ORIGINAL ARTICLE

Phytochemical and *in vitro* anticancer activity of *Cassia glauca* leaves extract

Y. Srinivas, Poornima Hosamath

Department of Pharmacognosy, BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot, Karnataka, India

Abstract

Aim: *Cassia glauca* leaves are extracted and studied for preliminary chemicals present in the extracts and studied for *in vitro* anticancer activity against human cell lines using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. **Results:** Preliminary phytochemical screening of ether extract shows the presence of glycosides, flavonoids, and phenols, and chloroform and methanol extracts show the presence of glycosides, flavonoids, and phenols, and alkaloids and methanol extract shows the presence of glycosides, alkaloids, steroids, and flavonoids. The viability of the extracts has shown significantly decreased at 1000 µg/ml after 72 h against cancer cells. Ether extract shown significant anticancer activity against MCF-7 (23.76%, half maximal inhibitory concentration [IC_{50}] – 479.63) and HT-29 (36.18%, IC_{50} – 583.71), and chloroform and methanol extracts show significant anticancer activity against A549 (26.04%, IC_{50} – 282.27) and A549 (35.52%, IC_{50} – 636.30) cancer cells. **Conclusion:** The results indicate that *C. glauca* leaves ether extract shows significant anticancer activity against MCF-7 and HT-29, and chloroform and methanol extracts show against A549, the activity may be due to the presence of flavonoids and polyphenols.

Key words: Anticancer, *Cassia glauca*, phytochemicals

INTRODUCTION

Cancer has been a constant battle globally with a lot of development in cures and preventative therapies. The disease is characterized by cells in the human body continually multiplying with the inability to be controlled or stopped. At present, treatment includes chemotherapy, radiotherapy, and chemically derived drugs. Treatment such as chemotherapy can put patient under a lot of strain and further damage their health and they cannot affordable.[1] The main disadvantage of synthetic medicine is associated with side effects, therefore, the focus on using alternative treatment and therapy against cancer. The herbal medicine treatment can also improve overall health and well-being. Medicinal plants have been used for thousands of years in folk medicines in developing and developed countries. According to the WHO, some nations still reply of plant-based treatments as their main source of medicine and developing nations are utilizing the benefit of naturally sourced compounds for therapeutic purpose.[2] Medicinal plants are playing an import role as a source of effective anticancer agents and it is significant that 60% of currently used anticancer agents are derived from natural source including plants. [3]

Many reports have shown the Cassia species possessing antimicrobial, antidiabetic, antimalerial, anticarcinogenic, hepatoprotective and cytotoxic effect against liver carcinoma cell line (Hep G2).[4,5]

Therefore, the aim of this study was to study the preliminary phytochemical and investigation of anticancer activity of crude extracts of *Cassia glauca* against MCF-7 (breast cancer cell line), HT-29 (human colon adenocarcinoma cell line), and A549 (adenocarcinomic human alveolar basal epithelial cells) cancer cell lines.

MATERIALS AND METHODS

Plant Material

C. glauca plant was collected around roadside of Bagalkot to Badami, Karnataka, in the month of July

Address for correspondence:
Dr. Y. Srinivas, Department of Pharmacognosy, BVVS Hanagal Shri Kumareshwar College of Pharmacy, Bagalkot, Karnataka - 587 101, India.
Phone: +91-9945872700. E-mail: yseenu_9@yahoo.com

Received: 06-05-2019
Revised: 16-07-2019
Accepted: 16-08-2019

2016. It was identified and authenticated by Dr. Kapali Professor and HOD Department of Botany, BVVS Science College Bagalkot-587101, Karnataka. The specimen voucher (HSK/2016/1/09) is stored in the department of pharmacognosy for further reference.

Preparation of Extracts and Preliminary Phytochemical Studies

The leaf part of *C. glauca* was dried in shades for about 1 month and it was made into a course powder. The powdered plant material is successively extracted with ether, chloroform, and methanol using Soxhlet apparatus. After extraction, the solvents were evaporated under reduced pressure to get concentrated residue, the residue is completely dried by lyophilization (Mini Lyotrap, LTE Scientific Ltd., Model No. I8199/5) and stored in an airtight container. The extracts thus obtained were subjected to preliminary phytochemical screening following the standard procedure.[6,7]

Tests for Carbohydrates

Molisch's test

To 2–3 ml of extract, added few drops of an α-naphthol solution in alcohol and shaken and added concentrated H_2SO_4 from the sides of the test tube were observed for violet ring at the junction of two liquids.

