British Journal of Pharmaceutical Research
11(6): 1-11, 2016, Article no.BJPR.26198
ISSN: 2231-2919, NLM ID: 101631759

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Phytochemicals, Antioxidant and Antiproliferative Studies of Some Medicinal Plants from Indian Sub-continent

Jamal Akhtar Ansari[1,2], Abdul Rahman Khan[1], Mohammad Kaleem Ahmad[2], Nishat Fatima[1,2], Namrata Rastogi[2], Mohammad Faheem Khan[3], Homa Jilani Khan[1,2] and Abbas Ali Mahdi[2*]

[1]Department of Chemistry, Integral University, Lucknow 226 026, U.P., India.
[2]Department of Biochemistry, Natural Products Research Lab, King George's Medical University, Lucknow 226 003, U.P., India.
[3]Department of Chemistry, University of Lucknow, Lucknow 226 007, U.P., India.

Authors' contributions

This work was carried out in collaboration between all authors. Author AAM designed the study and protocol. Author JAA managed the literature searches, analyses of the study performed and wrote the manuscript. Authors ARK, MKA, NF, NR, MFK and HJK managed the experimental process and helped in the study. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/26198
Editor(s):
(1) Rafik Karaman, Bioorganic Chemistry, College of Pharmacy, Al-Quds University, USA.
Reviewers:
(1) Antonello Santini, University of Napoli Federico II, Italy.
(2) Ozlem Sultan Aslanturk, Adnan Menderes University, Turkey.
(3) Zahrat El-Ola Mahmoud Mohamed, Food Technology Research Institute, Agricultural Research Center, Giza, Egypt.
Complete Peer review History: http://sciencedomain.org/review-history/14727

Original Research Article

Received 5th March 2016
Accepted 3rd May 2016
Published 22nd May 2016

ABSTRACT

Aims: The Indian sub-continent has rich culture and a vast botanical diversity with high potential abilities of traditional medicine. However, there is a paucity of information available in scientific literature regarding the chemical and pharmacological properties of the natural products as anticancer agents. The objective of the present study was to evaluate the phytochemicals potential, total phenolics content, the presence of rutin and quercetin, antioxidant and antiproliferative activity, of *Crotalaria juncea*, *Tinospora cordifolia* and *Rheum emodi* methanolic extracts.

**Corresponding author: E-mail: abbasalimahdi@gmail.com;*

Study Design: Phytochemicals screening, total phenolics content, rutin and quercetin determination, *in vitro* antioxidant activity and antiproliferative activity against human breast cancer MDA-MB-231 cells.
Place and Duration of Study: Natural Products Research Lab, Department of Biochemistry, King George's Medical University, May 2014 and August 2015.
Methodology: The phytochemicals, total phenolics content and antioxidant potential were assessed by qualitative phytochemicals screening, Folin-Ciocalteu reagent and DPPH assay, respectively. High Performance Liquid Chromatography was used to estimate the level of rutin and quercetin. The antiproliferative activity was evaluated against human breast cancer (MDA-MB-231) cells by MTT and colony formation assay. Hoechst 33342 staining was performed to examine apoptosis.
Results: All extracts showed antiradical activity and presence of phenolics content along with different phytochemicals. HPLC analysis showed presence of rutin and quercetin in *T. cordifolia* and *R. emodi*, however, *C. juncea* showed only presence of rutin. Antiproliferative activity revealed that *T. cordifolia* showed highest activity with lowest IC_{50} (51.74±3.14 to 21.7±2.5 µg /mL) as compared with other extracts. Hoechst 33342 staining showed that *T. cordifoli*a induced apoptosis in breast cancer cells.
Conclusion: Overall, this study demonstrated that methanolic extracts of above plants has potent antiproliferative and antioxidant activity along with different phytochemicals. This calls for further studies on the active components for proper assessment of their chemotherapeutic properties.

Keywords: Indian medicinal plants; phytochemicals; phenolics; HPLC; antioxidant antiproliferative activity.

1. INTRODUCTION

The Indian sub-continent has rich culture and widespread use of medicinal herbs, spices etc. and has a vast botanical diversity with high potential abilities of traditional medicine worldwide known as Ayurvedic, Unani and Siddha system of medicine. The traditional communities practicing here for thousands of years have built a precious knowledge base about the use of the rich bio-resources of herbal remedies. The scientific community has derived a large number of promising leads from them. Researchers have identified a number of compounds used in mainstream medicine which was derived from "ethno-medical" plant sources and are used medicinally in different countries and are a source of many potent and powerful drugs [1-3]. Moreover, among different phytochemicals, phenolics and flavonoids like quercetin and rutin are worthy compounds used mainly as antioxidants and for other benefits. Epidemiological studies have revealed the association between the consumption of phenolic-rich foods and prevention of oxidative stress-related diseases [4-6].

