GIAP Journals

Green Chemistry & Technology Letters
Vol 1, No 1, November 2015, pg 17-21
DoI: 10.18510/gctl.2015.113

QUANTITATIVE PHYTOCHEMICAL DETERMINATIONS AND IN-VITRO CYTOTOXIC ACTIVITY OF MEDICINAL PLANTS

[1]P. Ethalsha Robert [2]Dr. A. Malar Retna
[1]Research Scholar, [2]Assistant Professor, Department of Chemistry,
Scott Christian College (Autonomous), Nagercoil – 629 002
[1]ethalsharobert@gmail.com, [2]malarscott@gmail.com

Article History: 22nd July, Revised on 05th September, Published on 05th November 2015

Abstract

The present study evaluates in-vitro cytotoxic activity of active extracts of medicinal plants. It evaluates anti-cancer activity of most active extracts of I. pes-caprae and C. roseus. This study focuses on the total phenol content and the total flavanoid content of the plant and searches into its medicinal potency based on the total phenol and total flavanoid content. The anti-cancer activity of the extract is found to be more significant than one another. The cytotoxic activity of Ipomoea pes-caprae and C. roseus showed better results. Methanol extracts of aerial and root of Ipomoea pes-caprae possess maximum anticancer activity is found to be 61.77% and 65.55%. Chloroform extract of Catharanthus roseus root was also active against MCF- 7 cells by exhibiting 64.34% of activity. These plants may be a source of new antibiotic compounds.These findings enriches our knowledge of the chemical constituents that are responsible for the medicinal uses of the plant and the anti-cancer potential of selected plants.

Keywords: I.pes-caprae, C.roseus, phytochemicals, cytotoxic, MTT assay.

INTRODUCTION

Plants are considered as effective and important anti-cancer agents. According to an estimate more than 60 % of recently utilised anti-cancer agents have originated from natural products. Microorganisms, marine organisms and plants are the major natural sources from which products of anticancer activity are obtained [1]. Alkaloids, such as vinblastine, vincristine, paclitaxel, docetaxel, camptothecin, colchicine, demecolcine are the compounds obtained from the plants that have ability to stop the cell division are used for the chemotherapy. Similarly other important sources of chemotherapy are the compounds derived from plants that have ability to kill the cancer cells, these compounds are known as cytotoxic compounds [2].

Antitumour activity derived from medicinal plants can be the result of a number of mechanisms. These mechanisms include the effects on cytoskeletal proteins that have important role in mitosis, inhibition of topoisomerase enzymic activity, activation of the immune system, or antioxidant activity. Plant derived anticancer agents have great potential to be used as single agents or in combinational therapies. These agents have been used to treat localised or metastatic breast cancer. Paclitaxel is an example of anticancer compound first extracted from the Taxus brevifolia. It is complex diterpenoid compound [3]. Cancer is still a dreadful disease due to limited availability of effective drugs against cancer. Limitations linked with the present day chemotherapeutic agents to treat cancer are that they are highly expensive, mutagenic and sometimes carcinogenic. Their applications are therefore limited [4]. Therefore efforts are made to isolate and identify anticarcinogens that are naturally present in plants, which can effectively be used to prevent, slow or reverse cancer development.

Traditionally Ipomoea pes-caprae is used in different ways like; the juice from the succulent leaves has been used as a first aid to treat jellyfish stings. Some Indians use it in ritual baths to alleviate evil spells. Leaves are used in rheumatism, and as stomachic and tonic. The extract of the leaves have the astringent, diuretic and laxative properties. It has biological activity like anti-oxidant, analgesic and anti-inflammatory, antispasmodic, anti-cancer, antinociceptive, antihistaminic, insulogenic and hypoglycemic. It is also used in inhibition of platelet aggregation, diarrhoea, vomiting, and piles [5, 6].

Catharanthus roseus has historically been used to treat a wide variety of diseases. A decoction of all plant parts is used to treat malaria, diarrhoea, diabetes, cancer and skin diseases. The species is also well known as an oral hypoglycemic agent. Extracts prepared from leaves have been used as an antiseptic agent for healing wounds and as a mouthwash to treat toothache. The plant has long been used as a hypoglycaemic agent for the treatment of diabetes [123]. Commercial drugs have been developed from alkaloids (vinblastine and vincristine) extracted from C. roseus. Vinblastine sulphate (sold as Velban) is used to treat Hodgkin's disease.Vincristine sulphate (sold as Oncovin) is effective for treating acute leukaemia in children and lymphocytic leukaemia [7].

