INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

ISOLATION AND CHARACTERIZATION OF PHYTOCHEMICALS FROM STEM BARK OF *CASSIA FISTULA* LINN.

Arun K Sharma *, Mahaveer P Dobhal

Natural Products Laboratory, Department of Chemistry, University of Rajasthan, Jaipur, Rajasthan 302004, India

*Corresponding Author Email: draks2612@yahoo.com

Article Received on: 28/03/19 Approved for publication: 22/04/19

DOI: 10.7897/2230-8407.1004150

ABSTRACT

Different parts of the plant *Cassia fistula* Linn. are used in traditional medicine system due to their bioactive compounds. Phytochemical examination on the methanolic extract of *Cassia fistula* Linn. stem bark led to the isolation of 1,8-Dihydroxy-3-methylanthraquinone (A), Lupeol (B), *β*-sitosterol (C) and 1,8-dihydroxy-3-carboxylicanthraquinone (D) bioactive compounds. The structure of these compounds was elucidated on the basis of different type of spectroscopic techniques *i.e* ^{1}H NMR, ^{13}C NMR, IR and MS.

Keywords: Phytochemical, Fabaceae, *Cassia fistula,* stem bark, Biological Activity.

INTRODUCTION

Cassia fistula Linn. belong to Fabaceae family and commonly known as "Amaltas" or "Golden shower". It is extensively cultivated in various countries as an ornamental tree for its beautiful bunches of yellow flowers. The different parts of the plant have high therapeutic value and exert anti-Fungal activity[1], antibacterial activity[1], anti- inflammatory activity[2], antipyretic activity[3], antioxidant activity[4], antiulcer activity[5], anti-fertility[6], hepato- protective activity[7] and wound healing property[4]. It is also used in the treatment of haematemesis, pruritus, leucoderma and diabetes[8]. Phytochemical study of *C. fistula* revealed the presence of anthraquinones, anthraquinone glycosides, flavones and sterols from flowers, heartwood and leaves, anthraquinones and diterpene from pods, anthocyanins, flavanols, flavonol glycoside and isoflavone from fruits, anthraquinones and its glycoside from fruit pulp, flavonoid glycoside and anthraquinones from roots, leucoanthocyanidin, flavones and anthraquinone from sapwood, dimeric esters and anthraquinones from seeds, anthraquinones, coumarins, chromones flavonol glycosides, leucoanthocyanidin, long-chain hydrocarbons, sterols and terpenoids from stem bark[9,10].

MATERIALS & METHODS

General experimental procedures

Melting points were determined in soft glass capillaries in an electrothermal melting point apparatus. Qualitative TLC was conducted on aluminium sheet Kieselgel 60 F_{254} (E. Merck). Silica gel (E. Merck, 60-120 mesh, 550 gm) used for column (1.5m × 4.0cm) chromatography. The IR spectra were recorded on FTIR SHIMADZU 8400S spectrometer with KBr pellets. The ^{1}H and ^{13}C NMR spectra were collected on JEOL FX 400 FT NMR spectrometer in $CDCl_3$ at 400.4 MHz and 75.45 MHz frequencies for ^{1}H and ^{13}C NMR respectively using TMS as internal standard (From MNIT, Jaipur). FAB mass spectra were recorded on JEOL SX 102 /DA-6000 mass spectrometer using Argon /Xenon as FAB gas.

Plant Material

The plant material (stem bark), *Cassia fistula* Linn. was collected from Jaipur District of Rajasthan (India) and the authenticity was confirmed by Incharge of Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. A voucher specimen was submitted to the Herbarium of the University (voucher no. RUBL-220746).

Extraction and Isolation of the Constituents

The shade dried stem bark (2.0 kg) were fine powdered and extracted with methanol for 72 hrs on water bath. The extract was filtered hot and solvent was removed under reduced pressure where a semi-solid brown mass (18.0 gm) was obtained. The solvent free extract was chromatographed over silica gel column. The column was eluted with different solvents in order of increasing polarity where 04 compounds were isolated, purified and characterized.