Cancer is an abnormal growth and proliferation of cells. Cancer development is a multistep process including induction of genetic instability, abnormal expression of genes, abnormal signal transduction, angiogenesis, metastasis and immune evasion [7]. Carcinoma of the breast is one of the highly prevalent malignancies in both developed and developing countries. International Agency for Research on Cancer (IARC), January, 2014 estimated that 32.5 million people diagnosed with cancer within the five years previously were alive at the end of 2012. Most were breast cancer diagnosed (6.3 million). In India, around 555 000 people died of cancer in 2010 [8]. The absolute number of cancer deaths in India is projected to increase because of population growth and increasing life expectancy [9]. Fatality in breast cancer is generally due to metastasis and development of resistance to chemotherapy [10,11]. Metastasis and resistance to chemotherapy are mostly due to over expression of pro-metastatic, pro-angiogenic, multi-drug resistance and anti-apoptotic genes [12,10,13]. Chemotherapy is one of the commonly-used strategies in breast cancer treatment. This therapy is usually associated with adverse side effects, ranging from nausea to bone marrow failure [14] and development of multidrug resistance (MDR) [15]. Therefore, new chemotherapeutic drugs are needed for breast cancer treatment with least side effect. Medicinal plants have been accounted a rich source of bioactive compounds [16]. Discoveries of natural products derived vinca alkaloids, taxols and camptothecin agitated scientific community from focusing on medicinal plant to search new chemotherapeutic agents with least side effects.

Despite much awareness and known medical efficacy of ethno-medical plants of Indian system of medicine, only a relatively small number of these plants have been subjected to accepted scientific evaluation for their chemical and pharmacological potential anticancer effects. Therefore, in the present study, we have attempted to the evaluation of the phytochemicals potential, phenolics content, rutin and quercetin estimation, antioxidant and anticancer efficacy against breast cancer of some of the precious ethno-medical plants from Indian sub-continent origin.

2. MATERIALS AND METHODS

2.1 Reagents

Solvents, standard quercetin and rutin (HPLC grade) used in this study were purchased from Thermo Fischer, USA. Fetal bovine serum (FBS), phosphate buffered saline (PBS) and RPMI-1640 medium were purchased from Gibco BRL, USA. Penicillin and Streptomycin were procured from Lonza, USA. 3-[4, 5-Dimethyl-2- thiazolyl]-2, 5-diphenyltetrazolium bromide (MTT), Trypsin-EDTA, Hoechst 33342, Coomassie blue, DPPH (2,2-diphenyl-1-picrylhydrazyl) and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich, USA. Dimethyl Sulfoxide (DMSO) was purchased from Calbiochem. Double distilled water was used for cleaning of glasswares.

2.2 Plant Materials and Identification

Crotalaria juncea (seeds) (CJS), *Tinospora cordifolia* (stem) (TCS) and *Rheum emodi* (stem bark) (REB) were collected from authorized dealer of Ayurvedic plants, Lucknow. The authentication was done by Dr. M. M. A. A. Khan, Department of Botany, Shia P.G. College, Lucknow. Collected plants materials were air dried in shadow and grinded to fine powder for extraction.

2.3 Preparation of Extract

Air-dried powdered plant materials (5.0 kg each) were extracted with 95% MeOH overnight (4×10 L) at room temperature. The combined extracts were filtered and concentrated under reduced pressure at 40℃ on a rotatory evaporator to get dried methanol crude extracts. The crude extracts were completely dried under high vacuum and weighed at difference time interval until constant weight obtained. The dried crude extracts were kept in a desiccator for further studies.

2.4 Phytochemical Screening

Phytochemical screening was performed according to the method described by Trease and Evans, [17] with slight modifications for the presence of various types of phytochemicals.

