e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

IJP

Journal homepage: www.onlineijp.com

FATTY ACID COMPOSITION AND ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *CASSIA GLAUCA* SEED EXTRACTS

Deepak Kumar[1*], Shefali Arora[2], Ankit Verma[1]

[1]Deapartment of Pharmaceutical Chemistry, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun (UK), India.
[2]Departmant of Chemistry, University of Petroleum and Energy Studies, Dehradun (UK), India.

ABSTRACT

The objective of the study was to investigate phytochemical analysis, antimicrobial and antioxidant activity of seeds of different extracts of *Cassia glauca* as well as their fatty acid composition of petroleum ether extract. All extract were tested for presence of phytoconstituents i.e., alkaloid, carbohydrate, sterols, proteins, amino acids, saponin, and phenolic compounds in different extracts. Antimicrobial activity was done by well diffusion method at a concentration of 200 mg/ml. Antioxidant activity was done by DPPH method at a different concentration 50 µg/ml to 500 µg/ml. GCMS Analysis was done for petroleum ether extract with the help of PERKIN ELMER CLARUS-500 model coupled with CLARUS-500 Mass spectrometer. Phytochemical analysis showed the presence of alkaloid, carbohydrate, sterols, proteins, amino acids, saponin, and phenolic compounds in different extracts. Methanol extract was the richest extract for tested phytoconstituents. Different fatty acids were present in petroleum ether extract which analysed by GCMS analysis. Maximum antimicrobial activity of seeds of *Cassia glauca* showed in acetone and methanol extract in comparison to standard drug. From antioxidant studies, acetone extract showed maximum antioxidant activity (76.11 %) than other extract in comparison to standard drug ascorbic acid. From above studies it could be concluded that methanol and acetone extract showed maximum antimicrobial and antioxidant activity.

Key words: *Cassia glauca*, Antimicrobial activity, Antioxidant activity, Chloramphenicol, Ketoconazole.

INTRODUCTION

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age-related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders, etc. for which no modern medicine or only palliative therapy is available (Kamboj, 2000).

Cassia, is a large genus of around 500 species of flowering plants in the family Leguminosae and is widely distributed throughout Asia including India, Mauritius, China, East Africa, South Africa, America, Mexico, West Indies and Brazil. There are hundreds of species of Cassia which occurs with more than 1000 names. Some important species are Cassia fistula, Cassia grandis, Cassia hirsutica, Cassia sieberiena, Cassia alata, Cassia tora, Cassia occidentalis, C. auriculata, C. Nigricans. From a pharmaceutical perspective the presence of anthranoides with strong laxative and purgative effects is of particular interest and characteristic of this genus. Cassia species have been of medical interest due to their good therapeutic value in folk medicine (Mazumder *et al*., 2008). Various Pharmacological activities have been reported for different parts of this plant. *Cassia glauca* have been reported Ant-diabetic activity (Salahuddin *et*

Corresponding Author

Deepak Kumar
Email: deepsingh2304@gmail.com

al., 2010; Salahuddin, 2000), Hepatoprotective activity (El-Sawi *et al*., 2010; Farswan *et al*., 2009), antioxidant activity (Mahmoud Maher *et al*., 2010) and antimicrobial activity (Mortada M El-Sayed *et al*., 2011).

EXPERIMENTAL

Collection & Identification of Seeds of Cassia glauca

Seeds of *Cassia glauca* were collected from Dehradun (India). Seeds were authenticated by Dr. Manisha Thapliyal (Scientist-D & officer incharge Forest Tree Seed Laboratory), in Silviculture Division, Forest Research Institute, Dehradun, Uttarakhand, India**.**

Extraction of Seeds of Cassia glauca in different solvents

The Seeds (500 gm) of *Cassia glauca* were crushed. The crushed Seeds extracted with different solvents of increasing polarity viz. Petroleum ether (Pet. ether), Chloroform, Acetone and Methanol by hot percolation method using Soxhlet Apparatus. The extract was evaporated till dryness to obtain residue. These extracts were concentrated under reduced pressure. The percentage yield of Pet.ether, chloroform, acetone and methanol is 8.15%, 5.24%, 6.89% and 12.23% respectively.

Qualitative Phytochemical test

The different extract of Seeds of *Cassia glauca* were tested for various components by their specific tests viz. Mayer's test, Dragendroff's test, Wagner's test for alkaloids; Ferric chloride test, Vanillin hydrochloride test for tannins & phenolic compounds; Million test, Ninhydrin test, Xanthoproteic test for proteins and amino acids; Salkowski test, Sulfur powder test for sterols and triterpenoids; Molisch's test, Benedict's test, Barfoed's test, Bromine water test for carbohydrates and Foam test for saponins (Kokate, 2007).