1,8-Dihydroxy-3-methylanthraquinone (chrysophanol) (compound A): Dark orange colour compound A was isolated when column was eluted with Petroleum ether and Benzene in ratio 4:1. It showed melting point 187-88˚C. IR (KBr, cm^{-1}) 3405, 2970, 1680, 1625, 1600, 1270, 1200, 860 and 750; ^{1}H NMR (δ ppm, $CDCl_3$) 12.13 (s, 1H, C-1), 12.05 (s, 1H, C-8), 7.80 (dd, 1H, J = 7.5, 1.1 Hz, C-5), 7.65 (m, 1H, C-6), 7.30 (s, 1H, C-4), 7.26 (dd, 1H, J = 7.5, 1.1 Hz, C-7), 7.10 (s, 1H, C-2) and 2.46 (s, 3H, C-3, -CH_3); ^{13}C NMR (δ ppm, $CDCl_3$) 164.80 (C-1), 118.25 (C-2), 143.60 (C-3), 19.08 (-CH_3 at C-3), 121.83 (C-4), 134.25 (C-4a), 122.40 (C-5), 129.75 (C-6), 119.60 (C-7), 159.20 (C-8), 113.19 (C-8a), 188.50 (C-9), 108.00 (C-9a), 186.35 (C-10), 136.82 (C-10a). Mass (m/z) 254 (M^+), 239, 237, 226, 225, 198, 197, 152 etc. Molecular formula calculated as $C_{15}H_{10}O_4$.

Lupeol (compound B): Compound B was isolated when column was eluted with Petroleum ether and benzene in the ratio 1:1. The compound was crystallized from methanol as colourless powder (m.p. 220-21°C). IR (KBr, cm^{-1}) 3630(-OH stretching), 1650 (>C=C< stretching), 1380 and 1365 (C–H stretching of $>CMe_2$ group). ^1H NMR (δ ppm, $CDCl_3$) 4.65 (s, 1H, C-29), 4.50 (s, 1H, C-29), 3.17 (t, 1H, C-3), 2.35 (m, 2H, C-21), 1.65 (s, 3H, C-30), 0.95 (s, 3H, C-23), 0.92 (s, 3H, C-28), 0.86 (s, 3H, C-26), 0.82 (s, 3H, C-24), 0.76 (s, 3H, C-25), 0.74 (s, 3H, C-27), 1.25-1.75 (remaining 23 protons). ^{13}C NMR (δ ppm, $CDCl_3$) 37.70 (C-1), 26.45 (C-2), 79.42 (C-3), 39.20 (C-4), 55.30 (C-5), 18.34 (C-6), 33.38 (C-7), 41.20 (C-8), 50.42 (C-9), 37.20 (C-10), 20.90 (C-11), 24.90 (C-12), 38.30 (C-13), 43.08 (C-14), 27.40 (C-15), 35.57 (C-16), 42.80 (C-17), 48.00 (C-18), 48.02 (C-19), 151.30 (C-20), 29.10 (C-21), 40.10 (C-22), 28.20 (C-23), 15.40 (C-24), 16.02 (C-25), 16.15 (C-26), 14.50 (C-27), 18.00 (C-28), 109.70 (C-29), 19.30 (C-30). MS (m/z) 427 (M^+H), 426 (M^+). Molecular formula calculated as $C_{30}H_{50}O$.

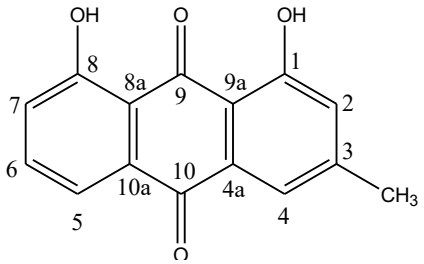
-sitosterol (compound C): Compound C was isolated on elution of column with benzene. On crystallisation with methanol white needle like crystals were obtained. It gave positive Liebermann-Burchard test. It showed melting point 138-39°C. IR (KBr, cm^{-1}) 3500-3440 (OH stretching), 1595 (C=C stretching), 1050 (C-O stretching); ^1H NMR (ppm, $CDCl_3$) 3.50 (m, 1H, C-3), 5.20 (t, 1H, C-6), 0.65 (s, 3H, C-18), 0.98 (s, 3H, C-19), 1.23 (d, 3H, C-21), 0.83 (d, 3H, C-26), 0.90 (d, 3H, C-27), 0.94 (t, 3H, C-29), 1.82 (m, 1H, C-25), 2.13 (dd, 2H, C-7), 1.44-1.86 (m, for remaining 26 protons); ^{13}C NMR (ppm, $CDCl_3$) 31.20 (C-1), 32.00 (C-2), 72.00 (C-3), 42.10 (C-4), 140.00 (C-5), 122.10 (C-6), 32.00 (C-7), 46.10 (C-8), 49.70 (C-9), 36.12 (C-10), 20.99 (C-11), 28.30 (C-12), 42.35 (C-13), 57.10 (C-14), 24.30 (C-15), 40.15 (C-16), 56.25 (C-17), 12.10 (C-18), 19.60 (C-19), 36.30 (C-20), 19.60 (C-21), 36.20 (C-22), 24.70 (C-23), 39.95 (C-24), 36.10 (C-25), 23.50 (C-26), 23.52 (C-27), 32.30 (C-28), 29.50 (C-29). MS (m/z) 414 (M^+), 397, 383, 369, 255 etc. Molecular formula calculated as $C_{29}H_{50}O$.

