Journal of Medicinal Plants Research Vol. 5(32), pp. 6955-6962, 30 December, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals
DOI: 10.5897/JMPR11.534

Full Length Research Paper

Screening of phytochemical compounds in selected medicinal plants of Deccan Plateau and their viability effects on Caco-2 cells

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Accepted 26 August, 2011

The aim of the present study was to investigate the presence of potential phytochemical compounds in medicinal plants of *Hemidesmus indicus*, *Canthium parviflorum* and *Canavalia gladiata* and to test their effect on Caco-2 cell viability. In *H. indicus* fresh roots were used where as in *C. parviflorum* and *C. gladiata* fresh leaves were screened for their potential phytochemical compounds. Phytochemical screening of the compounds was carried out with aqueous and methanolic extracts based on the standard identification methods. Results revealed the presence of tannins, alkaloids, flavonoids, saponins, steroids, anthraquinones and reducing sugars. The aqueous extract of all the screened plant materials contained tannins, alkaloids and flavonoids. Among this, flavonoids occupied a major portion in plant extracts. The methanolic extracts of all the plant extracts revealed the presence of flavonoids in them. Based on the screening results obtained by aqueous and methanolic extracts it is well understood that the plant extracts screened could be potentially used for a wide range of applications in medicine as well as for other industrial applications. These extracts were tested for their cytotoxic effect on the colon adenocarcinoma cell line (Caco-2). MTT assay was used to evaluate the viability of cells in the presence of the extracts. Methanolic extract of *C. parviflorum* extract showed to be a potent cytotoxic with an IC_{50} at 52 µg/ml. *H. indicus* showed an IC_{50} at 60 µg/ml and *C. gladiata* was non toxic.

Key words: Phytochemical, screening, medicinal plants, cytotoxicity.

INTRODUCTION

The use of medicinal plant extracts to cure various diseases is known since ancient times. In India, the practice of using plants for treating a wide variety of diseases is being carried out over ages and this practice is well explained and studied in Ayurveda (Siddharthan, 2007). Over one and half million practitioners of Indian system of medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications (Madhulika, 2010). Medicinal plants as a group comprises approximately 8000 species and accounts for more than 50% of all higher flowering plants species of India (Arjun et al., 2010). The booming of traditional medicine industry is increasing demand on medicinal plant products and 90% of the medicinal plants come from natural habitats (Raju, 2007). Studies on medicinal plants in South India (Deccan Plateau) are of interest for researchers and stakeholders to improve the conservation and to explain the prominence of Indian medicinal plants and their potentiality (Abayomi et al., 1993; Grover, 2002).

Medicinal plant wealth of Deccan Plateau contributes maximum to the medicinal plants diversity of India and apart from traditional systems of medicine, the modern medicine also utilizes the floral diversity and active

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Table 1. Ethano botanical Information of the medicinal plants used in the study with their family and common name and therapeutic use.

S/N	Plant species	Part used	Common name	family	Therapeutic advantages
1	*Hemidesmus indicus*	Root	Sarsaparilla	Asclepidaceae	Antihepatotoxicity, Arthritis,Anti epileptic activity
2	*Canavalia gladiata*	Leaf	Sword bean	Fabaceae	Anti cancer activity
3	*Canthium parviflorum*	Leaf	Negini	Fabaceae	Anti venom, Wound healing

principles with potential for treatment of disease are extracted from plants (Martins et al., 2001). During the past few decades enormous efforts were undertaken to introduce new chemical entities with potential medical applications in drug discovery research (Grover, 2002; Marcy, 2005).

The usage of medicinal plants to cure certain types of skin diseases were evaluated by various researchers (Arshad et al., 2010). Certain researchers evaluated their applications even in HIV (Bbosa et al., 2010). Studies on cultivation practices were undertaken for certain plants which were found to have potential market and which had a scope for commercial exploitation (Marcy, 2005). Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents but also because such information may be of value in disclosing new sources Lena 2010. The aim of this study was to determine the phytochemical properties of roots of *H. indicus* and fresh leaves of *C. parviflorum* and *C. gladiate* found in the Deccan plateau region of India and the possible use of its chemical composition in the industries and in medical sciences (Sofowara et al., 1982; Lilliana, 2010). Study on the Cytotoxicity effect of the extracts on Caco-2 cells and their property to reduce or induce permeability of the test compounds were carried out, as the extracts used for the present study were obtained from plant sources.

