Original Research Article **Biotechnology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

ANTICANCER ACTIVITY ON MCF-7 AND SSC-40 CANCER CELL LINES USING SUCCESSIVE SOLVENT EXTRACTS OF *PHYLLANTHUS ACIDUS* [LINN.] LEAVES

VIJAY D. MENDHULKAR* AND ASHWINI S. SHINDE[1]

Department of Botany, The Institute of Science, Madame Cama Road, Mumbai, India.

ABSTRACT

Phyllanthus acidus [Linn.] has diverse medicinal importance in Indian history and have high contents of Vitamin C. In terms of both variety and mechanism of action, medicinal plants provide an infinite source of anticancer drugs. The aim of the present study is to investigate cytotoxic potential of *P. acidus* leaves. Analysis is carried out by Successive Solvent Extraction (SSE) method using Petroleum Ether, Chloroform, Ethyl Acetate, Methanol and Aqueous as solvents. Total phenolic content (TPC) and total flavonoid content (TFC) was determined using gallic acid and quercetin, respectively as standards. The anti-proliferative activities of SSE were screened against two human cancer cell lines (Breast and Oral) representing different tissues using Sulforhodamine-B (SRB) assay. The results showed that the total flavonoid content (TFC) was highest (71.62 µg/ml) in methanol extract while, total phenolic content (TPC) was maximum (136.20 µg/ml) in Chloroform extract. The findings of SRB assay revealed that, Chloroform plant extract showed maximum anti-cancer properties against both Human Breast Cancer (MCF-7) andHuman Oral Cancer (SSC-40) Cell Line. While, Petroleum Ether, Ethyl acetate, Methanol and Aqueous plant extracts are cytotoxic only against MCF-7. These results indicate the potential of the medicinal herbs as chemopreventive agent and a good candidate for anti-neoplastic drug development. However, further studies are needed to isolate the active anti-cancer compounds responsible for this activity and evaluating their mode of action.

KEYWORDS: ***Phyllanthus acidus L., Phytochemical Analysis, Total Flavonoid Content, Total Phenolic Content and Anticancer Activity.***

VIJAY D. MENDHULKAR*

Department of Botany, The Institute of Science, Madame Cama Road, Mumbai, India.

Received on: 13-06-2017
Revised and Accepted on: 21-08-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.p36-42

INTRODUCTION

The process of programmed cell death is known as Apoptosis. It has been identified as key process in the regulation of homeostasis and tissue development. A normal state is needed to maintain homeostasis between cell death and cell proliferation. Disruption in this cellular balance or controlling mechanisms may cause human diseases including cancer. Thus deficient apoptosis or excessive apoptosis clinically leads many diseases.[1] Many studies have been focused on the associations between cancer and apoptosis. A large number of apoptosis-inducing agents are potent in treatment of different types of cancer which is considered as boon to mankind.[2] Both in developed and developing countries, Cancer is a major public health concern. Breast cancer is a large problem of public health all around the world. It is second most commonly diagnosed cancer types. In India, cancers of breast and cervix in females while lungs and oral cavity in males account for over 50% of all cancer deaths.[3] The investigations on the effect of best compatible drug formulations (natural, synthetic, biological or chemical) is a need of this hour to reverse, suppress or prevent carcinogenic progression in all the sectors of society. Chemoprevention is recognised as an important approach to control malignancy.[4] Plant-derived metabolites have recently become of great interest owing to their multi-facetated applications. For modern and traditional medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and synthetic drugs, medicinal plants are the richest bio-resource of drugs.[5] Plant Phenols and Flavonoids are potent natural anti-oxidants and anti-cancerous. They have triggered considerable interest recently because of their potential beneficial effects on human health in fighting diseases. For treating this Neoplastic cells, medicinal plants with their isolated lead molecules are also used as an alternative medicine. Diverse efficient compounds derived from natural products are isolated as anticancer agents. Some of the lead molecules isolated from different medicinal plants, that includes Vincristine, Vinblastin, Taxol, Podophyllotoxin and Camptothecin are already in use to treat Cancer and chemotherapeutic side effects.[6] *Phyllanthus acidus* L. of family Phyllanthaceae is commonly known as Star Gooseberry or Country Goosebery. It has an edible small yellow berries like fruits. Fruits bear in loose clusters. They are pale yellow or white, waxy, crisp and juicy and very sour. This tropical or subtropical species are observed throughout Asia and also has a home in the Caribbean region, Central and South America. The medicinal activities of *Phyllanthus* species are analgesic, anti-inflammatory, anti-pyretic, anti-viral, anti-hepatotoxic.[7]Fruits of the two well-known species, *P. acidus* and *P. emblica* contain high contents of vitamin C which is known for its anti-oxidant properties and have been used for improving vision deformitites and memory. These plants also have preventive action against Diabetes and relief of coughing.[8] The present work is aimed to investigate the potency of bioactive phytoconstituents for its medicinal utility with greater effectivity and biocompatibility. To this context, anticancer activity on MCF-7 and SSC-40 cancer cell lines have been assessed using Successive Solvent Extraction (SSE) of *P. acidus* L. leaves.

