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Research Article

QUANTIFICATION OF PHYTOCONSTITUENTS OF *Spermacoce Hispida* AND ITS INHIBITORY ACTIVITY AGAINST HCT 116 CANCER CELL LINES

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Abstract

Cancer is a leading cause of mortality and it strikes more than onethirdof the world's population and it is the cause of more than 20% of all deaths. Traditional herbs are being used and many researches are under progress toprovide an effective treatment for cancer without any adverse effects. In the present study, *Spermacoce hispida*, Rubiaceae family which has been traditionally claimed to be a useful herb and reported with many activities has been evaluated for its anticancer activity and an attempt has been made to investigate the phytochemical constituents present and quantified. Total phenolic content were estimated by Folin Ciocalteau method,total flavonoid, total tannin contents were also studied in the methanolic extract of *Spermacoce hispida. T*he results showed 31.73GAEmg/g, 99.48mgQE/g, 15.83TAEmg/g respectively. The antiproliferative activity of the methanolic extract in HCT 116 (Human Colon Cancer cell line) and its inhibitory activity were studied at 12 hrs and 24 hrs. The extract was preferently inhibiting the colon cancer cell line in dose dependent manner.

Keywords: *Spermacoce hispida*, Total phenolic content, Antiproliferative assay.

Introduction

Cancer can affect people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age. In 2007, cancer caused about13% of all human deaths worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world[1].Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%),radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental

pollutants. The allopathic physician of India is aware of the limitation of modern medicines and is simultaneously conscious of the strength of traditional medicine in certain areas. He may not turn to Ayurveda for treatment of acute infection, but tempted to try out therapies for chronic recurrent diseases, metabolic disorders or degenerative diseases where modern medicine has almost nothing to offer. The physician and patient prefer Ayurveda or alternative medicines for promotion of health as the concept of "Positive health" does not have in modern medicine.[2] S*permacoce hispida* is a traditional herb which is easily available in India. It is found throughout the country upto 900 m in the hills. It is a common weed found in all dry lands, road sides and open forests. Previous study indicated that the flavonoid , phenolic compounds, terpenoids were present in *Spermacoce hispida*. In the present study to quantify the phytoconstituents in the methanolic extract of the plant, and evaluate the *invitro* anticancer activity using cancer cell lines.

Materials and Methods:

Collection of the plant

The Aerial part of *Spermacoce hispida* was collected from in and around Trichy in the month of September 2013.The freshly collected plants were dried in shade and authenticated by Dr.Soosai Raj, Professor, Department of Botany, St.Joseph College, Tiruchirappalli, Tamil Nadu. The voucher specimen is maintained in the Department of Pharmaceutical Technology, Tiruchirappalli,Tamilnadu.

Preparation of the extract[3]

The plant extract was prepared by cold maceration process, 250g of the plant was coarsely powdered and macerated in 650 ml of methanol. It was left for a week and then filtered, the obtained filtrate is then concentrated using Rotary Vacuum Evaporator and the solvent was recovered. The concentrated extract was then kept at room temperature to remove the solvent completely from the extract. The extract obtained was stored for further use. The percentage yield of methanolic extract of the plant was 5.71%.

Chemicals required

Folin - Ciocalteau reagent solution (1N). Dilute commercial reagent (2N) with an equal volume of water on the day of use (2 ml of commercial reagent + 2 ml distilled water), Standard Quercetin, gallic acid, Aluminium chloride, Sodium carbonate. All the chemicals used were analytical grade (merck).

Detemination of Total Flavonoid content[4]

The aluminum chloride colorimetric method was used for the determination of the total flavonoid content of the sample. For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 50mg quercetin in 10 mL methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (5–200 μg/mL). An amount of 0.6 mL diluted standard quercetin solutions or extracts was separately mixed with 0.6 mL of 2% aluminum chloride. After mixing, the solution was incubated for 60 min at room temperature. The absorbance of the reaction mixtures was measured against blank at 420 nm wavelength with a Varian UV-Vis spectrophotometer (Cary 50 Bio UV-Vis Spectrophotometer, Varian). The concentration of total flavonoid content in the test samples was calculated from the calibration plot and expressed as mg quercetin equivalent (QE)/g of dried plant material.

Determination of Total Phenolic content[5,6]

The total phenolics of the methanolic extract were determined using the Folin and Ciocalteu method. The sample (200 μl) was mixed with 200 μl of Folin-Ciocalteau reagent (previously diluted 1:1 with distilled water) and allowed to stand at room temperature for 5 mins. A 2000 μl sodium bicarbonate solution (7% w/v) was added to the mixture. After 90 min at room temperature, absorbance was measured at 700 nm using a UV/Vis spectrophotometer. Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of gallic acid standard (10-50 μg/ml).The concentrations are expressed as mg of Gallic Acid Equivalents (GAE) per g of sample.