2.5 Total Phenolics Content

Total phenolics content was determined by method based on Folin- Ciocalteu reagent and Gallic acid as standard. The method is based on principle that Folin- Ciocalteu reagent show blue color produced by reaction with phenolics compound which is measured by absorbance at 765 nm. Briefly, 0.5 mL (1 mg/mL) solution of the plant extract in methanol was mixed with 5 mL of Folin-Ciocalteu reagent. The mixture was incubated for 3 minutes and 4 mL of 10% Na_2CO_3 solution was added and allowed to stand for 15 min with intermittent shaking. The absorbance of the blue color produced was measured with a double beam UV/Visible spectrophotometer (Systronics) at 765 nm. A standard curve was plotted using different concentrations of Gallic acid (standard, 0-1000 µg/mL). The Total Phenolics contents values of the extracts were expressed in terms of Gallic acid, equivalent (GAE) mg/g of powdered dry extract.

2.6 Determination of Rutin and Quercetin by High Performance Liquid Chromatography (HPLC)

Determination of rutin and quercetin was performed by an Agilent 1260 infinity Quaternary LC system consisting of 1260 infinity Diode Array Detector (DAD), quaternary solvent delivery system with thermostatted autosampler, thermostatted columns compartment and equipped with an Agilent Zorbax C-18 (4.6 mm×250 mm, 5 µm) column. Gradient elution was performed at 25°C with solution A (Water 0.3% HCOOH in water) and solution B (Methanol) in the following gradient elution program: 0–1 min— 90% of solution A and 10% of B, 1–5 min—30% of A and 70% of B, 5–7 min— 10% of A and 90% of B, 7-10 min— 90% of A and 10 of B, 10-11 min— 90% of A and 10% of B. Detection was conducted at wavelength of 280 nm. Flow rate and injection volume were set at 1.0 mL/min and 5 µL, respectively.

2.7 Free Radical-scavenging Activity: DPPH Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity was evaluated based on method given by Brand-Williams et al. [18]. It was measured a decrease in absorbance at 517 nm of a solution of colored DPPH in methanol brought about by the sample. A stock solution of 0.1 mM of DPPH was prepared. Ascorbic acid was used as reference material and a stock solution of 1.0 mg/mL in methanol of standard was prepared. Extracts were dissolved in methanol and prepared a concentration of 1 mg/mL. Series of reference standard of different concentrations (1-5 µg/mL) were prepared. Similarly, extracts were prepared of 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 400 µg/mL. Freshly prepared 1.0 ml of DPPH stock solution was added to each of the above sample tube and incubated to 20-30 minutes. The absorbance was recorded at 517 nm. DPPH radical scavenging activity was calculated as follows:

% inhibition = [Abs sample- Abs control) / (Abs of control)] X 100

Control = 1.0 mL Methanol + 1.0 mL DPPH

2.8 Cell Culture

Human Breast adenocarcinoma (MDA-MB-231, tumorigenic, and invasive) cell line was obtained from ATCC, USA. Cells were cultured with RPMI medium and penicillin gentamicin containing 10% FBS and grown at 37°C and 5% CO_2 in a humidified air.

2.9 Cell Viability Assay

The antiproliferative activity of extracts were evaluated by 3-[4, 5-Dimethyl-2- thiazolyl]-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. Briefly, the cells ($1x10^4$) were seeded in 100 µL complete medium in each well of 96-well culture plate for 24 hours at 37°C and 5% CO_2. Stocks of extracts were prepared in DMSO and diluted to the desired concentrations (400, 200, 100, 50, 25, and 12.5 µg/mL) in complete growth medium and added to the wells in triplicates. After 21 hrs of treatment, 10 µL of MTT (5 mg/mL in PBS) solution was added in each well and the plates were further incubated for 3 hrs at 37°C until formation of formazan blue crystal. Then the supernatant was discarded from each well and 100 µL of DMSO was added to solubilize formazan crystals for 10 min at 37°C. The absorbance was recorded at 540 nm by a microplate reader (Synergy HT; Biotek). The percentage viability was calculated by using the formula % Cell viability = [OD of treated) / (OD of control)] X 100.

2.10 Colony Formation Assay

Colony formation assay was performed to evaluate the efficacy of extract on the survival and proliferation of cells. In brief, cells at the initial density of $1x\ 10^3$ in 2 ml medium were seeded in 6-well plates. Cells were incubated for 16-18 h. Next day media was changed and cells were treated with different concentrations (12.5 µg/mL, 25 µg/mL, 50 µg/mL and 100 µg/mL) of extract. After 24 h media was removed and cells were grown in complete growth media for next 7-10 days until distinct colonies were formed. Thereafter colonies were washed with PBS and fixed with chilled 100% methanol for 10 min. Fixed cells were stained with 0.05% Coomassie blue stain for 10 min. Plates were air dried and the number of colonies was determined by counting them under an inverted phase-contrast microscope (Nikon Eclipse, Ti-S, Japan) at 20X magnification and a group of ~50 cells were counted as a colony.