Test for Glycosides

Hydrolysis of extracts

A minimum quantity of the extracts is hydrolyzed with hydrochloric acid for few minutes on water bath, and the hydrolysate is subjected to the following tests.

Legal's test

To the hydrolysate, 1 ml of the pyridine and few drops of sodium nitroprusside solution added and then are made alkaline with sodium hydroxide solution. Color change shows the presence of glycosides.

Test for Alkaloids

Dragendorff's test

To 1 ml of the extract, add 1 ml of Dragendorff's reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Mayer's test

To the 1 ml of extract, add 1 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish-yellow or cream-colored precipitate indicates the presence of alkaloids.

Hager's test

To 1 ml of the extract, add 1 ml of Hager's reagent (saturated aqueous solution of picric acid). A yellow-colored precipitated indicates the presence of alkaloids.

Wagner's test

To 1 ml of the extract, add 1 ml of Wagner's reagent (iodine in potassium iodide solution). Formation of reddish-brown precipitate indicates the presence of alkaloids.

Test for Phytosteroids

A small quantity of extract is dissolved in 5 ml of chloroform separately. The above-obtained chloroform solution is subjected to Salkowski and Liebermann–Burchard tests.

Salkowski test

To the 1 ml of the above-prepared chloroform solution, few drops of concentrated sulfuric acid are added. Formation of brown rind indicates the presence of phytosterols.

Liebermann–Burchard test

The above-prepared chloroform solution is treated with few drops of concentrated sulfuric acid followed by 1 ml of acetic anhydride solution. A bluish-green color solution shows the presence of phytosterols.

Test for Flavonoids

Shinoda test

To dried powder or extract, added 5 ml of 95% ethanol, few drops of concentrated HCl, and 0.5 g magnesium turnings. Pink color was observed.

Ferric chloride test

Test solution with few drops of ferric chloride solution shown intense green color.

Alkaline reagent test

Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

Test for tannins and phenolic compounds

To 2–3 ml of extract, add few drops of following reagents:

- 5% $FeCl_3$ solution: Deep blue-black color
- Lead acetate solution: White precipitate
- Dilute HNO_3: Reddish to yellow color.

In Vitro Anticancer Activity 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) Assay

The cytotoxicity is performed by MTT assay. This is a colorimetric assay that measures the reduction of yellow

MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and enters into the mitochondria, and it is reduced to insoluble, colored (dark purple) formazan product. The cells are then solubilized with an organic solvent dimethyl sulfoxide (DMSO) and the released, solubilized formazan reagent is measured spectrophotometrically using ELISA. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

The cell lines were cultured in Dulbecco's Modified Eagle's Medium (Invitrogen) which was supplemented with 10% fetal bovine serum (Gibco, Invitrogen) and 1% antibiotic – antimycotic ×100 solution (Thermo-Fisher Scientific). The cells were seeded at a density of approximately 5×10^4 cells/well in a 96-well flat-bottom microplate (NEST Biotechnology) and maintained at 37°C in 95% humidity and 5% CO_2 for overnight. Different concentrations (200, 100, 50, 25, 12.5, 6.5, and 3.125 µg/300 µL) of compounds were treated. Further, the cells were incubated for another 72 h and the cells washed twice with phosphate buffer solution and 20 µL of the MTT reagent (5 mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37°C. After 4 h, 100 µL of DMSO was added to each well to dissolve the formazan crystals and absorbance was recorded at 570 nm using microplate reader (LISA reader).[8] The half maximal inhibitory concentration (IC_{50}) values are calculated using GraphPad Prism Version 5.1. The surviving cells and inhibiting cells are calculated using the following formula:

Surviving cells (%) = Mean OD of test compound/Mean OD of negative control × 100

Inhibiting cells (%) = 100−Surviving cells. Using graphical (y = mx + c) method, the IC_{50} value was calculated.

RESULTS

C. glauca leaves extract was screened for phytochemical; ether extract shows the presence of glycosides, flavonoids, and phenols; chloroform and methanol extracts show the presence of glycosides, flavonoids, and phenols; and alkaloids and methanol extract shows the presence of glycosides, alkaloids, steroids, and flavonoids [Table 1].