MATERIALS AND METHODS

Collection and identification of plant materials

Fresh roots of *H. indicus* and fresh leaves of *C. parviflorum and C. gladiata* were collected from different regions of Deccan plateau (Hill et al., 1952; Harborne, 1973). The collected plant material was identified by the botany department. Plants were washed under the running tap water and air dried for two to three days and then homogenised to fine powder and stored in air tight container till further use. The information regarding the plants with their family name and parts used for the study with their therapeutic applications are given in Table 1.

Chemicals

Dulbecco's Modified Eagle's Medium (Invitrogen), Dimethyl sulfoxide (DMSO), 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyl tetrazolium bromide (Sigma-Aldrich), penicillin, streptomycin, fetal bovine serum (Invitrogen) and ethanol (Merk) were used for the present study.

Preparation of plant extracts

Aqueous extract

Twenty five grams of dried leaves and roots were weighed and taken and they were homogenized to fine powder and this powder was dissolved in 1000 ml of distilled water and mixed thoroughly for 15 to 20 min in a mixer (Akamine, 2009). Then the mixed extract was filtered using a cheese cloth vice versa followed by using a filter paper. The filtrate formed is then concentrated by using a vacuum rotary evaporator and then the concentrated filtrate is measured for dry weight of the plant extract (Trace and Evans, 1989).

Methanol extract

Ten grams of the dried leaves and roots were weighed and they were homogenised to fine powder and this fine powder was dissolved in 100 ml of 70% methanol and mixed thoroughly. Then the mixture is filtered by using a cheese cloth vice versa followed by using a vacuum rotary evaporator and then the concentrated filtrate is measured for dry weight of plant extract (Ayoola, 2008).

Phytochemical screening

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature (Egwaikhide et al., 2007; Jigna, 2007; Makut, 2008; Aiyelangbe, 2009).

Test for alkaloids

0.5 g of extract was stirred with ethanol containing 3% tartaric acid. The filtrate was taken in 3 beakers and tested for alkaloids by adding Hagar's reagent, Mayer's and Marquin's reagent were added. Precipitation in any of the 3 test indicates the presence of alkaloids.

Test for saponins

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test

Table 2. Results of Phytochemical screening of aqueous plant extracts by standard methods.

Test	*Hemidesmus indicus*	*Canavalia gladiata*	*Canthium parviflorum*
Alkaloids	-	+	+
Saponins	+	-	-
Tannins	+	+	+
Steroids	+	-	-
Flavonoids	-	+	+
Anthraquinones	-	-	-
Cardiac glycosides	-	+	+
Reducing sugars	-	+	-

- = Absent; + = present.

tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration Heslem 1989.

Test for steroids

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids

About 2 g of powdered leaf extract was completely detanned with acetone. The residual extractants in warm water after evaporating the acetone in a water bath. The mixture was filtered while, still hot. The filtrate was cooled and used. 5 ml of 20% NaOH were added to equal volume of the detanned water extract. Yellow solution indicates the presence of flavonoids.

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardioids.

Test for reducing sugars

The residue was dissolved in water and kept in the water bath. Two ml of the solution in a test tube was added with 1 ml each of Fehling's reagent A and B. The mixture was shaken and heated in a water bath for 10 min. A brick red precipitate indicates a reducing sugar.

Cell culture

Caco-2 cells were obtained from NCCS, Pune and cultured in DMEM medium with 1 mM L-Glutamine, 100 Units/ml penicillin and 0.1 mg/ml streptomycine, 20%FBS. Cells were maintained in a humidified incubator in an atmosphere of 95% air and 5% CO_2. Cells were culture for 3 days in the cell culture flasks and after reaching the confluence the cells were seeded in the 96 well plate with a seeding density of 5 x 10^4 cells/well (Bhuvan et al., 2009; Shaik, 2009).

Cell viability

Cell viability was determined by MTT (3-(4, 5 dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide) test method. MTT (5 mg/ml) was dissolved in PBS .The solution was filtered through a 0.2 µm filter and stored at 2 to 8°C. MTT assay was employed to assess cell viability. The 96 well culture plate with the seed cells in 100 µl of DMEM medium was incubated for 24 h prior to the treatment with the plant extracts. After 24 h incubation, the plant extracts were prepared freshly at various concentrations from 0.005 to 100 µg/ml in DMEM medium and were added to each well and incubated for 48 h. Control wells were only incubated with DMEM medium only .Wells containing only medium without cells are considered as blank wells (Chandra et al., 2009).