2.11 Morphological Changes by Hoechst 33342 Staining

To determine nuclear morphology change in cells we performed Hoechst 33342 staining and fluorescence microscopy (Nikon Eclipse, Ti-S, Japan). Cells were grown to 50% confluence on coverslip and left for 18-20 hours in the incubator until full morphology is attained. Next media was replaced with fresh growth media and cells were treated with different concentrations (12.5 µg/mL, 25 µg/mL and 50 µg/mL) of extract. After 24 hours cells were fixed with 4% paraformaldehyde and permeabilized with 0.3% Triton-X100 in PBS. Cells were then stained with Hoechst 33342 at a final concentration of (0.5 µg/ml) in PBS and incubated for 5 min at 25℃. Brightly stained, condensed nuclei with characteristic features of apoptotic cells were counted using a fluorescence microscope.

2.12 Statistical Analysis

The values are expressed as Means±SD. Experiments were repeated three times. Data

were analyzed using one way ANOVA followed by Dunnett's test for multiple comparisons using the graph pad prism v5.0 (Graph pad software, inc., USA). *P* values of less than 0.05 were taken to be significant in the experiments.

3. RESULTS

3.1 Phytochemicals Screening

Preliminary screening for the presence of different groups of compounds in methanolic extracts of CJS, TCS and REB is summarized in Table 1. As shown in Table 1, TCS and REB showed a positive test for terpenoids, alkaloids, glycosides, tannins, anthocyanins, saponins, steroids, flavonoids, polyphenols and lignans, however, CJS also showed a positive test for above phytochemicals except for steroids and lignans. The results showed that all the extracts hold different types of phytochemicals which may be accountable for different pharmacological activities of CJS, TCS and REB.

Table 1. Presence of different groups of phytochemicals in CJS, TCS and REB methanolic extracts

Phytochemicals	*C. juncea*	*T. cordifolia*	*R. emodi*
Terpenoids	+	+	+
Alkaloids	+	+	+
Glycosides	+	+	+
Tannins	+	+	+
Anthocyanin	+	+	+
Saponins	+	+	+
Steroids	-	+	+
Flavonoids	+	+	+
Polyphenolics	+	+	+
Lignans	-	+	+

3.2 Total Phenolics Content

Table 2 shows the total phenolics content in the methanolic extract of CJS, TCS and REB expressed as Gallic Acid Equivalent (in mg)/g of plant materials, (GAE). The total phenolics content of CJS extract demonstrated that 13.26±2.26 mg Gallic Acid Equivalent of per g dry extract, while TCS showed 65.25±4.22 mg GAE/g dry extract of TCS and REB 45.98±3.2 mg GAE/g dry extract of REB. Results were calculated from the standard gallic acid calibration curve (R2 = 0.9980).

3.3 HPLC Analysis

Various kinds of flavonoids and phenolics compound present in many medicinal plants. These compounds are responsible for different pharmacological activity. Owing to the presence of phenolics contents we also estimated two potent compounds rutin and quercetin in CJS, TCS and REB methanolic extracts with the help of HPLC. HPLC analysis demonstrated that CJS, TCS and REB showed presence of rutin and quercetin. As shown in Table 3, TCS demonstrated most abundant amount of rutin 12.5±1.10 mg/g followed by quercetin 0.4±0.05 mg/g while REB had 2.1 2.10±0.8 mg/g of rutin with 0.28±0.07 mg/g of quercetin. CJS demonstrated the lower amount of rutin 0.0033±0.001 mg/g with the absence of quercetin (Table 3 and Fig. 1).

Table 2. Total phenolics content in CJS, TCS and REB methanolic extracts

Extracts	Total phenolics content (mg) GAE/g of plant extract
CJS	13.26±2.26
TCS	65.25±4.22
REB	45.98±3.22

All the values are expressed as mean±SD (n=3)

3.4 Free Radical-scavenging Activity: DPPH Assay

The free radical DPPH scavenging activity to demonstrate antioxidant potential has been widely developed. Results showed that methanolic extracts of CJS, REB and TCS inhibits 50% of the DPPH free radical at a concentration of 383.0±4.25 µg/mL, 98.3±1.2 µg/mL and 29.7±3.5 µg/mL, respectively. These results were compared with ascorbic acid as standard with IC_{50} value of 2.09 µg/mL. The significant percent inhibition of DPPH radical was initiated at 12.5 µg/mL extract concentration of CJS with 11.98%±1.65, REB 15.65%±2.65 and TCS 20.53%±3.6 However, highest inhibition 57.12%±3.65, 93.88%±2.6 and ≅100%±3.5 was recorded at 400 µg/mL of CJS, 400 µg/mL of REB and 100 µg/mL for TCS, respectively (Fig. 2).