GC-MS Analysis of Petroleum ether extract (Oil) of Cassia glauca Seed

The extracted petroleum ether oil was subjected to GC-MS analysis. The GC-MS analysis of oil was carried out on a PERKIN ELMER CLARUS-500 model coupled with CLARUS-500 Mass spectrometer (it is equivalent to DB-5) with a RTX-5 column (60m X 0.32mm X 0.25µm). Injection volume was 0.1µl in the split mode (split flow 50ml/minute) Helium as a carrier gas at a flow rate of 1ml/min , inlet temperature were 210˚C.Oven temperature was held at 60˚C to 220˚C for 10 min followed by linear temperature programming at the rate of 3˚C temperature rise per minute .The column was coupled directly to the Perkin Elmere Clarus mass spectrometer operated in the electron ionization mode at 70ev, ion source temperature 150˚C. Mass spectral identification was made by matching the mass against the NIST library software and the Retention time comparison with the publisher data of Wiley.

Antimicrobial activity

The antimicrobial activity of the Seeds of *Cassia glauca* was carried out. The Seed extracts were screened for anti bacterial and anti fungal activities.

Antibacterial activity of seed extracts of Cassia glauca

The bacterial cultures used in the study were *E. coli, Klebsiella pneumonia, Bacillus subtilus, Salmonella typhi, S. aureus.* These bacteria's were provided by Department of Microbiology, Dolphin Institute of Biomedical and Natural Sciences, Manduwala, Dehradun and checked for purity by convention biochemical methods. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity. Fresh culture were obtained by transferring a loop full of culture into nutrient broth and then incubated at 37ºC overnight. To test antibacterial activity, the well diffusion method was used. The drug (extract) concentration used for antibacterial activity is 200 mg/ml.

The microbiological media prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA).

Well diffusion method

25 ml of pre autoclaved Mueller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri plates. These petri plates were allowed to solidify at room temperature. After the plates solidified the freshly prepared microbial broth culture suspension (about 0.1 ml) was spreaded over the Mueller-Hinton agar (MHA) media using L-shaped sterilized glass spreader separately under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 8 mm diameter. In these well, about 0.1 ml of each leaves extracts individually was loaded. This method depends upon the diffusion of leaves extracts from hole through the solidified agar layer of petri dish to such an extent that the growth of added microorganism is prevented entirely in a circular area or zone around the hole containing leaf extract. Petri plates were incubated for 24 hrs at 37ºC in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well or holes were measured in mm and compared with standard drug.

Antifungal activity of seed extracts of Cassia glauca

In this study, the antifungal activity was studied against the microorganism viz. *Aspergillus niger. Penicillum chrysogenum. Sacchomyces cerevesi*, *Candida*

albicans. These cultures were obtained from the standard cultures maintained in the Microbiology Department of DIBNS, Dehradun. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25ºC for about 72-96 hours and then stored at 4ºC as stock cultures for further antifungal activity. Fresh cultures were obtained by transferring a loop full of cultures into sabouraud dextrose broth and then incubated at 25ºC for 72 hrs. To test antifungal activity, the well diffusion method was used. Here culture media preparation in sabouraud dextrose agar (SDA) and incubation period is 72 hours at 25ºC rest the method is same as that of antibacterial activity. The drug (extracts) concentration used for antifungal activity is 200 mg/ml.

Antioxidant activity

DPPH Method

DPPH is a highly oxidisable compound. It oxidized in light, so DPPH is prepared in dark. Weigh accurately 20 mg DPPH and dissolved in solvent. Generally Methanol and for some cases Ethanol is used as a solvent for DPPH. Ascorbic acid is a strong antioxidizing agent. It is taken as standard. Standard solution of ascorbic acid is prepared. Different concentrations of the test sample which is to be examined for antioxidant activity is prepared. *viz.* 50 µg/ml, 100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml. 3 ml of different concentration of test sample *Cassia glauca* extract was mixed with 1 ml of DPPH solution in dark. 3 ml of different concentration of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in dark. The prepared solution of ascorbic acid and test sample was incubated for 1/2 half an hour. When procedure is done than absorbance is taken with the help of U.V. Spectrophotometer at 517 nm (Md. Sikder Almin *et al*., 2010, Molyneux P, 2010).

Calculation

We calculate the % activity of individual concentration of individual extract from the following formula:-

$$\% \, Activity = \frac{Abs.of\ controll - Abs.\ of\ individual\ concentration}{Abs.of\ controll} \times 100$$

Abs. = Absorbance.