Exposure of plant extracts to cells

After removal of 100 µl of medium with plant extracts, 50 µl of MTT was added to the plate and incubated for 4 h in the humidified incubator. After that 100 µl of DMSO was added to all wells and mixed thoroughly to dissolve the dye crystals. The absorbance was measured using spectrophotometer plate reader .The absorbance was measured at 570 nm with the reference wavelength of 630 nm.High optical density readings corresponds to a high intensity of dye color, that is to a high number of viable cells (Papiya, 2010).

RESULTS

The presence of phytochemical compounds in the plant extracts of *H. indicus* were evaluated by aqueous and methanolic extracts. The investigation through aqueous extraction method revealed the presence of saponins, tannins and steroids.

Study of phytochemical compounds by methanolic extracts revealed the presence of saponins, tannins, flavonoids and reducing sugars (Tables 2 and 3). In case

Table 3. Results of Phytochemical screening of Methanolic plant extracts standard methods.

Test	*Hemidesmus indicus*	*Canavalia gladiata*	*Canthium parviflorum*
Alkaloids	-	+	-
Saponins	+	-	+
Tannins	+	-	+
Steroids	-	-	-
Flavonoids	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	-	+	+
Reducing sugars	+	-	-

- = Absent; + = present.

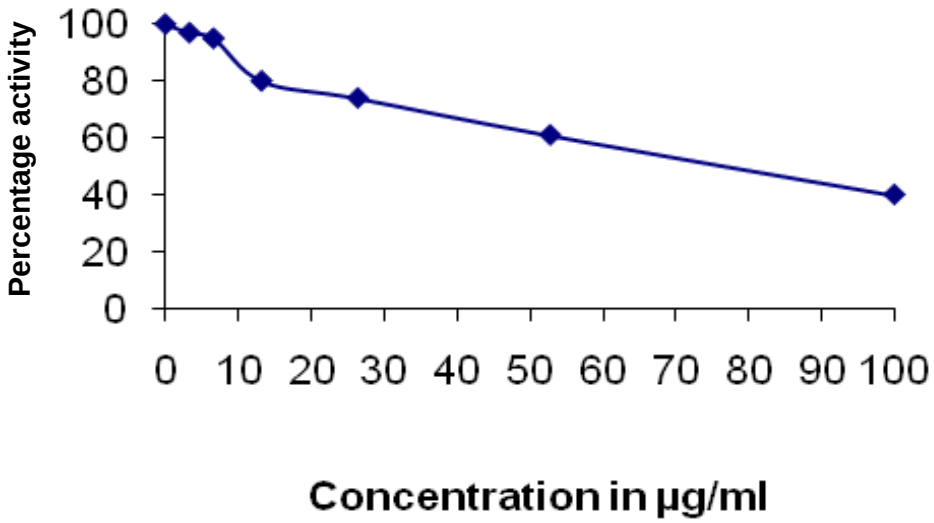

Figure 1. Cytotoxicity of *Hemidismum indicus* aqueous extract.

of plant extracts of *C. gladiata* subjected to aqueous and methanolic extracts the investigation through aqueous extraction method revealed the presence of alkaloids, tannins, flavonoids, cardiac glycosides and reducing sugars. Study of phytochemical compounds by methanolic extracts revealed the presence of alkaloids, flavonoids and cardiac glycosides (Tables 2 and 3). Whereas, on the other hand, the results from plant extracts of *C. parviflorum* when evaluated, the investigation through aqueous extraction method revealed the presence of alkaloids, tannins, flavonoids and cardiac glycosides and with methanolic extracts revealed the presence of saponins, tannins, flavonoids and cardiac glycosides (Tables 2 and 3).

The cytotoxicity assay of *H. indicus* plant extract by aqueous plant extract material showed an inhibitory concentration (IC_{50}) at 78 µg/ml (Figure 1). Whereas the inhibitory concentration (IC_{50}) by methanolic extract was at 60 µg/ml (Figure 2). In case of cytotoxicity assay of *C. gladiata* plant extract by aqueous plant extract material showed an Inhibitory concentration (IC_{50}) at 95 µg/ml (Figure 3). Whereas, the inhibitory concentration (IC_{50}) by methanolic extract was at 82 µg/ml. (Figure 4) and in case of *C. parviflorum* plant extract by aqueous plant extract material showed an inhibitory concentration (IC_{50}) at 71 µg/ml (Figure 5). Whereas, the inhibitory concentration (IC_{50}) by methanolic extract was at 52 µg/ml (Figure 6).