MATERIAL AND METHODS

Plant Material

The plant material for proposed study was collected from Botanical garden, The Institute of Science, Mumbai, India. Authentication of the experimental plant was done at Blatter herbarium, St. Xavier's College, Fort, Mumbai. The leaves were separated from other parts, washed, cleaned, shade dried and finely powdered for further use.

Preparation of Successive Solvent Extracts (SSE)

The 10 gm of finely powered plant material of *P. acidus* L. leaves was subjected with 100 ml of petroleum ether (i.e. in ratio of 1:10) and extracted successively with chloroform, ethyl acetate, methanol and aqueous solvents for about 8 hours each, using soxhlet apparatus. The extract was filtered through a Whattman filter paper (No. 1). The extract was evaporated under reduced pressure using rotary evaporator. The dried extract was sterilized by overnight UV-irradiations and stored at 4°C in refrigerator for further screening of phyto-constituents and to check its anticancer activity. We have used successive solvent extracts so as to understand the status of the secondary metabolites that have been extracted accordingly from the leaves of experimental system.

Phytochemical Screening

The leaf extract prepared by successive solvent extraction method was tested for the presence of bioactive compounds like tannins, flavonoids, terpenoids, phenols, saponins (Foam test), steroids (Salkowski test), gylcosides (Liebermann's test) and alkaloids (Dragendroff's test).[9,10] Stock solution of concentration 5 mg/ml of SSE was prepared for analysis.

Determination of Total Flavonoid and Phenolic Content (TFC & TPC)

The total flavonoids, present in SSE, were estimated by Aluminium chelating method, using quercetin as standard. While, the total phenolic compounds were determined by Folin-Ciocalteau method, using gallic acid as standard.[11]

Sulforhodamine-B (SRB) Assay

The Anticancer activity of extracts was studied at Advance Centre for Treatment, Research and Education in Cancer (ACTREC), Mumbai, India.The cancer cell lines MCF-7 and SSC-40 were grown in RPMI 1640 medium containing 10% Fetal Bovine Serum and 2 mM L-glutamine in the cell culture laboratory of ACTREC. The SRB colorimetric assay is used to determinethe effect of different concentrations of *P. acidus* successive solvent leaves extracts.[12] In brief, cells were seeded to 96 well plates and treated with different concentrations of SSE (10, 20, 40, 80 µg/ml) in Dimethyl sulfoxide (DMSO) solvent for 24 and 48 hours and fixed with 10 % TCA (w/v) for 60 min at 4°C. The supernatant was discarded, the plates were washed five times with tap water and air dried. At room temperature, SRB solution

(50 µl) at 0.4 % (w/v) in 1% acetic acid was added to each of the wells, and plates were incubated for 20 minutes.After staining, unbound dye was recovered and the residual dye was washed with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base. The absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. For each experiments, a known anticancer drug Adriamycin (Doxorubicin) was used as a positive control. The studied SRB assay parameter was subjected to statistical analysis using Minitab 17 software. The data was expressed as mean ± S.D of three independent experiments. One–way ANOVA test was carried and the difference is considered to be significant, when $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical Analysis

The phytochemical analysis was performed to identify the chemical nature of the active principles. The qualitative tests of the plant extracts reveals the presence of alkaloids, phenols, flavonoids, tannins, terpenoids, saponins, steroids and glycosides (Table 1).