Determination of total tannins

Tannin content in the sample was determined using insoluble Polyvinyl-Polypirrolidone (PVPP) as described by Makkaret al.1 mL of extract dissolved in methanol (1%), was mixed with 100 mg PVPP, vortexed, left for 15 min at 4°C. Then it is centrifuged for 10 min at 3,000 rpm. In the clear supernatant the non-tannin phenolics were determined in the way similar to the total phenolics content. Tannin content was calculated as a difference between total phenolic and non-tannin phenolic content in the extract[7].

Invitro Evaluation of Anti-Cancer Activity[8]

The in-vitro anti-proliferative activity studies were performed with mammalian cell lines HCT-116 (Human colon carcinoma) using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method. The cells were

purchased from NCCS, Pune, India and maintained in monolayers in 100-mm2 flask at 37ºC under humidified 5% CO2 - 95% air. The cells were cultured in Minimum Essential Medium (MEM) supplemented with Fetal Bovine Serum (FBS 10% final concentration), antibiotic antimycotic solution 100X 1%. Cells were harvested during the log phase with a solution of 0.05% trypsin and 0.02% EDTA, and resuspended in MEM to a final concentration of 1X104cells/well in 96 well culture plates [34]. The seeded culture plates were incubated for overnight at 37ºC under humidified 5% CO2 - 95% air. Various concentrations of plant extract were loaded in duplicates with positive and negative controls. After 12 and 24 hrs incubation the wells were added with MTT and left for 3 hours. All wells were removed the content using pipette and 100µl SDS in DMSO were added to dissolve the Formosan crystals, absorbance are read in Lark LIPR-9608 micro plate reader at 540 nm.[9]

Results and Discussion

Phytochemical Studies

The freshly prepared methanolic extract of *Spermacoce hispida was* subjected to qualitative analysis, it showed the presence of alkaloids, flavonoids, tannins, sapponins, terpenoids and sterols.The total flavonoid content were estimated by using Alumiium chloride reagent. Thus the amount of flavonoids present in the *Spermacoce hispida* methanolic plant extract was found to 99.483 mg QE/ g of extract. (r2 = 0.9752).

Phenolic compounds constitute one of the most numerous and ubiquitous group of plant metabolites, and are an integral part of the human diet. It was found that in addition to their primary antioxidant activity, many studies demonstrated this group of compounds displays a wide variety of biological functions which are mainly related to modulation of carcinogenesis.

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (r2 = 0.9934). The values obtained for the concentration of total phenols was 31.73mgGAE/g.

The total Tannin content was also estimated and the concentration of tannins in the methanolic extract was found to 15.899mg GAEmg/g.

The in-vitro anti-proliferative activity studies were proved that cancer cell lines were inhibited their proliferation significantly with the increasing of drug concentration. In HCT-116 cell line more cytotoxicity effect was observed in *Spermacoce hispida* plant extract in 24 hours treatment, it also revealed the increased concentration of the extract showed

good toxicity over the cancer cell. This effect is due to the drug having more specific activity to cancer cell line. InHCT-116 cell lines expressed the anti-proliferative effect of drug in accountable levels. It was recorded with maximum of 87.9% cell viability for 100 µg of the plant extract. In same way the drug has shown its minimum of 12.58% cell viability for 500 µg. The IC_{50} value of this extract against HCT-116 cell line was observed 100µg/ml after 24 hours of incubation. The extract inhibits the cancer colon cancer cell line in the dose dependent manner.

Table-1: Concentration and absorbance of quercetin standard.

S.No.	Concentration (µg/ml)	Absorbance (no unit)
1.	0	0
2.	25	0.253
3.	50	0.307
4.	75	0.449
5.	100	0.585
6.	125	0.703
7.	150	0.861

Table-2: Concentration and absorbance of Gallic acid standards.

S.No.	Concentration (µg/ml)	Absorbance (no unit)
1.	0	0
2.	10	0.0982
3.	20	0.2041
4.	30	0.2721
5.	40	0.3476
6.	50	0.4414

Conclusion:

Based on these results, it is possible to conclude that methanolic extracts of *Spermacoce hispida* has higher Percentage of flavonoid, phenolic compopunds. These are complex secondary metabolites having various medicinal properties. Presence

of high content of flavonoid and phenolic compounds contribute the antiproliferative effect of HCT-116 cancer cell. The results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate its anticancer activity.

Figure-1: Calibration curve of Quercetin.