In the present study, the most potent plant extracts [8] were screened for quantitative phytochemical determinations and anticancer activity.

Green Chemistry & Technology Letters
Vol 1, No 1, November 2015, pg 17-21
DoI: 10.18510/gctl.2015.113

MATERIALS AND METHODS

Quantitative Determinations

Total phenol

Total phenolic content of the extracts was determined by Folin Ciocalteu reagent method with some modifications. Plant extract (1 ml) was mixed with Ciocalteu reagent (0.1 ml, 1 N), and allowed to stand for 15 min. Then 5 ml of saturated Na_2CO_3 was added. The mixtures were allowed to stand for 30 min at room temperature and the total phenols were determined spectrophotometrically at 760 nm. Gallic acid was used as a standard. Total phenol values are expressed in terms of gallic acid equivalent (mg g^{-1} of extracted compound) [9].

Total flavonoid

Aluminium chloride colorimetric method with some modifications was used to determine flavonoid content. Plant extract (1 ml) in methanol was mixed with 1 ml of methanol, 0.5 ml aluminium chloride (1.2 %) and 0.5 ml potassium acetate (120 mM). The mixture was allowed to stand for 30 min at room temperature; then the absorbance was measured at 415 nm. Quercetin was used as standard. Flavonoid content is expressed in terms of quercetin equivalent (mg g^{-1} of extracted compound) [10].

Invitro anticancer activity (Cytotoxicity assay on MCF-7 cell lines)

Chemicals and reagents: MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) invitrogen, Sigma, USA. Acridine orange were obtained from Sigma, USA. All other chemicals were obtained from Sigma–Aldrich, St. Louis.

Cell culture: MCF-7 cells obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose well Park Memorial Institute medium (RPMI), supplemented with 10 % fetal bovine serum, penicillin/streptomycin (250 µg/ml), gentamycin (100 µg/ml) and amphotericin-B (1 mg/ml) were obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37°C in a humidified atmosphere of 5 % CO_2. Cells were allowed to grow to confluence over 24 h before use.

Cell growth inhibition studies by MTT assay: Cell viability was measured with the conventional MTT reduction assay, as described previously with slight modification. Briefly, MCF-7 cells were seeded at a density of 5×10^3 cells/well in 96-well plates for 24 h, in 200 µl of RPMI with 10 % FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.11–100 µg/ml) of test compound was added and incubated for 48 h. After treatment cells were incubated with MTT (10 µl, 5 mg/mL) at 37 °C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. Data represented the mean values for six independent experiments [11]. Cell viability (%) = Mean OD/Control OD x 100

RESULTS AND DISCUSSION

Total flavanoids and phenols

Quantitative phytochemical estimation of flavonoids and phenols were carried with four selected most active extracts among all the extracts. The results are presented in Figure 1 and 2. Plants are able to make a wide range of aromatic compounds; more common among them are phenols or their derivatives having oxygen substitution. Flavonoids are the largest group of polyphenolic compounds. They are widely distributed throughout the plant kingdom. There are four major groups of flavonoids; anthocyanins, flavones, flavonols and isoflavones. The results of total phenolic and flavonoid content showed that the total phenolic content was higher in methanol extract of aerial parts of *C. roseus* is found to be 1889.44 µg GAE/g) than chloroform extracts of *C. roseus* root is found to be 531.63 µg GAE/g. Total flavonoid content of methanol extract of aerial parts of *C. roseus* is found to be 97.41 µg QE/g and chloroform extract of *C. roseus* roots is found to be 130.72 µg QE/g respectively. Total flavonoid content was higher in methanol extract of aerial parts of *I. pes-caprae* is found to be 187.91 µg QE/g than methanol extract of the *I. pes-caprae* roots is found to be 111.23 µg QE/g. Total phenolic content of methanol extracts aerial and roots of *I. pes-caprae* is found to be 1581.08 µg GAE/g and 1541.81 µg GAE/g respectively.

Green Chemistry & Technology Letters
Vol 1, No 1, November 2015, pg 17-21
DoI: 10.18510/gctl.2015.113