Table 1
Phytochemical Analysis of P. acidus leaf Extracts in Successive Solvent System.

Sr. No.	*Phyto – constituents*	*Petroleum Ether Extract*	*Chloroform Extract*	*Ethyl Acetate Extract*	*Methanol Extract*	*Aqueous Extract*
1.	Tannins	+	+	+	+	+
2.	Phenols	+	+	+	+	+
3.	Flavonoids	+	+	+	+	+
4.	Terpenoids	+	-	+	+	+
5.	Saponins (Foam Test)	-	+	-	+	+
6.	Steroids (Salkowski Test)	+	-	+	+	+
7.	Glycosides (Liebermann's Test)	+	+	-	-	+
8.	Alkaloids	+	-	+	+	-

+ Present; - Absent

The phytochemical screening in the present study reveals that, the secondary metabolites like tannins, phenols and flavonoids are present in all SSE of *P. acidus* leaves. Terpenoids and Steroids are present in SSE except the chloroform leaf extract. Saponins are present in Chloroform, Methanol and Aqueous extracts. Glycosides are present in Petroleum ether, Chloroform and Aqueous extracts. Finally, Alkaloids are present in Petroleum ether, Ethyl acetate and Methanol extracts. The ethanolic extract, showed the presence of phenols, flavonoids, glycosides, saponins and phytosterols while, the aqueous extract showed the presence of proteins, amino acids, carbohydrates, flavonoids, phenols, saponins and glycosides in *P. acidus*.[13] The curative properties of the medicinal plants are due to the presence of the various secondary metabolites. For the detection of the bioactive molecules, the preliminary phytochemical screening tests may be useful. This may subsequently lead to the drug discovery and development.

Total Flavonoid and Phenolic Content (TFC & TPC)

The TFC for SSE in *P. acidus* were measured with Aluminium Chloride colorimetric assay using quercetin as standard. The quercetin solution of concentration 10 - 90 ppm confirmed to Beer's Law at 415 nm with regression coefficient (R^2) = 0.9946. For TPC, the gallic acid solutions of concentrations 10 – 90 ppm confirmed to Beer's Law at 765 nm with regression coefficient (R^2) = 0.9981. Both obeying the $y = mx + c$ linearity equation. The chemical composition of *P. acidus* leaves extracts showed that the plant is rich in phenolic compounds as compared to flavonoid compounds. These compounds, act as UV filters, protecting some cell structures, like chloroplasts, from harmful effects of UV radiations.[14] The TFC was observed highest (71.62µg/ml) in the methanolic extract and the lowest (21.81µg/ml) in ethyl acetate extract (Table-2) with descending order as: methanol > chloroform > aqueous > petroleum ether > ethyl acetate. While, the TPC was highest (136.20µg/ml) in chloroform extractand the lowest (72.60 µg/ml) in petroleum ether extract (Table-2) and they decrease in the following order: chloroform > ethyl acetate > methanol > aqueous > petroleum ether.The variation in the content of phenols and flavonoids during the extraction process depends upon the solvent with varying polarity, pH, temperature, extraction time and composition of sample.

Table 2
Chemical composition of P. acidus leaf extracts – TFC and TPC.