DISCUSSION

The results obtained in our aqueous extraction method revealed the presence of tannins in all of the screened medicinal plant extracts (Table 2) and the presence of tannins could be extremely helpful as a diuretic agent (Heslem, 1989). Medically, tannins are used as antidotes to poisoning by alkaloids depending on their capacity to

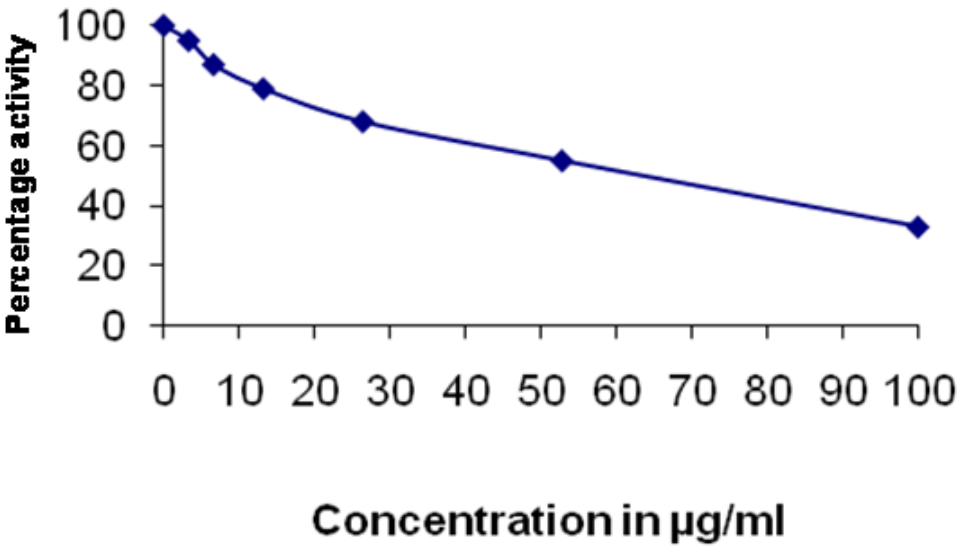

Figure 2. Cytotoxicity of *Hemidismum indicus* methanolic extract.

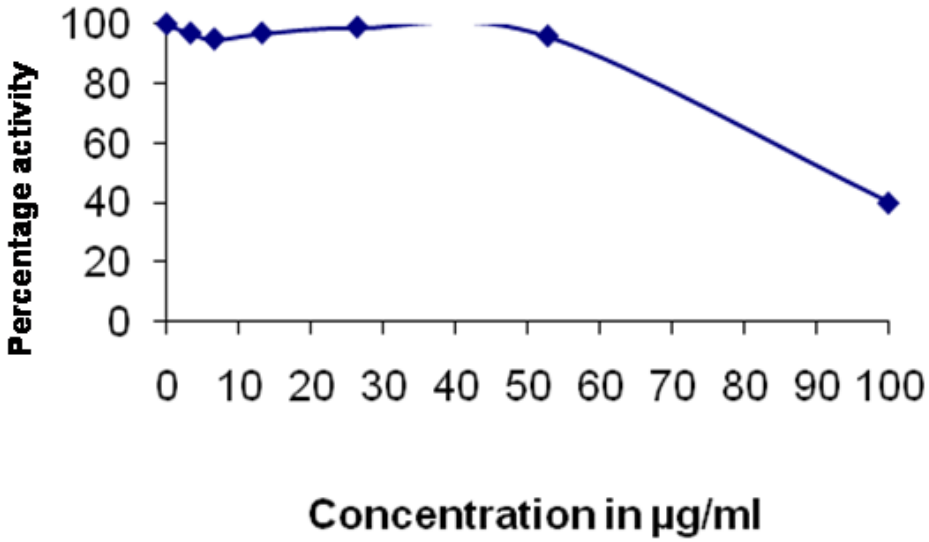

Figure 3. Cytotoxicity of *Canavalia gladiata* aqueous extract.

form insoluble tannates. However, only dilute solutions of tannins are applied for this work, tannins help to draw out all irritants from the skin. These properties impart medicinal qualities to tannin which is applied on the skin to pull out poisons from bee stings or poison oak bringing in instant relief. The other remedial values of tannins include application on burns to heal the injury and on cuts to stop bleeding. Tannin's ability to form a strong 'leather' resistance on the exposed tissues helps in protecting the wounds from being affected further. While it stops infection from the foregoing, internally tannin continues to heal the wound.