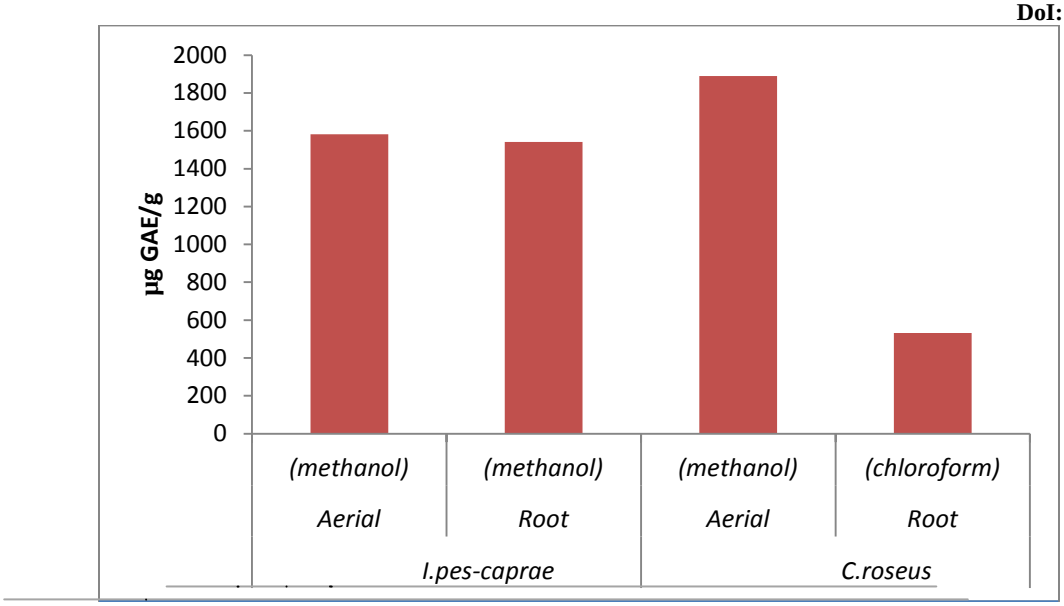


Figure 1: Total phenols

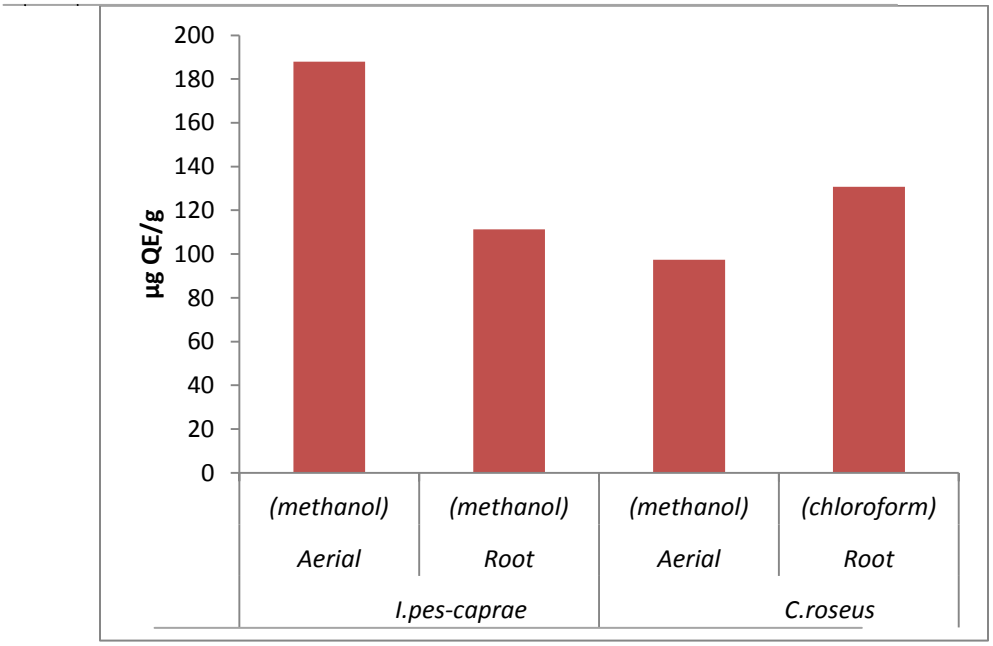


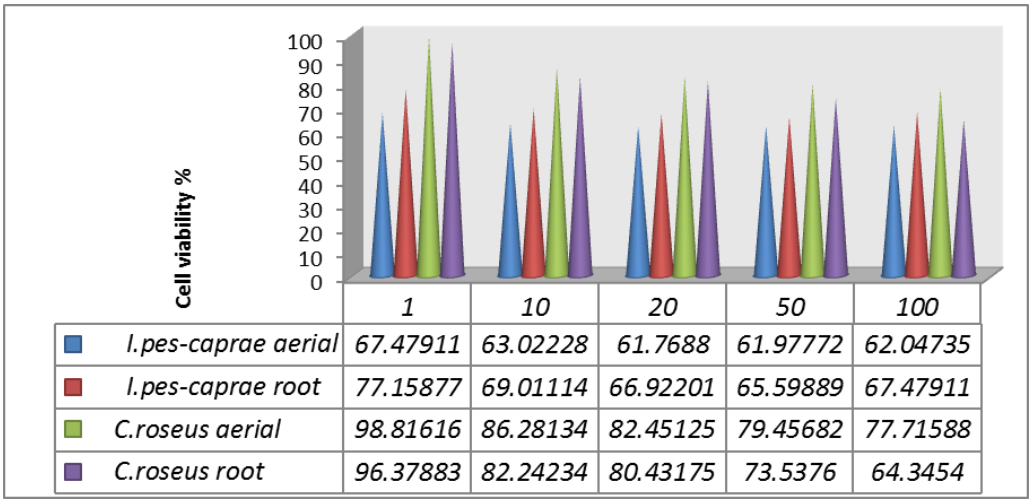
Figure 2: Total flavonoids

Anticancer activity

MTT assay was performed to test the activity of crude plant extracts against MCF-7 human breast adenocarcinoma cell line. Human MCF-7 cells act as a model for breast cancer cell growth during various experiments. It was first derived from 69 years patient with metastatic breast cancer in 1970. MCF-7 cell line is the first cell line, that response positively to estrogens. Expression of receptors for estrogen and progestron is different in MCF-7 cells therefore their sensitiveness to anti-estrogens and anti-progestrone is exhibited by different ways [12]. MTT assay is used for the determination of cytotoxicity. Cytotoxicity screening model provides important information regarding the activity of crude extracts and fractions on the basis of which future work can be planned [13]. Fotakis and Timbrell (2006) reported that MTT assay shows statistically significant difference between the treated cells and the controls and is the most sensitive cytotoxicity assay, especially in detecting early toxicity [14]. The results were presented in Figure 3 and 4.

GIAP Journals

Green Chemistry & Technology Letters
Vol 1, No 1, November 2015, pg 17-21
DoI: 10.18510/gctl.2015.113



	1	10	20	50	100
I.pes-caprae aerial	67.47911	63.02228	61.7688	61.97772	62.04735
I.pes-caprae root	77.15877	69.01114	66.92201	65.59889	67.47911
C.roseus aerial	98.81616	86.28134	82.45125	79.45682	77.71588
C.roseus root	96.37883	82.24234	80.43175	73.5376	64.3454

Figure 3: Anticancer activity of best screened crude extracts

Methanol extract of aerial and roots of *I.pes-caprae* shows significantly active against MCF-7 cell line with the maximum of inhibition at lower concentrations of samples. Most active methanol extract of aerial parts of *C.roseus* and *I.pes-caprae* inhibits the cell in maximum level. Most active chloroform extract of *C.roseus* roots exhibits considerable activity against the MCF-cell line assay. Methanol extract shows the maximum cytotoxic activity; it indicates the polar nature of active compounds present in the plant extract [15].