RESULTS

From Phytochemical analysis the extracts of seeds of *Cassia glauca* undergoes various qualitative chemical tests. They showed the presence and absence of phytoconstituents in the different solvent systems which is summarized in table 1. From the phytochemical studies we can find out that methanol extract was the richest extract for phytoconstituents. It contains phytoconstituents viz. Alkaloids, carbohydrate, saponins and Protein and amino acids. Acetone extract contain carbohydrates, alkaloid only. Chloroform extract contains Phenolic compound and sterols. Sterols are also present in Pet. ether extract.

Fatty acid composition

The Pet. Ether extract of seed of *Cassia glauca* contained o-Xylene (0.07%), Octadecane (CAS) n-Octadecane (0.07%), Octadecane (CAS) n-Octadecane (0.11%), Heneicosanoic acid, methyl ester (CAS) Methyl heneicosanoate (0.16%). Octadecane (CAS) n-Octadecane (0.07%), 2-Pentadecanone,6,10,14-trimethyl (0.11%), Octadecane (CAS) n-Octadecane (0.07%), 9-Hexadecanoic acid, methyl ester, (Z) (0.33%), Hexadecanoic acid, methyl ester (CAS) Methyl palmitate (24.57%), Octadecane (0.12%), 9-Octadecenal (Z) (0.07%), Hexadecanoic acid, 14-methyl-, methyl ester (0.07%), 9,12-Octadienoic acid (Z,Z)-, methyl ester (40.23%), 9-Octadecenoic acid (Z)-, methyl ester (25.88%), Octadecanoic acid, methyl ester (CAS) Methyl stearate (3.51%), Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (1.25%), Eicosanoic acid, methyl ester (CAS) Arachidic acid methyl ester (1.10%), 9,12-Octadecadienoyl chloride, (Z,Z)-, (0.89%), Di-(9-octadecenoyl)-glycerol (0.77%), Docosanoic acid, methyl ester (0.53%) (Table 1).

Anti - microbial activity

From antibacterial studies we can find out that only two extracts methanol and acetone showed antibacterial activity against all bacterial culture. But the most active extracts which showed highly antibacterial activity is Acetone shown in table 2 and figure 1. Acetone extract showed inhibition zone 13 mm against E. coli, 26 mm against Klebsella pneumonia, 13 mm against Bacillus, 12 mm against Salmonella typhii, and 18 mm against S.Aureus. Methanol extract showed inhibition zone 12 mm against E. coli, 10 mm against Klebsella pneumonia, 11 mm against Bacillus, 18 mm against Salmonella typhii, and 25 mm against S.Aureus. Standard drug chloramphenicol showed antibacterial activity against E. coli, Klebsella pneumonia, Bacillus, Salmonella typhii and S.Aureus. The inhibition zone of Chloramphenicol, 30 mm against E. coli, 40 mm against Klebsella pneumonia, 28 mm against Bacillus, 28 mm against Salmonella typhii, and 26 mm against S.Aureus.

From antifungal studies, we can find out that only two extracts methanol and acetone extracts showed antifungal activity against all fungal culture shown in table 3 and figure 2.

Acetone extract showed inhibition zone 22 mm against *Aspergillus niger*, 27 mm against *Penicillum chrysogenum*, 25 mm against *Sacchomyces cerevesi* and 27 mm against Candida albicans. Methanol extract showed inhibition zone 24 mm against *Aspergillus niger*, 22 mm against *Sacchomyces cerevesi* and 23 mm against

Penicillium chrysogenum and 21 mm against Candida albicans. Standard drug ketoconazole showed antifungal activity inhibition zone 30 mm against *Aspergillus niger*, 27 mm against *Sacchomyces cerevesi* and 26 mm against *Penicillium chrysogenum* and 32 mm against Candida albicans.

Table 1. Showing Fatty acid composition of petroleum ether extract (oil) from seeds of *Cassia glauca* linn.