3.5 Cell Viability Assay

The cytotoxic effect of *C. juncea, T. cordifolia* and *R. emodi* methanolic extracts on proliferation of MDA-MB-231 cell line assessed in time and dose dependent manner. Cells were exposed to increasing doses of methanolic extracts for 24, 48, and 72 hours and cell viability and percentage inhibition were determined by the

MTT assay. Comparing the results of the control group of untreated cells with treated cells, treated cells exhibited a dose and time dependent decline in viability. TCS showed remarkable initiation in decline in cells viability at a concentration of 12.5 µg/mL with 10.6% inhibition while REB at 50 µg/mL with 11.02% inhibition and CJS showed at 100 µg/mL with 12.7% inhibition after 24 hours of extract exposure (Fig. 3). Half-maximal inhibitory concentration (IC_{50}) values are commonly used to evaluate the potency of a compound, lower the IC_{50} value, the more potent the compound is. According to the results obtained from MTT assay, the IC_{50} values for CJS, TCS and REB against MDA-MB-231 cells were summarized in Table 4. The results showed the tendency of MDA-MB-231 cells to decrease sharply upon treatment with TCS followed by REB extract, with tapering response intensity as the concentrations of the extract was increased. However, MDA-MB-231 cells similarly treated with CJS extract did not show as much reduction in viability as TCS and REB.

3.6 Colony Formation Assay

Preliminary screening on cell viability revealed that TCS showed lowest IC_{50} value indicating most antiproliferative effect on MDA-MB-231 cells as compared with other extracts. Therefore, we evaluated the effect of TCS on colony formation and proliferation of MDA-MB- 231 cells. As evident in Fig. 4 (A-B), treatment of MDA-MB-231 cells with different concentrations of TCS resulted in a significant decrease in colony formation when compared with untreated MDA-MB-231 cells. A decrease of colonies from 12.4% to 80.6% was noted after treatment with12.5 µg/mL to 100 µg/mL concentrations of TCS extract when compared with respective control. These finding confirmed that TCS possess significant antiproliferative activity against breast cancer MDA-MB-231 cell line.

3.7 Nuclear Morphology by Hoechst 33342 Staining

The results obtained from cell viability and colony formation assay demonstrated TCS extract inhibitory potential against MDA-MB-231 cells. Hence, we further explored that whether TCS extract induces apoptosis in breast cancer cells we performed Hoechst 33342 staining after treatment of MDA-MB-231 cells with TCS extract. Morphological changes in the nucleus were observed with the help of a fluorescence microscope. TCS treated cells showed distinctive character of apoptosis such as nuclear fragmentation, chromatin condensation, cell shrinkage and loss of membrane symmetry. However, the untreated cells showed normal morphology of nucleus such as round, homogenous and intact chromatin (Fig. 4, C-D).

Table 3. Concentration of rutin and quercetin and rutin in CJS, TCS and REB methanolic extracts as estimated by HPLC

Extracts	Rutin (mg/g of extract±SD)	Quercetin (mg/g of extract±SD)
CJS	0.003±0.001	ND
TCS	12.5±1.10	0.40±0.05
REB	2.10±0.8	0.28±0.07

All the values are expressed as mean±STDEV (n = 3). ND-Not detected

Table 4. Comparison of IC_{50} values between methanolic extracts of CJS, TCS and REB after the exposure to MDA-MB-231 cells

Plants	IC_{50} (µg/mL±SD)		
	24 h	48 h	72 h
C. juncea	>400±8.65	388±7.35	355±7.34
T. cardifolia	51.7±3.14	37.5±2.12	21.7±2.5
R. emodi	269±5.28	193±4.65	158±5.25

4. DISCUSSION

Phytochemicals such as terpenoids, alkaloids, glycosides, polyphenols and flavonoids present in extracts exhibit a broad range of therapeutic effects for diabetes, anaemia, osteoporosis, hypercholesterolemia, cardiopathy, asthma, constipation, microbial infections, indigestion, and inflammation including cancer [19]. Our results showed the presence of different categories of phytochemicals in methanolic extracts of CJC, REB and TCS. Different types of phytochemicals distributed widely in plants and microbes have been recognized as potent bioactive compounds and used in various human ailments [20]. Natural product derived alkaloids, terpenoids, polyphenols and flavonoids have been reported to possess significant pharmacological activities among different groups of phytochemicals [21-22]. In our study, we found that all the extracts showed the presence of alkaloids, terpenoids, polyphenols and flavonoids. The presence of different category of phytochemicals may be correlated

