water (4:4:3), *BEA: Benzene: Ethyl acetate: Acetic acid (75:24:1), $^{++}$BA: Benzene: Acetic acid (1:1), BN: Brown, OR: Orange, LT: Light, YW: Yellow

Table 2: Spectral studies of isolated glycosidic anthraquinone from selected *Cassia* species

Name of compound	UV light absorption band	IR : v cm^{-1} max KBr	^{1}HNMR	^{13}C NMR
Emodin	264 sh, 295 sh, 345 sh, 510 sh	3245(-OH), 1677, 1627(C = O), 1625, 1580(C = C) cm^{-1}	2.35 (H_1), 7.02 (H_2), 2.35 (H_3) 5.25 (H_4), 2.35 (H_5), 7.07 (H_6) 2.35 (H_7), 7.24 (H_8), 5.53 (H_9) 7.24 (H_{10}), 3.48 (H_{11}), 3.94 (H_{12})	22.3 (C_1), 135 (C_2), 129.6 (C_3), 137.4 (C_4) 138 (C_5), 22.23 (C_6), 123.3 (C_7), 138.4 (C_8) 137.4 (C_9), 129.6 (C_{10}), 135.0 (C_{11}), 124.4 (C_{12}) 134.6 (C_{13}), 148.3 (C_{14}), 101.3 (C_{15})
Rhein	208 sh, 245 sh, 355 sh, 510 sh	3410 (OH),1627 (C = O),1625, 1580 (C = C) cm^{-1}	2.35 (H_1), 7.07 (H_2), 2.35 (H_3) 5.25 (H_4), 7.27 (H_5), 7.1 (H_6) 7.44 (H_7), 7.27 (H_8)	173.0 (C_1), 135.0 (C_2), 137.4 (C_3), 138.4 (C_4) 22.2 (C_5), 123.3 (C_6), 141.4 (C_7), 137.5 (C_8) 128.9 (C_9), 125.8 (C_{10}), 123.7 (C_{11}), 134.7 (C_{12}) 148.3 (C_{13}), 134.6 (C_{14}), 124.4 (C_{15})

Table 3: Isolated anthroquinones content (mg gdw^{-1}*) in different plant part of selected *Cassia* species

	C. nodosa				
Anthraquinones type	Root	Stem	Leaves	Flower	Pods
Emodin	0.13	0.23	0.66	0.10	0.14
Rhein	0.22	0.25	0.30	0.13	0.17
Total anthraquinones	0.35	0.48	0.96	0.23	0.31

While assessing the levels of anthraquinones in different plant parts of *C. nodosa*, maximum levels of anthraquinones was measured in leaves (0.96 mg gdw^{-1}) and minimum levels in flowers (0.23 mg gdw^{-1}) as shown in Table 3. According to the nonadaptive hypothesis, the distribution of secondary metabolites within organs may be roughly equivalent to the distribution of the primary metabolic pathways responsible for the creation of the secondary metabolite (as a byproduct) and thus they do not necessarily have an adaptive function in each organ (Eriksson and Ehrlen, 1998). The allocation of emodin and rhein in various plant organs and the unequal distribution of their concentration among them may in itself provide circumstantial evidence for their multifunctionality in plants. Previously also the plant *C. nodosa* has reported the presence of many usual and novel anthraquinones (El-Sayyad and Ross, 1983; Singh and Singh, 1988, 2008; Yadava *et al.*, 1998; Sanghi *et al.*, 1999).

The isolated anthraquinones are also effective against all test organisms. But emodin shows maximum activity against *F. moniliforme* (IZ = 26.00 mm) and minimum activity was recorded against *S. aereus* (IZ = 6.50 mm). Similarly the rhein was more active against *A. flavus* (IZ = 21.00 mm) and less activity against *S. aureus* (IZ = 9.00 mm). But emodin was inactive against *S. typhi* (Table 4).

The antimicrobial activity of isolated two compounds were carried out by microdilutiom method against selected fungal and bacterial microorganisms and compared with commercially available antibiotics. Table 5 shows the MIC basically, the MIC value indicates the potential of each extract to inhibit the microbial growth at lowest concentration for isolated anthraquinones against test micro organisms recorded in mg $disc^{-1}$ of the diametrical sections of the respective zones of inhibition for each metabolite. Emodin and Rhein MIC value 2×10^{3} mg $disc^{-1}$ was recorded against *E. coli*, *S. aureus*, *P. aeurginosa*, *A. flavus*, *A. niger* and *F. monilifome* but MIC value of 3×10^{3} mg $disc^{-1}$ was recorded for *S. typhi* and *R. bataticola*. It has been demonstrated that anthraquinones, extracted from different species of Aloe, exhibit antibacterial activity by inhibition of nucleic acid synthesis in *Bacillus subtilis* (Levin *et al.*, 2006). Anthraquinones or quinones are aromatic are in nature with two ketone substitution. They are ubiquitous in nature and are highly