1,8-dihydroxy-3-carboxylicanthraquinone (rhein) (compound D): This compound was isolated when column was eluted with benzene and chloroform in ratio 1:3. It showed melting point 352-53°C. IR (KBr, cm^{-1}) 3422-3206 (OH stretching), 1691 (>C=O stretching), 1629 (>C=C< stretching); ^1H NMR (δ ppm $CDCl_3$) 12.05 (s, 1H, C-1, –OH), 11.98 (s, 1H, C-8, –OH), 11.03 (s, 1H, C-3, –COOH), 8.20 (s, 1H, C-2,) , 7.92 (s, 1H, C-4), 7.82 (d, 1H, J = 7.5 Hz, C-7), 7.81 (m, 1H, C-6), 7.35 (d, 1H, J = 8.2 Hz, C-5). ^{13}C NMR (δ ppm, $CDCl_3$) 161.27 (C-1), 124.20 (C-2), 138.20 (C-3), 191.10 (C-3, -COOH), 119.05 (C-4), 124.70 (C-5), 138.54 (C-6), 124.72 (C-7), 161.51 (C-8), 187.27 (C-9), 181.25 (C-10), 130.00 (C-4a), 118.92 (C-8a), 118.76 (C-9a), 133.31 (C-10a). MS (m/z) 284 (M^+), 267, 256, 239, 228, 211, 183, 155, 142, 126 etc. Molecular formula calculated as $C_{15}H_8O_6$.

RESULT & DISCUSSION

Compound A

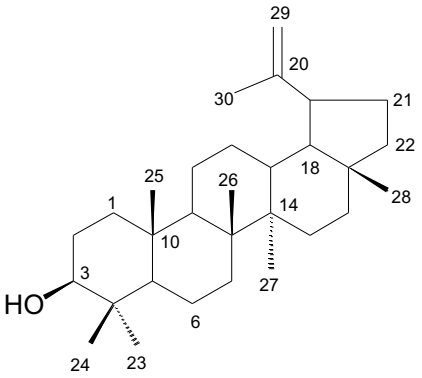
In the mass spectrum, molecular ion peaks was observed at m/z 254 (M^+). Other prominent peaks appeared at 239, 237, 226, 198 etc. The elemental and mass spectral analysis of compound A indicated its molecular formula to be $C_{15}H_{10}O_4$. The colour reactions with methanolic NaOH and magnesium acetate indicated its anthraquinone nature. The compound when treated with alkaline formamide gave a dark red colour which showed the presence of 1,8-dihydroxy system which was further confirmed by the appearance of two carbonyl peaks at 1680 and 1625 cm^{-1} in the IR spectrum (KBr, cm^{-1}). Other important peaks appeared at 3405 (-OH stretching) and 1680, 1625 cm^{-1} (chelated and non-chelated C=O groups). In the ^1H NMR spectrum, two broad singlets were observed at δ 7.10 and 7.30 due to *meta* coupled C-2 and C-4 protons. The C-5 and C-7 protons appeared as double doublets at δ 7.80 and 7.26 (J = 7.5, 1.1 Hz). The C-6 proton was observed as a multiplet at δ 7.65. A singlet at δ 2.46 revealed the presence of a methyl group. Two singlets observed at 12.05 and 12.13 for two hydroxyl groups at C-8 and C-1 respectively. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) showed absorption at 188.50 (C-9), and 186.35 (C-10) which indicated the presence of two carbonyl groups and these values were assigned on the basis of reported values[11,12]. Two hydroxyl groups attached at carbon atom C-1 and C-8 position showed absorptions at 164.80 and 159.20 respectively. An Absorption observed at 19.08 indicates the presence of methyl group. The attachment of methyl group at C-3 position was confirmed by a signal observed at 143.60. Other absorptions observed at 118.25 (C-2), 121.83 (C-4), 134.25 (C-4a), 122.40 (C-5), 129.75 (C-6), 119.60 (C-7), 113.19 (C-8a), 108.00 (C-9a) and 136.82 (C-10a). From the above evidences, compound A was characterized as 1,8-dihydroxy-3-methylanthraquinone (chrysophanol).