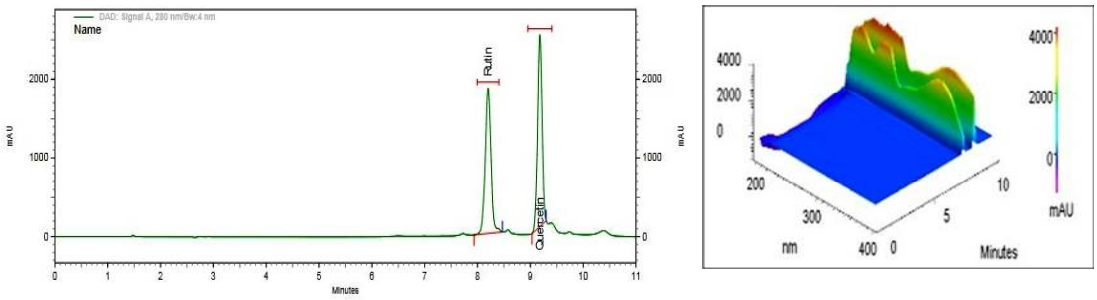

Fig. 1. HPLC chromatogram of rutin and quercetin standard and 3D view of the chromatogram
Detection at 280 nm with Zorbax Eclipse C-18 column (4.6 x 150 mm, 5 µm), gradient eluents methanol/water, flow rate 1.0 mL/min with injection volume 5.0 µL

with different biological properties of CJS, TCS and REB. Hamendra et al. 2011, reported that the higher fatty acids content such as linoleic acid, oleic acid, steric acid, palmatic acid from CJS could be responsible for its anti-inflammatory activity [23]. Isoquinoline an alkaloid, clerodane glycosides isolated from TCS have been reported to have anticataract, antioxidant, cytotoxic properties [24]. Rashid et al. [25], reported that anthraquinone derivatives from of *R. emodi* showed evidence of antifungal, antimicrobial, anti-Parkinsonism effect. Our findings are in concordance with the earlier reports [20-25] which demonstrated the presence of diverse phytochemicals in CJS, TCS and REB and highly linked with different pharmacological properties.

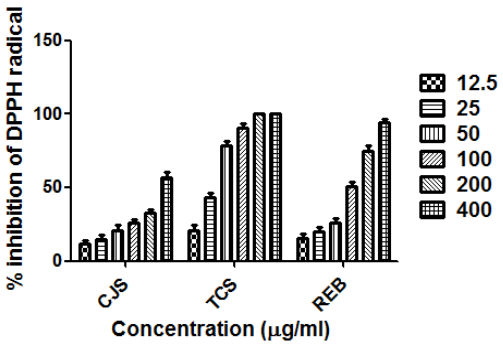

Fig. 2. Percent inhibition of DPPH free radicals after treatment with methanolic extracts of CJS, TCS and REB
Values are expressed as mean ±SD (n=3)