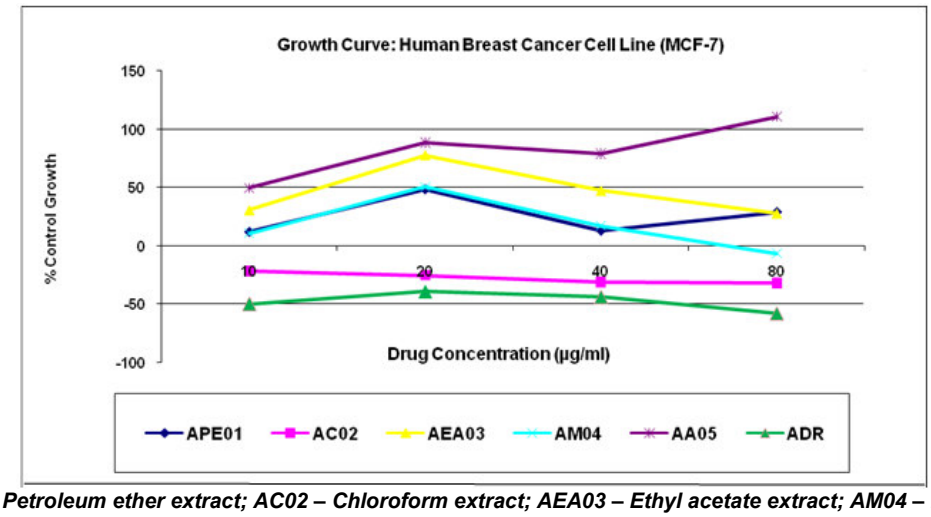
Sr. No.	*Plant Extract*	*Flavonoids (QE) µg/ml*	*Phenols (GAE) µg/ml*
1.	Petroleum Ether	26.06 ± 0.54	72.60 ± 0.34
2.	Chloroform	27.98 ± 0.19	**136.20± 0.42**
3.	Ethyl Acetate	21.81 ± 0.43	120.75 ± 0.52
4.	Methanol	**71.62± 0.55**	113.96 ± 0.56
5.	Aqueous	26.22 ± 0.41	75.68 ± 0.39

QE – Quercetin Equivalent; GAE – Gallic Acid Equivalent

The TPC inchloroform extract (136.20 µg /mg), ethyl acetate (120.75 µg /mg) and methanol (113.96µg /mg) is higher than that of other extracts. This may be due to the complex formation of some phenolic compounds in the extract that are soluble in chloroform, ethyl acetate and methanol. These phenolic compounds may contain more phenol groups or may have higher molecular weights than the phenolics in other extracts. Based on the results of TPC, chloroform, ethyl acetate and methanol are best extracting solvents. The TFC of *P. acidus* in ethanolic and aqueous extract was reported to be as 65.82 µg /mg and 341.75 µg/mg, respectively while, TPC in ethanolic and aqueous plant extract was found to be 152.12 µg /mg and 219.81 µg/mg, respectively.[15] Thus, confirming high contents of flavonoids and phenols in this plant. Edible berry fruits produced by *P. acidus* known as Malay gooseberry, Otaheite gooseberry or Tahitian gooseberry.This fruit contains a lot of polyphenols such as caffeic acid adenosine, kaempferol, 4-hydroxybenzoic acid and hypogallic acid. According to previous reports, the presence of quercetin and kaempferol is 795 and 216 µg/g, respectively in the aqueous extract of *Moringa oleifera* leaf extract.[16] In *Goniothalamus velutinus*, 68 and 78 mg GAE/g of total phenolic content was reported in bark and leaf methanolic extract, respectively. Similarly, the total flavonoid content was also evaluated by these workers in the same experimental system and estimated 43 and 71 mg QE/g in bark and leaf methanolic extract, respectively.[17] Medicinal and dietary plants containing phenolic compounds are bioactive and play an important role in prevention of cancer. They have a complementary and overlapping mode of action, including antioxidant activity and carcinogen metabolism modulation that alter important cellular and molecular mechanisms related to carcinogenesis, a multistep process involving the transformation, survival, proliferation, invasion, angiogenesis, and metastasis of the tumor cells.[18]