Compound A

Compound B

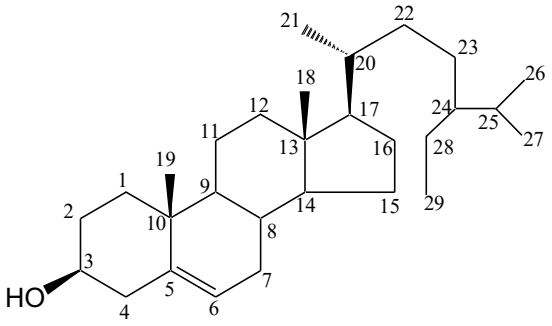
The mass spectrum of compound B showed molecular ion peak at m/z 426 (M^+). The molecular formula for compound B was assigned as $C_{30}H_{50}O$. Its triterpenoid nature was confirmed with Liebermann-Burchard and Noller's reagents test. The IR spectrum (KBr, cm^{-1}) showed strong absorption at 3630 suggested the presence of hydroxyl group. The presence of >C=C< was confirmed by the characteristic absorption at 1650. The sharp absorptions observed at 1380 and 1365 are characteristic for bending vibrations of gem dimethyl group ($>CMe_2$). In the proton NMR spectrum (ppm, $CDCl_3$) the presence of six tertiary methyl groups were observed at 0.74 (s, 3H, C-27), 0.76 (s, 3H, C-25), 0.82 (s, 3H, C-24), 0.86 (s, 3H, C-26), 0.92 (s, 3H, C-28) and 0.95 (s, 3H, C-23). The methyl group attached to olefinic carbon was observed as a singlet at 1.65 for three protons. The vinylic protons attached at C-29 were observed as a pair of broad singlets at 4.65 and 4.50 for one proton each. The proton present at C-3 position was observed at 3.17 as a triplet. A multiplet at 2.35 was assigned for two protons present at C-21 position in pentacyclic ring. The remaining twenty-three protons were observed in the region from 1.25 to 1.75. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) showed characteristic absorptions for olefinic carbon atoms at 109.70 and 151.30. The attachment of hydroxyl group at C-3 position was confirmed by a signal observed at 79.42. Other absorptions observed at 37.70 (C-1), 26.45 (C-2), 39.20 (C-4), 55.30 (C-5), 18.34 (C-6), 33.38 (C-7), 41.20 (C-8), 50.42 (C-9), 37.20 (C-10), 20.90 (C-11), 24.90 (C-12), 38.30 (C-13), 43.08 (C-14), 27.40 (C-15), 35.57 (C-16), 42.80 (C-17), 48.20 (C-18), 48.02 (C-19), 29.10 (C-21), 40.10 (C-22), 28.20 (C-23), 15.40 (C-24), 16.02 (C-25), 16.15 (C-26), 14.50 (C-27), 18.00 (C-28) and 19.30 (C-30) and their assignment has been done accordingly as shown in parentheses. These spectral data are in good agreement with those reported for lupeol in the literature[13-15]. On the basis of these observations compound B was identified as lupeol.