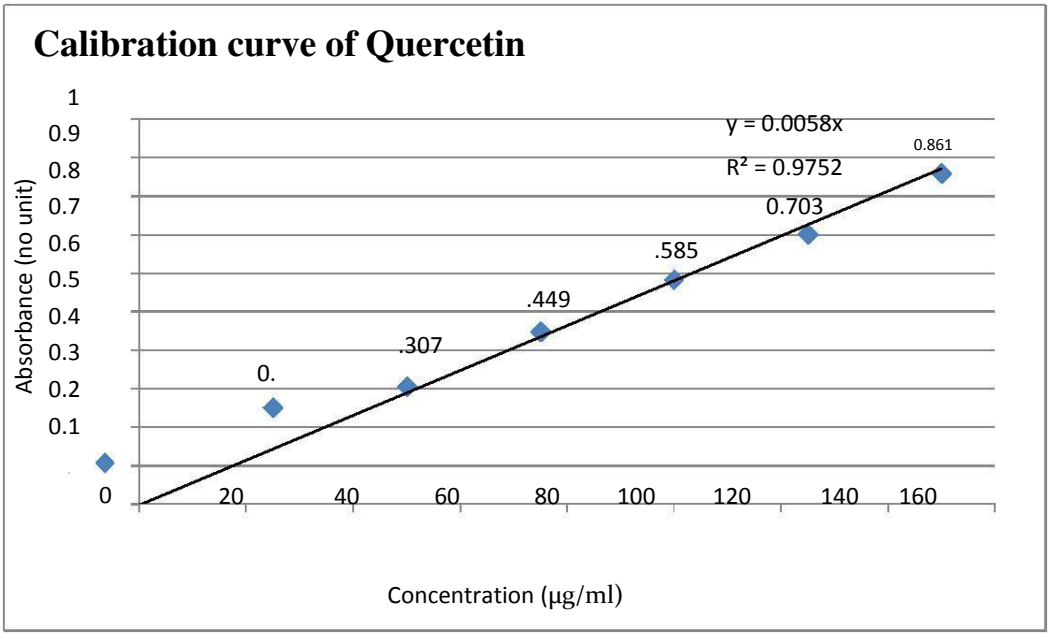
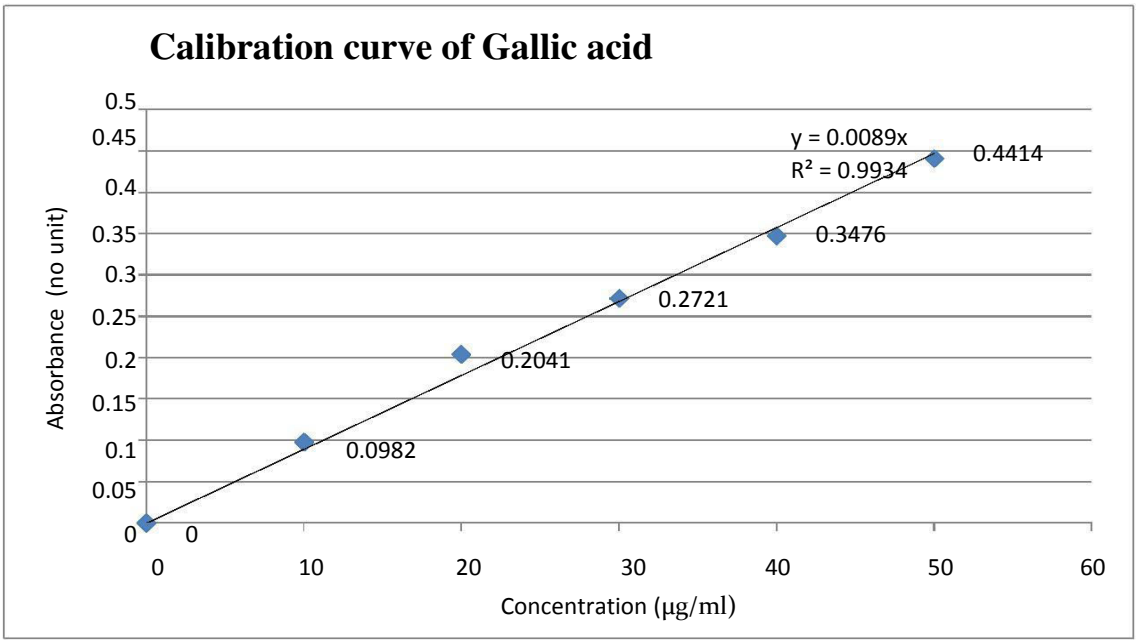


Fig-2: Calibration curve of Gallic acid.



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