Table 4: Inhibition zone (mm) of isolated bioactive compounds from *Cassia nodosa* against different bacterial and fungal strains

	Anthraquinone	
Test organisms	Emodin	Rhein
A. Bacterial		
E. coli		
IZ	21.00	21.00
AI	0.74	0.74
S. aureus		
IZ	6.00	9.00
AI	0.20	0.36
P. aeruginosa		
IZ	23.00	14.00
AI	0.91	0.50
S. typhi		
IZ	-	16.00
AI	-	0.42
B. Fungi		
A. flavus		
IZ	21.00	10.00
AI	0.85	0.50
A. niger		
IZ	9.00	10.00
AI	0.56	0.60
R. bataticola		
IZ	8.00	18.00
AI	0.40	0.64
F. moniliforme		
IZ	26.00	28.00
AI	1.06	1.30

Table 5 : Zones of inhibition of different concentration (MIC) of anthraquinones (mg mL^{-1})

	Emodin (mg mL^{-1})					Rhein (mg mL^{-1})				
Tested microorganisms	1×10^3	2×10^3	3×10^3	4×10^3	5×10^3	1×10^3	2×10^3	3×10^3	4×10^3	5×10^3
Bacteria										
E. coli	-	+	+	+	+	-	+	+	+	+
S. aureus	-	+	+	+	+	-	+	+	+	+
P. aeurginosa	-	+	+	+	+	-	+	+	+	+
S. typhi	-	-	-	+	+	-	-	±	+	+
Fungi										
A. flavus	-	+	+	+	+	-	+	+	+	+
A. niger	-	+	+	+	+	-	+	+	+	+
R. bataticola	-	-	-	+	+	-	-	±	+	+
F. moniliformae	-	+	+	+	+	-	+	+	+	+

reactive. The potential range of quinine for antimicrobial effect is excellent as there occur switch between diphenols and diketone occur easily through redox reactions. Quinines are known to complex irreversibly with nucleophillic amino acids in proteins often leading to inactivation of protein and loss of function (Sher, 2009). Probable targets of anthraquinones in the microbial cell

are surface exposed adhesions; membrane bound enzymes and cell wall polypeptides. Quinones also render substrates unavailable to enzymes. Anthraquinones act to the gastrointestinal tract to increase peristaltic action.

World Health Organization (WHO) encourages examining traditional medicine with a view to identifying and exploiting aspects that provide safe remedies for different diseases (Akinyemi *et al*., 2005; Uwumarongie *et al*., 2007). The investigated results obtained from this study support the WHO recommendations as it provides scientific evidence that the various plant parts of *Cassia nodosa* have antimicrobial activity. The anthraquinones viz., emodin and rhein isolated from this have demonstrated powerful effects against selected pathogenic bacterial and fungal strains.

CONCLUSION

The phytochemical screening of the different parts of the plant *Cassia nodosa* revealed the presence of two major anthraquinones viz. Emodin and Rhein. The antimicrobial activities of these crude compounds showed a broad spectrum action towards range of bacterial and fungal strains with the MIC values ranging from 1×10^{-3} to 1×10^{-5} mg mL^{-1}. The ability of these compounds to inhibit the growth of tested microbial isolates has confirmed the effectiveness of *C. nodosa* for the treatment of various human diseases caused by these pathogenic strains.

There are still many *C. nodosa* anthraquinones and their derivatives, whose pharmacological activities have not yet been investigated. It is possible that they may contain beneficial pharmacological properties. Therefore, *in vitro* and *in vivo* investigations regarding their effects could provide insight into benefits of *C. nodosa* for future clinical management of many human diseases. The synergistic effects of these different anthraquinones may partly explains their use in combinational traditional medicines in Palestine against a number of infections for generations (Sakagami *et al*., 2001; Ayo and Amupitan, 2004; Ayo *et al*., 2007).

REFERENCES

Abo, K.A., A.A. Adeyemi and I.A. Jegede, 2000. Spectrophotometric estimation of anthraquinone content and antimicrobial potential of extracts of some *Cassia* species used in herbal medicine in Ibadan. Sci. Forum., 3: 57-63.

Ahuja, A., R. Parshad and J.P. Kaushik, 1988. Anthraquinones from callus culture of *Cassia fistula* L. Fitoterapia, 59: 496-497.