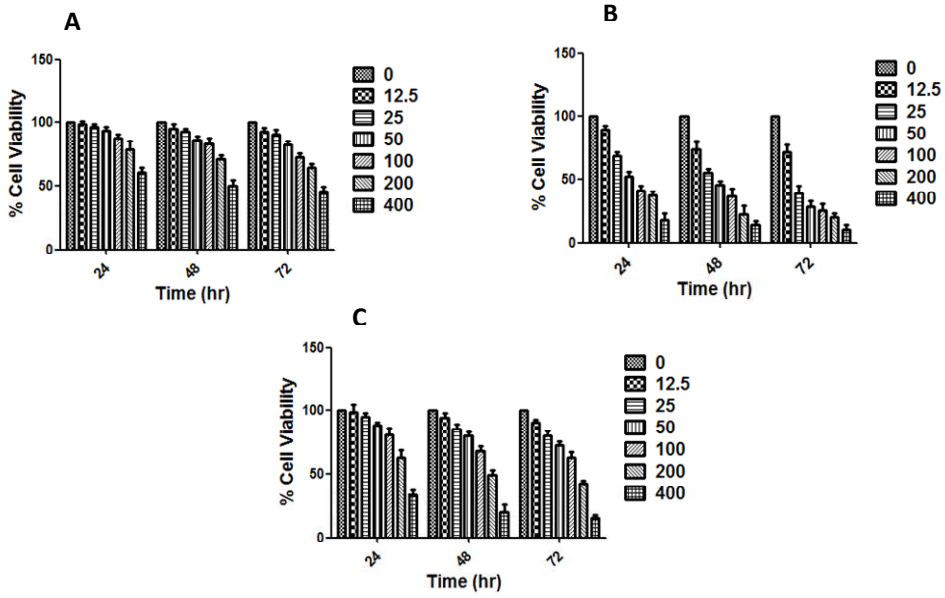

Fig. 3. Anti-proliferative effect of different extracts against human breast cancer cells
Human Breast cancer MDA-MB-231 cells were treated with methanolic extracts of CJS (A), TCS (B) and REB (C) for 24, 48 and 72 h, and cell viability was determined by MTT assay

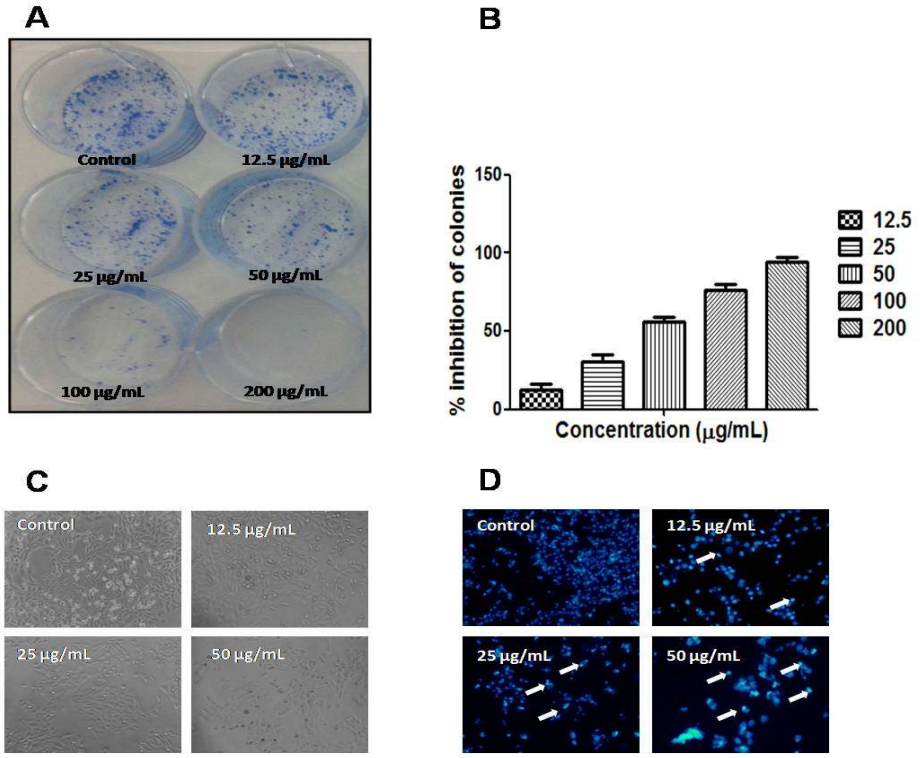

Fig. 4. (A, B) Effect of TCS on growth assay estimated by colony formation

Human breast cancer MDA-MB-231 cells were grown in media along with different concentrations of the TCS. The number of colonies was recorded after 7 days of treatment. Data represents the mean±SD of three different assays. P ≤ 0.05 versus control

(C) Microscopic examination of human breast cancer MDA-MB-231 cells, after treatment with TCS for 24 h. (D). Induction of nuclear fragmentation by TCS in MDA-MB-231

2×10^4 cells/well were seeded in 6-well culture plate and allowed to grow for 24 h and then treated with different concentrations of TCS for 24 h and stained with Hoechst 33342 stain following standard protocol and the image was captured by inverted microscopy at 20X magnification. Arrows indicate the cells with DNA fragmentation and apoptotic nuclei

Polyphenols are an important class of antioxidants due to their ideal structural chemistry for free-radical scavenging activity. They can act as reducing agents, metal chelators and free radical quenchers by donating an electron or hydrogen atom to free radicals [26]. Hence, we estimated Total Phenolics Content (TPC) in CJS, REB and TCS methanolic extracts. Results showed that the highest amount of TPC was present in the case of TCS methanolic extract in comparison with REB and CJS extracts. There are many different phenolics and flavonoids compounds present in extract and it is difficult to measure each separately. However, rutin and quercetin are two very important compounds which belong to these categories and play a vital role in determining various therapeutical roles of plant extracts. Therefore, we measured rutin and quercetin by HPLC in CJS, TCS and REB methanolic extracts and found that TCS and REB showed a significant amount of rutin and quercetin, however, CJS had comparatively lower amount.