In the present study, the results indicate that the viability of MCF-7 and HT-29 cancer cells was significantly decreased at 1000 µg/ml concentration with ether extract, i.e., MCF-7 (23.76% and IC_{50} – 479.63) and HT-29 (36.18% and IC_{50} – 583.71) after 72 h. Similarly, viability of A549 cancer cell was significantly decreased to 1000 µg/ml concentration with chloroform and methanol extracts (26.04% and IC_{50} – 282.27) and (35.52% and IC_{50} – 636.30) respectively [Table 2].

DISCUSSION

C. glauca leaves ether and chloroform extract shows the presence of flavonoids and polyphenols. Flavonoids are polyphenolic compounds that are ubiquitously in plants. They have showed to possess a variety of biological activities at non-toxic concentrations in organism. Compelling data from laboratory studies, epidemiological investigations, and human clinical trials indicate that flavonoids have important effects on cancer chemoprevention and chemotherapy. Many mechanisms of action have been identified, including carcinogen inactivation, antiproliferation, cell cycle arrest, induction of apoptosis and differentiation, inhibition of angiogenesis, and antioxidation. Based on these results, flavonoids may be promising anticancer agents.[9]

The study involves in developing the effective cancer preventive approaches for utilization of natural bioactive compounds that are able to induce selective cancer cells.[10] Researchers have reported that anticancer activity of some

Table 1: Phytochemical evaluation of *C. glauca* extract

Phytochemical constituents	Name of the tests	Ether extract	Chloroform extract	Methanol extract
Carbohydrates	Molisch's test	++	−−	−−
Glycosides	Legal test	++	−−	++
	Balget test	++	++	−−
Alkaloids	Dragendorff's test	−−	++	++
	Hager's test	−−	−−	−−
	Mayer's test	−−	−−	++
	Wagner's test	−−	−−	++
Steroids	Salkowski test	−−	++	++
	Liebermann–Burchard test	−−	++	++
Flavonoids	Shinoda test	−−	++	++
	Alkaline acetate test	++	++	−−
	Ferric chloride test	++	++	−−
Tannins and phenolic compounds	5% Ferric chloride solution	++	++	−−

++ (Positive), −− (Negative). *C. glauca: Cassia glauca*

Table 2: Anticancer of *C. glauca* leaves extract against MCF-7, HT-29, and A549 cell lines

Extracts	Conc. (µg/ml)	% of cell viability		
		MCF-7	HT-29	A549
CGL EX extract	1000	23.76	36.18	39.52
	500	44.92	51.09	50.09
	250	56.92	60.02	63.03
	125	72.78	63.02	69.09
	62.5	75.22	73.08	82.97
IC_{50} (µg/ml)		479.63	583.71	655.85
CGL CH extract	1000	44.55	41.08	26.04
	500	51.20	46.77	29.07
	250	53.56	56.62	32.87
	125	58.94	64.83	66.35
	62.5	70.58	72.36	71.40
IC_{50} (µg/ml)		158	608.67	282.27
CGL ME extract	1000	50.57	46.91	35.52
	500	56.17	51.22	53.75
	250	59.65	61.09	58.12
	125	62.84	77.51	68.38
	62.5	63.33	86.20	89.66
IC_{50} (µg/ml)		655.85	774.47	636.30

CGL EX: *C. glauca* leaves ether, CGL CH: *C. glauca* leaves chloroform, CGL ME: *C. glauca* leaves methanol. *C. glauca: Cassia glauca*, IC_{50}: Half maximal inhibitory concentration

Cassia species for different cell lines such as *Cassia fistula* flowers was studied for two types of cell lines, namely, VERO and COLO 320 MD. Rhein showed notable toxicity toward COLO320 DM cell line.[11] *Cassia auriculata* leaves showed anticancer activity against HepG2.[12] *Cassia italica leaves extract displayed more than 50% anticancer activity against HepG2 cell line*.[13] Purified flavonoids have shown anticancer activity against other human cancers including hepatoma (HepG2), cervical carcinoma (Hela), and breast cancer (MCF-7).[14]

In our studies, *C. glauca* leaves extract was studied for anticancer activity against three cell lines – MCF-7, HT-29, and A549. Ether extract shows a significant anticancer activity against MCF-7 and HT-29 cell lines at 1000 µg/ml at 72 h, whereas chloroform and methanol extracts shown anticancer activity only against A549.