From figure 4 it is found that the most active methanol extract of aerial parts of *I.pes-caprae* shows the maximum activity against MCF-7 cell line. The result indicates a dose dependent response of extract. Percentage of cell viability varied from 67.47 % (1 µg) to 62.04 % (100 µg). This result reveals the increasing concentration decreases the cell viability. Active methanol extracts of aerial parts of *I.pes-caprae* shows maximum inhibition of 61.77 % in 20 µg of sample. The most active methanol extract of *I.pes-caprae* roots shows the maximum activity against MCF-7 cell line. The result indicates a dose dependent response of extract. Percentage of cell viability varied from 77.15 % (1 µg) to 67.47 % (100 µg). This result reveals the increasing concentration decreases the cell viability. Active methanol extract of *I.pes-caprae* roots shows maximum inhibition of 65.55 % in 20 µg of sample. The most active methanol extract of aerial parts of *C.roseus* shows the maximum activity against MCF-7 cell line. The result indicates a dose dependent response of extract. Percentage of cell viability varied from 98.81 % (1 µg) to 77.77 % (100 µg). This result reveals the increasing concentration decreases the cell viability. Active methanol extracts of aerial parts of *C.roseus* shows maximum inhibition of 77.77 % (100 µg) in 20 µg of sample. *C.roseus* roots show highest activity against MCF-7 cell line. Percentage of cell viability varied from varying the concentrations like 96.37 % (1µg) to 64.34 % (100 µg). This result suggests that the increasing concentration decreases the cell viability. Roots of *C.roseus* shows maximum inhibition is 64.34 % in 100 µg of sample. These findings are consistency with Mothana et al., (2010) according to him strong antibacterial as well as cytotoxic activity was exihibited by crude extracts of plants [16].