The stable free radical DPPH has been widely used to determine the primary antioxidant activity. The DPPH free scavenging assay is based on the ability of DPPH, a purple color stable free radical, to decolorize to yellow color in the presence of an antioxidant. A dose-dependent radical scavenging activity was observed with all the extracts investigated, when the concentration of the extracts was decreased, the free radical inhibition was also found decreased. There have been many reports about the correlation of phenolics contents with the

antioxidant activity of plant extracts [27-29]. These results suggested that phenol content is responsible for the highest DPPH radical scavenging activity. Moreover, the high antioxidant activity may also be due to the presence of phenolics components. The results obtained from antioxidant assay supported that there is a positive co-relation between phenols and antioxidant activity. Typical phenolics and polyphenols are the molecules contained with an aromatic ring, bearing hydroxyl group and other functional derivatives, which are well recognized for their antioxidant, antimutagenic and anti-tumor activities [30].

The MTT assay for cytotoxic activity of the methanolic extracts derived from CJS, TCS and REB demonstrated a decrease in MBA-MB-231 cells survival in a dose and concentration dependent manner. Our results illustrated that TCS methanolic extract exhibited a most cytotoxic effect on MDA-MB-231 cells among three extracts. The methanolic extract obtained from REB showed second highest antiproliferative effect, however, CJS extract showed a cytotoxic effect after TCS and REB. Furthermore, growth inhibition potential of TCS extract was also proved by reduction and growth inhibition of size and colonies of MDA-MB-231 cells. These result suggested that *T. cordifolia* possess significant anti-proliferative activity against breast cancer cells. The comparison between IC_{50} of these extracts showed that the methanolic extract of TCS has lower IC_{50} value when compared with others after incubation of 24 to 72 hours. According to the United States National Cancer Institute plant screening program, a plant extract is generally considered to have active cytotoxic effect if the IC_{50} value, following incubation between 24 to 72 hours, is 30-40 µg/mL or less [31,32,24]. This finding suggested that the reduction observed in the viable cells following treatment with extracts is due to cell death. The preventive mechanisms of tumor promotion by extract range from the inhibition of genotoxic effects, antioxidant effects and anti-inflammatory effects, inhibition of proteases and cell proliferation, protection of intracellular communications to modulate apoptosis and signal transduction pathways [33]. Our results demonstrated that exposure of MDA-MB-231 cells with TCS extract exhibited significant changes in nuclear morphology representing antiproliferation activity through induction of apoptosis which is accounted for cell death. The extracts or agents that induce apoptosis in cancer cells gained considerable attention in development of anticancer drugs [34,35]. Natural chemopreventive agents exhibiting anti-proliferative and apoptotic effects on cancer cells usually showed high antiradical and antioxidant property that regulate signaling molecules, protecting cells from damage or transform into cancer cells [36,37].

The efficiency of these extracts in our study is unconditionally attributed to the presence of rutin and quercetin along with the presence of other different phytochemicals which could be attributed to their pharmacological properties including antiproliferative activity against MDA-MB-231 cells. It is obvious from above study and results that antioxidant properties and presence of phenolics compounds in the extracts may prevent progression of cancer; while the cytotoxic potential against cancer cells, directing towards induction of apoptosis and cell death.

5. CONCLUSIONS

In conclusion, it may be stated that the present observations provide preliminary data on CJC, TCS and REB methanolic extracts cytotoxicity. TCS had potent cytotoxic activity against MDA-MB-231 cancer cells followed by REB and CJS. These extracts contained substantial amounts of quercetin and rutin and this is the first such report. The presence of quercetin and rutin and other compounds may be involved in growth inhibition of MDA-MB-231 cancer cells and DPPH free radicals. The antiproliferative property of TCS is attributed to induction of apoptosis. These findings indicate that TCS may be a potential source of anti-carcinogenic therapeutic compounds. This calls for further studies on the active components for proper assessment of their chemotherapeutic properties as well as their possible development as promising anticancer drugs.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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http://sciencedomain.org/review-history/14727