CONCLUSION

In the present study, medicinal herb *C. glauca* leaves ether extract showed anticancer activity against two cell lines MCF-7 and HT-29, and chloroform and methanol extracts show anticancer activity against A549 the cell viability at 1000 µg/ml concentration after 72 h with dose-dependent manner [Table 2]. The results indicate that *C. glauca* leaves ether extract shows anticancer activity in MCF-7 and HT-29 cell lines, whereas chloroform and methanol extracts show anticancer activity in A549 cell lines, the anticancer activity of the extracts is due to the presence of flavonoids and polyphenols. It is observed from our studies that effective drugs produced from *C. glauca* leaves lead to support the traditional medicinal use of the plant in the treatment of cancer.

ACKNOWLEDGMENT

We are thankful to Rajiv Gandhi University of Health Sciences, Karnataka, Bengaluru for its financial support under research grant. RGU:Adv. Res.: proposal-P-178:2015-16, Date:06-01-2016.

REFERENCES

1. Greenwell M, Rahman PK. Medicinal plants: Their use in anticancer treatment. Int J Pharm Sci Res 2015;6:4103-12.
2. Rao BR, Singh K, Sastry KP, Singh CP, Kotari SK, Rajpur DK, *et al*. In: Reddy KJ, Bahadur B, Bhadraiah B, Rao ML, editor. Cultivation Technology for Economicaly Important Medicinal Plant Advances in Medicinal Plants. Hyderabad: University Press; 2007. p. 112-22.
3. Kumar RS, Rajkapoor B, Perumal P. *In vitro* and *in vivo* anticancer activity of *Indigofera cassioides* Rottl. Ex. DC. Asian Pac J Trop Med 2011;4:379-85.
4. Kittur BS, Srinivas Y, Deshpande SR. Evaluation of leaf and stem extracts for *Cassia glauca* L for antimicrobial activity. Int J Pure Appl Zool 2015;3:98-102.
5. El Sayed MM, Mabher MA, Mohfourz MA, El-Sayed SA, Wofaa SA, Exat EA. Chemical constituents and cytotoxiic activity of *Cassia glauca* Linn leaves. Life Sci 2013;10:1617-25.
6. Kokate CK. Practical Pharmacognosy. 4th ed. New Delhi: Vallabh Prakashan; 2003. p. 122-6.
7. Harborne JB. Phytochemical Methods. A Guide to Modern Technique of Plant Analysis. 3rd ed. New York: Chapman and Hall; 1998.
8. Samundeeswari S, Chougala B, Holiyachi M, Shastri L, Kulkarni M, Dodamani S, *et al.* Design and synthesis of novel phenyl -1, 4-beta-carboline-hybrid molecules as potential anticancer agents. Eur J Med Chem 2017;128:123-39.
9. Ren W, Qiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. Med Res Rev 2003;23:519-34.
10. Anushia C, Sampathkumar P, Ramkumar L. Antibacterial and antioxidant activity in *Cassia auriculata*. Glob J Pharmacol 2009;3:127-30.
11. Duraipandiyan VB, Albert A, Ignacimuthu S, Muthukumar C, Al-Harbi NA. Anti cancer activity of rhein isolated from *Cassia fistula* L. flower. Asian Pac J

Trop Dis 2012;2:S517-23.
12. Padmalochana K. Antibacterial, anticancer and antioxidant activity of *Cassia aericulata* leaves. Methanol petrolium ether and ethyl acetate extracts. Int J Pharm Res Health Sci 2018;6:2610-14.
13. Ahmed MA, Abu EE, Shalaby E, Shemy HE. Potent anticancer and antioxidant activity of active ingredients seperated from *Solanum nigrum* and *Cassia italica* extracts. J Arid Land Stud 2014;24:145-52.
14. Wen L, Wu D, Jigan Y, Prasad KN, Lin S, Jiang G, *et al*. Identification of flavanoids in *Litchi* (*Litchi chinensis* soon) leaf and evaluation of anticancer activity. J Funct Foods 2014;6:555-63.

Source of Support: Nil. **Conflict of Interest:** None declared.