S. No.	Identified compound	Percentage
1	o-Xylene	0.07
2	Octadecane (CAS) n-Octadecane	0.07
3	Octadecane (CAS) n-Octadecane	0.11
4	Heneicosanoic acid, methyl ester (CAS) Methyl heneicosanoate	0.16
5	Octadecane (CAS) n-Octadecane	0.07
6	2-Pentadecanone,6,10,14-trimethyl	0.11
7	Octadecane (CAS) n-Octadecane	0.07
8	9-Hexadecanoic acid, methyl ester, (Z)	0.33
9	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	24.57
10	Octadecane	0.12
11	9-Octadecenal (Z)	0.07
12	Hexadecanoic acid, 14-methyl-, methyl ester	0.07
13	9,12-Octadienoic acid (Z,Z)-, methyl ester	40.23
14	9-Octadecenoic acid (Z)-, methyl ester	25.88
15	Octadecanoic acid, methyl ester (CAS) Methyl stearate	3.51
16	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	1.25
17	Eicosanoic acid, methyl ester (CAS) Arachidic acid methyl ester	1.10
18	9,12-Octadecadienoyl chloride, (Z, Z)	0.89
19	Di-(9-octadecenoyl)- glycerol	0.77
20	Docosanoic acid, methyl ester	0.53

Table 2. Antibacterial activity of different extracts of seeds of *Cassia glauca* and standard drug Chloramphenicol

S. No.	Test organism	Inhibition zone in mm				
		Pet. ether	Chloroform	Acetone	Methanol	Chloramphenicol
1	E. coli	-	-	13	12	30
2	Klebsella pneumonia	-	-	26	10	40
3	Bacillus	-	-	13	11	28
4	Salmonella typhii	-	-	12	18	28
5	S.Aureus	-	-	18	25	26

Table 3. Antifungal activity of different extract of seeds of *Cassia glauca* and standard drug Ketoconazole

S.No.	Test organism	Inhibition zone in mm				
		Pet. ether	Chloroform	Acetone	Methanol	Ketoconazole
1	*Aspergillus niger*	-	-	22	24	30
2	*Penicillum chrysogenum*	-	-	27	22	26
3	*Sacchomyces cerevesi*	-	-	25	23	27
4	*Candida albicans*	-	-	27	21	32

Table 4. Showing absorbance of different concentration at 517 nm

S. No.	Extracts	Absorbance of Concentration						
		50 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml	Control
1	Pet. ether	0.497	0.492	0.486	0.469	0.474	0.431	0.507
2	Chloroform	1.678	1.669	1.589	1.627	1.483	1.392	1.695
3	Acetone	0.859	0.630	0.315	0.250	0.266	0.268	1.122
4	Methanol	1.109	1.011	0.941	0.863	0.845	0.828	1.122
5	Ascorbic acid	0.032	0.032	0.031	0.032	0.034	0.030	0.959

Table 5. Showing % antioxidant activity of all extract in comparison to standard drug Ascorbic acid and control

S. No.	Extracts	% Antioxidant activity					
		50 µg/ml	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml	500 µg/ml
1	Pet. ether	1.97	2.95	4.14	7.49	6.50	14.99
2	Chloroform	1.0	1.53	6.25	4.01	12.50	17.87
3	Acetone	23.44	43.85	71.92	77.71	76.29	76.11
4	Methanol	1.15	9.89	16.13	23.08	24.68	26.20
5	Ascorbic acid	96.66	96.66	96.76	96.66	96.45	96.87

Figure 1. Showing antibacterial activity of extracts of seeds of *Cassia glauca* linn

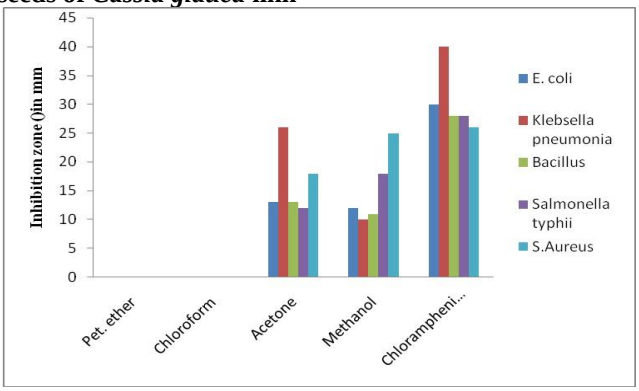
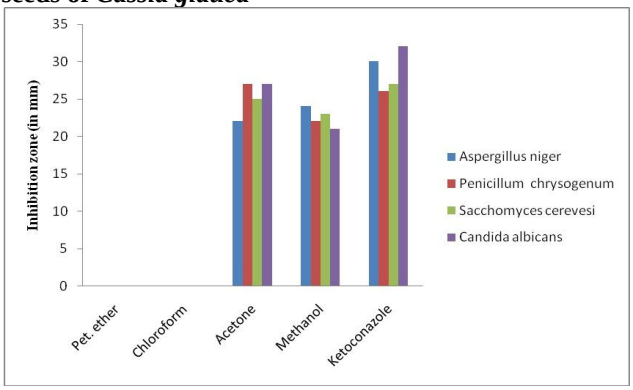