MCF 7-MTT assay- activity

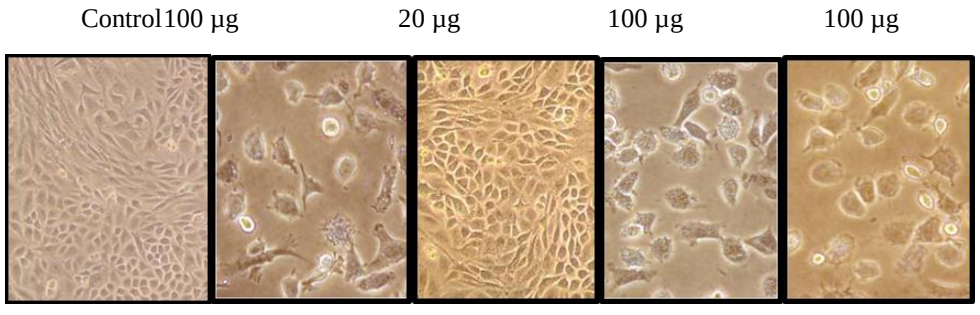


Figure 4: MTT ASSAY- Activity of sample

Green Chemistry & Technology Letters
Vol 1, No 1, November 2015, pg 17-21
DoI: 10.18510/gctl.2015.113

CONCLUSIONS

The present study was evaluated quantitative phytochemical determinations and in-vitro cytotoxic activity of crude active extracts of aerial and roots of *I.pes-caprae* and *C.roseus*. Preliminary phytochemical quantitative analysis is used find out the presence of quantitity of phytochemicals in the each sample. It shows primary data of the active compounds present in the aerial and root parts of *Ipomoea pes-caprae* and *Catharanthus roseus* which is responsible for the in-vitro pharmacological activity. These plants showed significant inhibition activity, so further the compound isolation, purification and characterisation which is responsible for inhibiting activity, has to be done for the usage of anti-cancer agent. Regarding cytotoxic activity of most potent extracts of *Ipomoea pes-caprae* and *C.roseus* showed better results which showed better cytotoxic activity. Overall both the species, though now belong to different family, they are potential candidates in the field of drug development. This in-vitro study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. This study gives an indication of the efficacy of the plants obtained from the traditional healers. The results from this study form a basis for further studies of the potent plants so as to isolate the compounds responsible for the anticancer activity.

REFERENCES

1. Newman DJ, Cragg GM., J. Nat. Prod., 70, 461-77, (2007).
2. Haridas C, Arntzen J, Gutterman A., PNAS., 98, 11557-11562, (2001).
3. Mantle D, Lennard TW, Pickering AT., Adverse Drug Reaciont.Toxicol. Rev., 19, 223-40, (2000).
4. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT., J. Am. Chem. Soc., 93, 190-198, (1971).
5. Premanathan M, Nakashima H, Kathiresan K, Rajendran N, Yamamoto N., Indian J. Medical Research, 130, 276-279, (1996).
6. Parekh KK, Patel AM, Modi AJ, Chandrashekhar HR., Pharmacologia, 3, 377-386, (2012).
7. Aslam J, Khan SH, Siddiqui ZH, Fatima Z, Maqsood M, Bhat MA., Int. J. Comp. Pharm., 4, 1−16, (2010).
8. Ethalsha P and Malar Retna A, International Journal of Natural Products Research, 4, 58-64 (2014).
9. Mc Donald S, Prenzler PD, Autolovich M, Robards K., Food Chemistry, 73, 73-84, (2001).
10. Chang C, Yang M, Wen H., Journal of Food and Drug Analysis, 10, 178-182, (2002).
11. Mosmann T., J. Immun. Methods, 65, 55–63, (1983).
12. Hamelers IHL, Richard FMA, Schaik V, Sussenbach JS, Steengergh PH., Cancer cell. Int., 3, 10, (2003).
13. Ferguson PJ, Kurowska E, Freeman DJ, Chambers AF, Koropatnick DJ., J. Nutr., 134, 1529-1535, (2004).
14. Fotakis G, Timbrell JA., Toxicol . Lett., 160, 171-177, (2006).
15. Bibi Y, Nisa S, Waheed A, Zia M, Sarwar S1, Ahmed S, and Chaudhary MF., Afr. J. Biotech., 9, 5611-5615, (2010).
16. Mothana RAA, Abdo SAA, Hasson S, Althawab FMN, Alghbari SAZ, Lindequist U., eCAM., 7, 323-330, (2010).