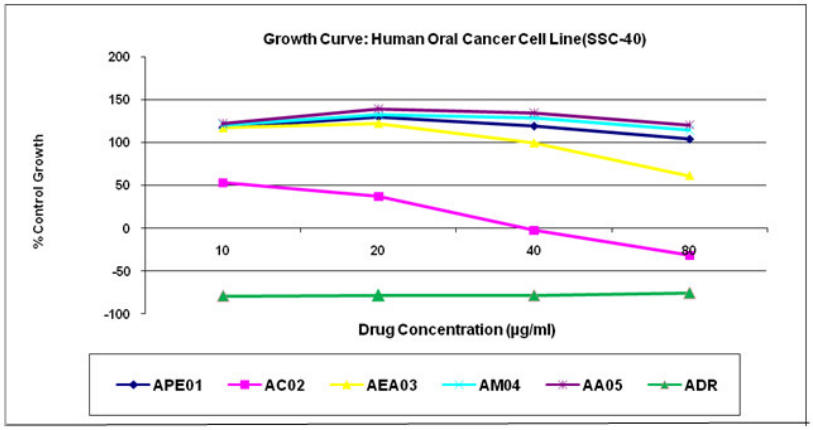
Anticancer Activity

In terms of both variety and mechanism of action, medicinal plants has provided an infinite source of anticancer drugs. In many diverse process ranging from development and stress responses, apoptosis is major form of cell death that plays an important role. The central objective of cancer is the inactivation of apoptosis. Thus, induction of apoptosis can be effective strategy against tumor progression.[19] Present study was focused on the ability of *P. acidus* leaf extracts in influencing the process of apoptosis in MCF-7 and SSC-40 cancer cell lines and to check its anti-proliferative activity. We evaluated the cytotoxicity of SSE using SRB assay (Figure 3 and 4) against 2 different human cancer cell lines - MCF-7 and SSC-40. In moderated acid conditions, SRB binds stoichiometrically to basic amino acids. In the present assessment, the decrease in the optical density was noted in SSE treated samples. It may be co-related to the decrease in the cell number and changes in protein moiety. The decrease in cell number and protein turnover is associated with the cell viability.[11] These biochemical and metabolic variations might have taken place in the treated cell samples thereby causing retardation in the cell growth of cancer cells. The results depicted in Figures 1 and 2, shows the graph of percent control growth of cancer cells with increase in drug concentration (µg/ml). For MCF-7 cancer cell line, the growth of cells was controlled after the treatment of Petroleum ether, Chloroform, Methanol and Aqueous plant extracts as compared to ADR (Figure 1). While for SSC-40 cancer cell line, the growth of cells was controlled after the treatment of Chloroform plant extract as its concentration increased (Figure 2).After cell exposure to toxicants, the most readily observed effect is morphological alteration in the cell layer and cell shape in monolayer culture.[20] SSE treatment induced alterations in cell morphology. The untreated control cells of MCF-7 and SSC-40 grow as colonies, have irregular morphology and were stained homogenously, with the nucleus more stained than the cytoplasm and the nucleolus plainly visible. On the other hand, when these cancer cell lines exposed to SSE, cells were shrivelled, became rounded and individually separated, morphologically deformed with wrinkled cytoplasmic membrane and nuclear condensation indicating cytoskeleton disruption (Figure 3 and 4). This leads to inhibition of the process of mitotic cell division, thereby indicating mitotic depression activity.



APE01 – Petroleum ether extract; AC02 – Chloroform extract; AEA03 – Ethyl acetate extract; AM04 – Methanol extract; AA05 – Aqueous extract; ADR – Adriamycin

Figure 1
Growth Curve – Human Breast Cancer Cell (MCF-7) after SSE treatment of P. acidus.



APE01 – Petroleum ether extract; AC02 – Chloroform extract; AEA03 – Ethyl acetate extract; AM04 – Methanol extract; AA05 – Aqueous extract; ADR – Adriamycin

Figure 2
Growth Curve – Human Oral Cancer Cell (SSC-40) after SSE treatment of P. acidus.