Figure 2. Showing antifungal activity of extracts of seeds of *Cassia glauca*



DISCUSSION

In recent years, the search for phytochemicals possessing antimicrobial and antioxidant properties have been on the rise due to their potential use in the therapy of various chronic and infectious diseases. Preliminary phytochemical screening of different extract of Seeds of *Cassia glauca* reveals the presence of carbohydrates, alkaloids, saponins in methanol and acetone extract. This is well known, since tannins and saponins are important plant metabolites which is majorly responsible for antimicrobial activity. DPPH is a highly oxidisable compound. The molecule of 1,1-diphenyl-2-picrylhydrazyl (α,α-diphenyl-β-picrylhydrazyl; DPPH:1) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 517 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (2) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present) (Molyneux P, 2010). Plant phenolics constitute one of the major groups of compounds acting as a primary antioxidant free radical terminators. These compounds possess a wide spectrum of chemical and biological activities including radical scavenging properties. Natural extracts with proven antioxidant activity are usually present with their phenolic moiety, for example flavonoids, coumarins, proanthocyanidins and tocopherols (Ponmari Guruvaiah *et al*., 2012).

CONCLUSION

From the above study it is concluded that the methanol and acetone extracts showed the maximum antimicrobial activity in comparison to other extracts. From antioxidant studies, it is concluded that acetone extract showed maximum antioxidant activity as comparison with other extracts. Therefore, it is suggested that further work be performed on the isolation and identification of the bioactivecompounds, which may be useful for therapeutic purpose.

ACKNOWLEDGEMENT

Authors are thankful to Management and Department of Microbiology, Dolphin (PG) Institute of Biomedical and Natural Sciences, Dehradun for providing necessary facilities and all the technical assistance.

REFERENCES

El-Sawi SA and Sleem AA. Flavonoids and Hepatoprotective activity of Leaves of *Senna Surattensis* (Burm.f.) In CCl4 Induced Hepatotoxicity in Rats. *Australian Journal of Basic and Applied Sciences*, 4(6), 2010, 1326-1334.

Farswan Mamta, Mazumder Papiya Mitra, Parcha V. Protective effect of Cassia glauca Linn. on the serum glucose and hepatic enzymes level in streptozotocin induced NIDDM in rats. *Indian J Pharmacol*, 41(1), 2009, 19-22.

Kamboj VP. Herbal Medicine. *Current Science*, 78(1), 2000, 35-39.

Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy, 39th edition, 2007, Nirali Prakashan, 108-109.

Mahmoud Maher, et al. Antioxidant Properties of Methanolic Extracts of The Leaves of Seven Egyptian *Cassia* Species. *Acta Pharm*, 60, 2010, 361-367.

Mazumder Papiya Mitra, Parcha V, Farswan Mamta, Upaganlawar Aman. Cassia: A Wonder Gift to Medical Sciences. *International Journal of Community Pharmacy*, 1(2), 2008, 16-38.

Md. Salahuddin, Jalalpure Sunil S, Gadge Navneet B. Antidiabetic activity of aqueous bark extract of *Cassia glauca* in streptozotocin-induced diabetic rats. *Canadian Journal of Physiology and Pharmacology*, 88(2), 2010, 153-160.

Md. Sikder Almin, Md. Rahman Arifur, Md. Islam Rashedul, Md. Kaisar Abul Mohmad S, Invitro-anti-oxidant,Reduing Power, Free Radical Scavanging and Membrane Stabilizing activities of *Spilanthes carva. Bangladesh Pharmaceutical Journal*, 13, 2010, 63-67.

Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin Journal Sci. Technol*, 26(2), 2010, 211-219, 67.

Mortada M El-Sayed, et al. Evaluation of antioxidant and antimicrobial activities of certain Cassia species. *Australian Journal of Basic and Applied Sciences*, 5(9), 2011, 344-352.

Ponmari Guruvaiah, Annamalai Arunachalam and Lakshmi Palanisamy Thanga Velan. Evaluation of phytochemical constituents and antioxidant activities of successive solvent extracts of leaves of Indigofera caerulea Roxb using various in vitro antioxidant assay systems. *Asian Pacific Journal of Tropical Disease*, 2012, S118-S123.

Rafi khan Pathan, et al. *In vitro* Antimicrobial Activity of Citrus aurantifolia and its Phytochemical screening. *Asian Paicfic Journal of Tropical Disease*, 2012, S328-S331.

Salahuddin M and Jalalpure SS. Evaluation of Antidiabetic Activity of Cassia Glauca Lam. Leaf in Streptozotocin Induced Diabetic Rats. *Iranian Journal of Pharmacology and Therapeutics*, 9, 2009, 29-33.