Compound B

Compound C

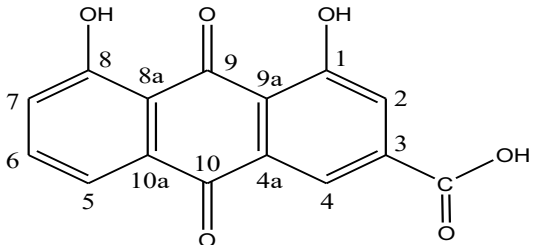
In the mass spectrum molecular ion peak was observed at m/z 414 (M^+). Other prominent ions were observed at m/z 397, 383, 369, 255 etc. On the basis of mass spectrum the molecular formula of the compound was established as $C_{29}H_{50}O$. In the IR spectrum (KBr, cm^{-1}) strong absorptions at 3500-3440 (O-H stretching) indicated the presence of hydroxyl group. The absorption at 1595 confirmed the presence of olefinic group (C=C stretching) whereas the absorption at 1050 was assigned for C-O stretching. The proton NMR spectrum (δ ppm, $CDCl_3$) of compound C showed a singlet at 0.65 for three protons accounted for tertiary methyl group present at C-18 position. The absorption at 0.83 and 0.90 as a doublets confirmed the presence of methyl protons at C-26 and C-27 positions respectively. A triplet observed at 0.94 was assigned for three protons of two methyl groups present at C-29 position. Methyl protons present at C-19 position showed the absorption at 0.98 as a singlet. The three protons of methyl group present at C-21 position was assigned as a doublet at 1.23. A multiplet was observed at 1.82 for methine proton present at C-25 position. Methylene protons at C-7 appeared as double doublets at 2.13. The olefinic proton present at C-6 was assigned as a triplet at 5.20 with coupling constant J = 2.8 Hz. A multiplet observed at 3.50 accounted for one proton and was assigned for a methine proton at C-3 position where the hydroxyl group is attached. The chemical shift and coupling constant J =5.60 Hz of methine proton supported *β*-orientation of hydroxyl (–OH) group at C-3 position. Absorption at 72.00 in ^{13}C NMR spectrum (δ ppm, $CDCl_3$) also confirmed the presence of hydroxyl group at C-3 position. Olefinic carbon atoms were confirmed by the absorptions at 140.00 and 122.10 which were assigned to C-5 and C-6 carbon atoms respectively. Thus confirming the presence of C=C between carbon atom five and six. Other signals were obtained at 31.20 (C-1), 32.00 (C-2), 42.10 (C-4), 32.00 (C-7), 46.10 (C-8), 49.70 (C-9), 36.12 (C-10), 20.99 (C-11), 28.30 (C-12), 42.35 (C-13), 57.10 (C-14), 24.30 (C-15), 40.15 (C-16), 56.25 (C-17), 36.30 (C-20), 36.20 (C-22), 24.70 (C-23), 39.95 (C-24), 36.10 (C-25), 32.30 (C-28), 12.10 (C-18), 19.60 (C-19), 19.60 (C-21), 23.50 (C-26), 23.52(C-27) and 29.45 (C-29) and their arrangements was done according to the reported values. The above data were found to be similar with those reported for *β*-sitosterol[16]. On the basis of above spectral studies compound C was characterized as *β*-sitosterol.



Compound C

Compound D

In the mass spectrum molecular ion peak was observed at m/z 284 (M^+). Other prominent ions were observed at m/z 267, 256, 239, 228, 211, 183, 155, 142, 126 etc. On the basis of mass spectrum the molecular formula of the compound was established as $C_{15}H_8O_6$. In the IR spectrum (KBr, cm^{-1}) strong absorptions at 3422-3206 (O-H stretching) indicated the presence of hydroxyl group. The absorption at 1691 confirmed the presence of carbonyl group whereas the absorption at 1629 was assigned for olefinic group (C=C stretching). In the ^1H NMR spectrum (δ ppm, $CDCl_3$), three singlets were observed at δ 12.05, 11.98 and 11.03 due to two hydroxyl (s, 1H, C-1, –OH), (s, 1H, C-8, –OH) and one carboxylic proton (s, 1H, C-3, –COOH). The C-2 and C-4 protons appeared as singlets at δ 8.20 and 7.92 respectively. A peak was observed as multiplet at δ 7.81 for C-6 proton. Two doublets were observed at δ 7.82 and 7.35 with J = 7.5 Hz for C-7 and C-5 protons. The ^{13}C NMR spectrum (δ ppm, $CDCl_3$) showed absorption at 187.27 (C-9), and 181.25 (C-10) which indicated the presence of two carbonyl groups and these values were assigned on the basis of reported values[17,18]. The carbon atom C-1 and C-8 position showed absorptions at 161.27 and 161.51 respectively. The attachment of carboxylic group at C-3 position was confirmed by a signal observed at 138.20 and 191.10 (C-3, -COOH). Other absorptions observed at 124.20 (C-2), 119.05 (C-4), 130.00 (C-4a), 124.70 (C-5), 138.56 (C-6), 124.72 (C-7), 118.92 (C-8a), 118.76 (C-9a) and 133.31 (C-10a). From the above evidences, compound D was characterized as 1,8-dihydroxy-3-carboxylicanthraquinone (rhein).