Tannins can also be effective in curbing hemorrhages as well as restrict bare swellings. While tannins are proved haemostatic's, they are also beneficial when applied on mucosal coating in mouth. Conventionally, tannins have also been used to cure diarrhea (De Wet, 2010). Tannin has several industrial uses as preservatives. In dry wood and leather, tannin averts rotting, changing of shape and decay by bacteria and fungi (Arshad, 2010). So, the identification of tannins in medicinal plants screened could be very advantageous

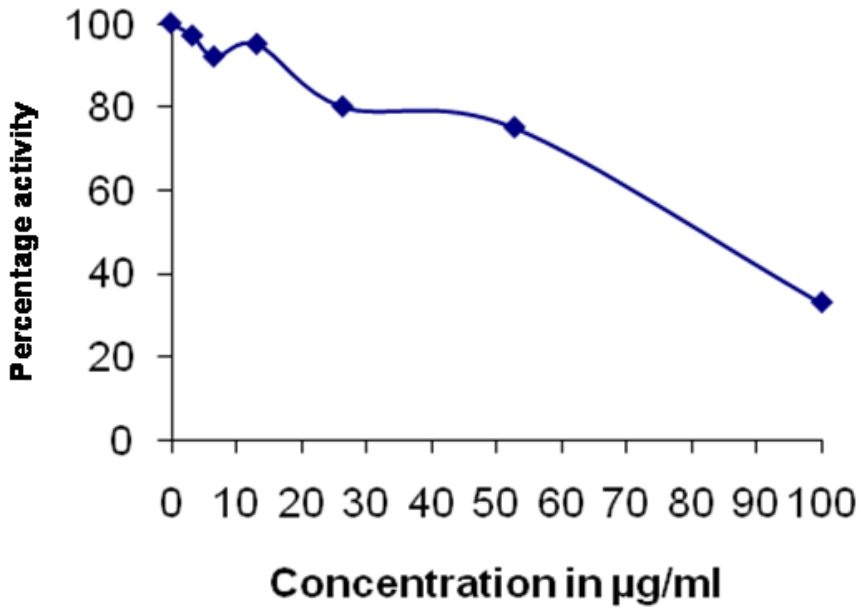

Figure 4. Cytotoxicity of *Canavalia gladiata* methanolic extract.

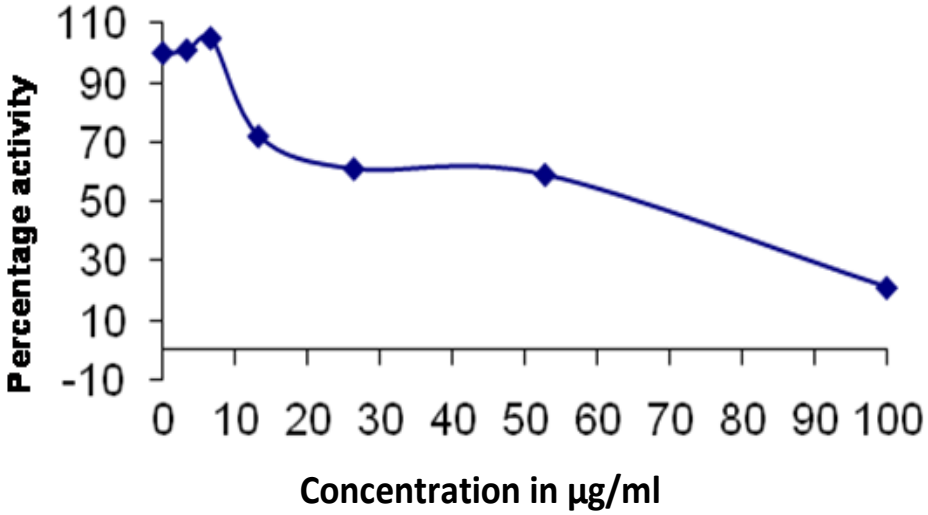

Figure 5. Cytotoxicity of *Canthium parviflorum* aqueous extract.

in healing various diseases and these plants could be potentially used for varied range of applications.

The results obtained in our methanol extraction method revealed the presence of flavonoids in all of the screened medicinal plant extracts (Table 3) and the presence of flavonoids could be extremely helpful as flavonoids possess antiallergic, antiinflammatory, antiviral and antioxidant activities (Bbosa et al., 2010). Moreover, acting by several different mechanisms, particular flavonoid can exert significant anticancer activity including anti carcinogenic properties and even a pro differentiative activity, amongst other modes of action. Certain flavonoids possess potent inhibitory activity against a wide array of enzymes (Lena et al., 2010). Evidence suggests that only activated cells are susceptible to the modulating effects of flavonoids that is, cells which are responding to a stimulus. So the presence of this type of phytochemical compounds in the screened medicinal plants has a wide range of applications and could be certainly used for a variety of applications (Lena 2010).