Akinyemi, K.O., O. Oladapo, O. Okwara, C.C. Ibe and K.A. Fasure, 2005. Screening of crude extracts of six medicinal plants used in South West Nigeria Unorthodox medicine for antimethicillin resistant *Staphylococci aureus* activity. Complemen. Alter. Med., Vol. 5, 10.1186/1472-6882-5-6

Arya, V. and J.P. Yadav, 2010. Antioxidant activity and total phenolics in leaves extract of *Cassia tora* L. Pharmacologyonline, 2: 1030-1036.

Arya, V., S. Yadav, S. Kumar and J.P. Yadav, 2010. Antimicrobial activity of *Cassia occidentalis* L (leaf) against various human pathogenic microbes. Life Sci. Med. Res., 2010: 1-11.

Arya, V., S. Yadav, S. Kumar and J.P. Yadav, 2011. Antioxidant activity of organic and aqueous leaf extracts of *Cassia occidentalis* L. in relation to their phenolic content. Nat. Prod. Res., 25: 1473-1479.

Ayo, R.G. and J.O. Amupitan, 2004. Antimicrobial activity screening of crude extract from leaves of *Cassia nigricans* Vahl. Chem. Class J., 1: 24-26.

Ayo, R.G., J.O. Amupitan and Y. Zhao, 2007. Cytotoxicity and antimicrobial studies of 1,6,8-trihydroxy-3-methyl-anthraquinone (emodin) isolated from the leaves of *Cassia nigricans* Vahl. Afr. J. Biotechnol., 6: 1276-1279.

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

El-Sayyad, S.M. and S.A. Ross, 1983. A phytochemical study of some *Cassia* species cultivated in Egypt. J. Nat. Prod., 46: 431-432.

Eriksson, O. and J. Ehrlen, 1998. Secondary metabolites in fleshy fruits: Are adaptive explanations needed? Am. Naturalist, 152: 905-907.

Fernandes, P., C.K. Kokate and H.P. Tipnis, 1982. Anthraquinones from tissue culture of *Cassia angustifolia* Vahl. Indian Drugs, 19: 442-445.

Harborne, J.B., 1973. Phytochemical Methods. Chapman and Hall Ltd., London, Pages: 80.

Hatano, T., H. Uebayashi, H. Ito, S. Shiota, T. Tsuchiya and T. Yoshida, 1999. Phenolic constituents of *Cassia* seeds and antibacterial effect of some naphthalenes and anthraquinones on methicillin-resistant *Staphylococcus aureus*. Chem. Pharm. Bull., 47: 1121-1127.

Jiang, T.F., Z.H. Lv and Y.H. Wang, 2005. Separation and determination of anthraquinones in *Cassia obtusifolia* (Leguminosae) by micellar electrokinetic capillary electrophoresis. J. Sep. Sci., 28: 2225-2229.

Jones, R.N. and A.L. Barry, 1987. The antimicrobial activity of A-56268 (TE-031) and roxithromycin (RU965) against *Legionella* using broth microdilution method. J. Antimicrob. Chemother., 19: 841-842.

Kitanaka, S. and M. Takido, 1985. Anthraquinoids from *Cassia nomame*. J. Nat. Prod., 48: 849-849.

Kitanaka, S., F. Kimura and M. Takido, 1985. Studies on the constituents of the seeds of *Cassia obtusifolia* Linn. The structures of two new anthraquinone glycosides. Chem. Pharm. Bull., 33: 1274-1276.

Kumavat, U.C., S.N. Shimpi and S.P. Jagdale, 2012. Evaluation of hypoglycaemic activity of *Cassia nodosa* leaves in normal and streptozotocin-induced diabetic rats. Int. J. Green Pharmacy, 6: 9-13.

Lemli, J., J. Cuveele and E. Verhaeren, 1983. Chemical identification of Alexandrian and tinnevelly senna: Studies in the field of drugs containing anthracene derivatives XXXIV. Planta Med., 49: 36-37.

Levin, H., R. Hazenfratz, J. Friedman, D. Palevitch and M. Perl, 2006. Partial purification and some properties of an antibacterial compound from *Aloe vera*. Phytothe. Res., 2: 67-69.

Malhotra, S. and K. Misra, 1982. Anthraquinones from *Cassia sophera* heartwood. Phytochemistry, 21: 197-199.

Mohtar, M. and K. Shaari, 2000. Antimicrobial activity of *Senna alata* and *Cassia javanica* subsp. *nodosa* extracts against micro-organisms related to skin infections. J. Trop. For. Prod., 6: 218-221.