Compound D

CONCLUSION

All parts of *Cassia fistula* Linn. are rich source of phytochemicals. These natural compounds can be used to synthesis medicines. In this paper we have isolated four different phytochemicals from stem bark and all have medicinal values. This paper is useful to researchers who are working in the field of natural products chemistry and medicinal chemistry.

ACKNOWLEDGEMENTS

Authors are thankful to Head, Department of Chemistry, University of Rajasthan for providing lab facilities.

REFERENCES

1. Kuo YH, Lee PH, Wein YS. For new compounds from the seeds of *Cassia fistula*. Journal of Natural Products 2002; 65: 1165-1167.
2. Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of *Cassia fistula* L.: an ethnomedicinal plant. Journal of Ethnopharmacology 2007; 112: 590-594.
3. Govindarajan M, Jebanesan A, Pushpanathan T. Parasitology Research 2008; 102: 289-292.
4. Siddhuraju P, Mohan PS, Becker K. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chemistry 2002; 79: 61-67.
5. Nirmala A, Eliza J, Rajalakshmi M, Edel P, Daisy P. Effect of hexane extract of *Cassia fistula* barks on blood glucose and lipid profile in streptozotocin diabetic rats. International Journal of Pharmacology 2008; 4: 292-296.
6. Navanath MS, Naikwade N, Mule S, Krishna PP. Evaluation of anti-inflammatory activity of *Cassia fistula* and *Ficus benghalensis*. Journal of Pharmacy Research 2009; 2: 1304-1306.
7. Pradeep K, Raj Mohan CV, Gobianand K, Karthikeyan S, Protective effect of *Cassia fistula Linn.* on diethylnitrosamine induced hepatocellular damage and oxidative stress in ethanol pretreated rats. Biological Research 2010; 43: 113-125.
8. Pawar AV, Patil SJ, Killedar SG. Uses of *Cassia fistula* Linn as a Medicinal Plant. International Journal of Advance Research and Development 2017; 2: 85-91.
9. Sharma DK. Enumerations on phytochemical, pharmacological and ethnobotanical properties of *Cassia fistula* Linn: yellow shower. Journal of Phytopharmacology 2017; 6: 300-306.
10. Bahorun T, Neergheen VS, Aruoma OI. Phytochemical constituents of *Cassia fistula*. African Journal of Biotechnology 2005; 4: 1530-1540.
11. Khanna RK, Chandra S. Forest/Domestic waste as a source of natural dyes. Journal of Economic and Taxonomic Botany 1996; 20: 497-500.
12. Mahesh VK, Sharma R, Singh RS. Anthraquinones and kaempferol from *Cassia fistula* species. Journal of Natural Products 1984; 47: 733-751.
13. Alam MS, Chopra N, Ali M, Niwa M. Normethyl pentacyclic and lanostane-type triterpenes from *Adiantum venustum*. Phytochemistry 2000; 54: 215-220.
14. Bhattacharyya J, Barros CB. Triterpenoids of *Cnidoscolus urens*. Phytochemistry 1986; 25: 274-276.
15. Gupta R, Kachhawa JBS, Gupta RS, Sharma AK, Sharma MC, Dobhal MP. Phytochemical evaluation and antispermatogenic activity of *Thevetia peruviana* methanol extract in male albino rats. Human Fertility 2011; 14: 53–59.
16. Sharma AK, Sharma MC, Dobhal MP: Isolation of Natural Chemical Constituents from aerial part of *Solanum surattens.* Der Pharmacia Lettre 2013; 5: 355-361.
17. Wei Y, Zhang T, Ito Y. Preparative separation of rhein from Chinese traditional herb by repeated high-speed counter-current chromatography. Journal of Chromatography A 2003; 1017: 125-130.
18. Danielsen K, Aksnes DW, Francis GW. NMR Study of Some Anthraquinones from Rhubarb. Magnetic Resonance in Chemistry 1992; 30: 359-363.

Cite this article as:

Arun K Sharma and Mahaveer P Dobhal. Isolation and characterization of phytochemicals from stem bark of *Cassia fistula* Linn. Int. Res. J. Pharm. 2019;10(4):209-212 http://dx.doi.org/10.7897/2230-8407.1004150

Source of support: Nil, Conflict of interest: None Declared