The results obtained in our aqueous and methanolic

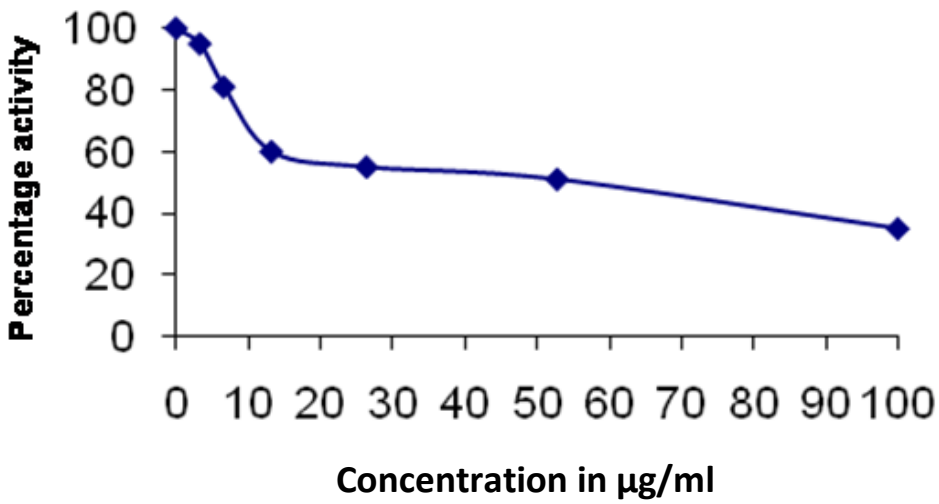

Figure 6. Cytotoxicity of *Canthium parviflorum* methanolic extract.

Table 4. IC_{50} Values (µg/ml) of the methanolic and aqueous extracts of the three medicinal plants on Cac-2 cell lines.

S/N	Plant name	IC 50 (µg/ml)	
		Methanol extract	Aqueous extract
1	*Hemidesmus indicus*	60	78
2	*Canavalia gladiate*	82	95
3	*Canthium parviflorum*	52	71

extract method revealed the presence of saponins, cardiac glycosides and alkaloids also saponins are a class of chemical compounds. Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative saponins are also used as dietary supplements and nutriceuticals (Yu-Fen, 2010) saponins in human therapy are used as adjuvants in the production of vaccines, toxicity associated with sterol complexation remains a major issue for attention Even in the case of digoxin, therapeutic benefit from the cardiotoxin is a result of careful administration of an appropriate dose. Very great care needs to be exercised in evaluating or acting on specific claims of therapeutic benefit from ingesting saponin-type and other natural products.

Cardiac glycosides are used therapeutically mainly in the treatment of cardiac failure. These effects are caused by the ability to increase cardiac output by increasing the force of contraction by increasing intracellular calcium as described below, increasing calcium-induced calcium release and thus contraction. Normally, sodium-potassium pumps in the membrane of cells (in this case, cardiac myocytes) pump potassium ions in and sodium ions out. Cardiac glycosides inhibit this pump by stabilizing it in the E2-P transition state, so that sodium cannot be extruded: intracellular sodium concentration therefore increases (Ghrahem, 2010). A second membrane ion exchanger, NCX, is responsible for 'pumping' calcium ions out of the cell and sodium ions in (3Na/Ca); raised intracellular sodium levels inhibit this pump, so calcium ions are not extruded and will also begin to build up inside the cell. Increased cytoplasmic calcium concentrations cause increased calcium uptake into the sarcoplasmic reticulum via the SERCA2 transporter. Raised calcium stores in the SR allow for greater calcium release on stimulation, so the myocyte can achieve faster and more powerful contraction by cross-bridge cycling. The refractory period of the AV node is increased, so cardiac glycosides also function to regulate heart rate. Our study describes investigations into the anticancer potential of medicinal plants by screening for cytotoxic activity against caco-2 cell lines.

The Cytotoxicity assay of both the extracts of the three plant extracts showed carrying ranges of Inhibitory concentrations (IC_{50}). Among all the three extracts the methanolic extracts exhibited IC_{50} at lower values compared to aqueous extracts (Table 4). This shows that methanolic extract is more cytotoxic than the aqueous extract.

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