Noriaki, K., S. Satoshi, M. Kyo and F. Tokio, 2005. *In vitro* antifungal and antiviral activities of.GAMMA. and DELTA. lactone analogs utilized as food flavoring. Biocontrol. Sci., 10: 31-36.

Rai, P.P. and M. Shok, 1982. Anthracene derivatives in tissue cultures of *Cassia* species indigenous to Nigeria. Proceedings of the 5th International Congress of Plant Tissue and Cell Culture, July 11-16, 1982, Tokyo, Japan, pp: 277-278.

Rai, P.P., T.D. Turner and G. Greensmith, 1974. Anthracene derivatives in tissue culture of *Cassia senna* L. J. Pharm. Pharmacol., 26: 722-726.

Rai, P.P., 1978. The production of anthraquinones in callus cultures of *Rheum palmatum*. Lioydia, 41: 114-116.

Rizk, A.M. and H.T. Heiba, 1990. Anti-inflammatory and cytotoxic activities of extracts of 30 indigenous plant species growing in Qatar. Intl. J. Crude Drug Res., 28: 89-95.

Roia, F.C. and R.A. Smith, 1977. The antibacterial screening of some common ornamental plants. Econ. Bot., 31: 28-37.

Sakagami, Y., H. Murata, T. Nakanishi, Y. Inatomi and K. Watabe *et al*., 2001. Inhibitory effect of plant extracts on production of verotoxin by enterohemorrhagic *Escherichia coli* O157: H7. J. Health Sci., 47: 473-477.

Sanghi, R., R. Singh and J. Singh, 1999. Two new O-β-D-glycosides from the stem bark of *Cassia javanica*. Indian J. Chem., 38: 521-524.

Sher, A., 2009. Antimicrobial activity of natural products from medicinal plants. Gomal J. Med. Sci., 7: 72-78.

Singh, J., 1981. Phytochemical investigation of *Cassia multijuga* seeds. Isolation and characterisation of new anthraquinone pigments. Planta Med., 41: 397-399.

Singh, J., A.R. Tiwari and R.D. Tiwari, 1980. Anthraquinones and flavonoids of *Cassia laevigata* roots. Phytochemistry, 19: 1253-1254.

Singh, J. and J. Singh, 1987. Two anthraquinone glycosides from *Cassia marginata* roots. Phytochemistry, 26: 507-508.

Singh, J. and J. Singh, 1988. Isolation and characterization of two new anthraquinones from stem bark of *Cassia javanica*. Indian J. Chem., 278: 858-859.

Singh, R. and J. Singh, 2008. A bianthraquinone and anthraquinone from *Cassia javanica* heart wood. J. Indian Chem. Soc., 85: 1159-1162.

Sinha, R.K. and R.P. Verma, 1994. A new anthraquinone from the pods of *Cassia grandis* Linn. J. Indian Chem. Soc., 71: 707-708.

Takahashi, S., M. Takido, S. Yeh, H. Otsuka and Z. Shoyakugaku *et al*., 1981. Formation of anthraquinones, hydroanthracene and flavonoid by the callus culture of *Cassia torosa*. Jpn. J. Pharmacogn., 35: 22-25.

Tiwari, R.D. and M.N. Sharma, 1981. Anthraquinone pigments from *Cassia javanica* seeds. Planta Med., 43: 381-383.

Uddin, M.G., A. Jabbar, M.A. Rashid and C.M. Hasan, 2003. Cytotoxic activity of the ethanolic extract of *Cassia tora* roots and an anthraquinone derivative isolated from the extract. Dhaka Univ. J. Biol. Sci., 12: 95-97.

Uwumarongie, O.H., D.N. Onwukaeme and O. Obasuyi, 2007. Antimicrobial activity of the methanolic leaf extract of *Tabernaemontana pachysiphon* stapf. (Apocynaceae). Niger. J. Nat. Prod. Med., 11: 23-25.

Verma, R.P. and K.S. Sinha, 1996. Anthraquinone β-D-glucoside from *Cassia grandis*. Int. J. Pharmacognosy, 34: 290-294.

Verma, R.B., B.K. Ambasta, G. Prasad and K.S. Sinha, 1997. Isolation and characterisation of new anthraquinnone derivative from *Cassia grandis*. J. Indian Chem. Soc., 74: 428-428.

Yadav, J.P., V. Arya, S. Yadav, M. Panghal, S. Kumar and S. Dhankhar, 2010. *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia, 81: 223-230.

Yadava, N.K., B.K. Singh and A. Kumar, 1998. A new anthraquinone from the root bark of *Cassia nodosa*. Asian J. Chem., 10: 204-206.