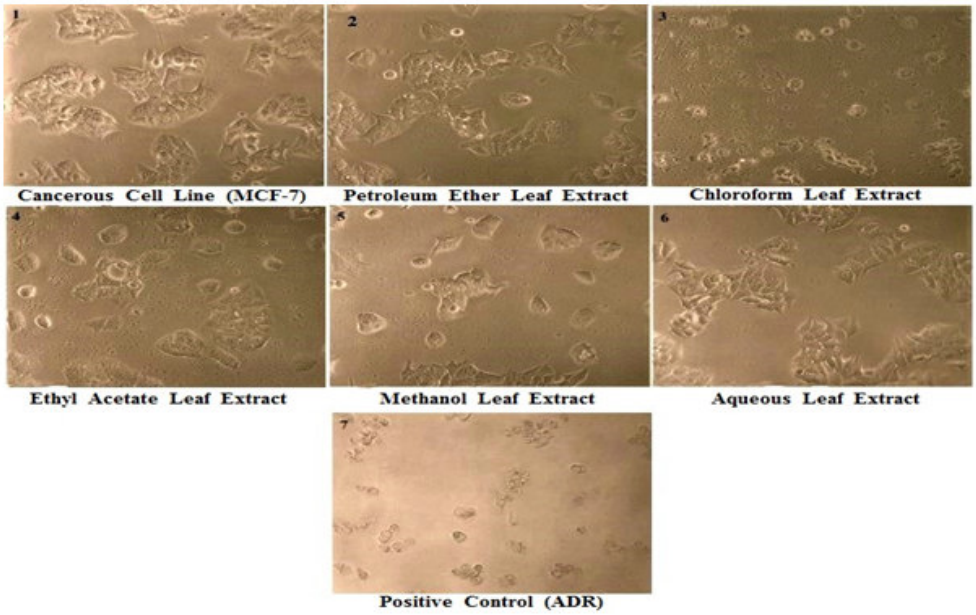


Figure 3
P. acidus Leaf Extract (SSE) Treatment to MCF-7 Cancer Cell Line.

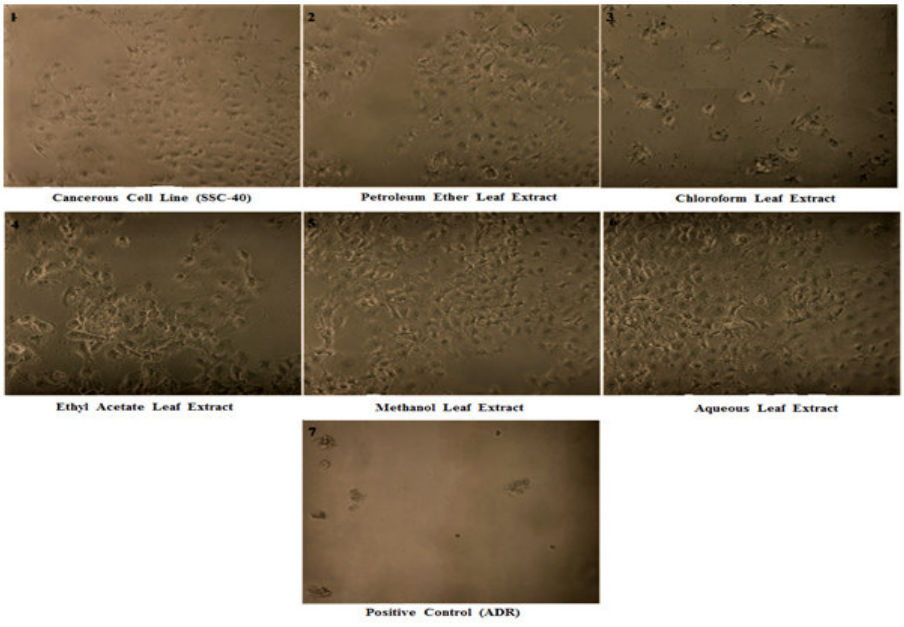


Figure 4
P. acidus Leaf Extract (SSE) Treatment to SSC-40 Cancer Cell Line.

The GI50 for SSE is cited in Table 3. Petroleum Ether, Chloroform, Methanol, Aqueous extract (< 10 µg/ml) showed maximum anti-cancer activity against MCF-7 cell line. While only Chloroform extract (7.3 µg/ml) showed anti-cancer activity against SSC-40 cell line. In the present investigation, Adriamycin (ADR), was used as positive control and showed 100% anticancer activity against both cell lines. Thus, the mean values of cancer cell viability treated with different concentrations of SSE have meaningful differences. When the extract concentration is increased, the mean value of cell viability has been decreased meaningfully ($p < 0.05$).

Table 3
Median Growth Inhibition (GI50) values for test extracts (µg/ml).

Sr. No.	*Plant Extracts*	*Drug concentrations (µg/ml) calculated from graph : GI50**	
		MCF-7	*SSC-40*
1.	Petroleum Ether	**<10**	>80
2.	Chloroform	**<10**	**7.3**
3.	Ethyl Acetate	24.7	>80
4.	Methanol	**<10**	>80
5.	Aqueous	**<10**	>80
6.	ADR (Positive Control)	**<10**	**<10**

The plant *P. acidus* is reported to be an excellent anti-oxidant source.[8] Free radicles are scavenged from the tissues by the antioxidant enzymes superoxide dismutase and glutathione peroxidase which we believe in contributes in the decrease in protein content, degradation of protein components, structural changes in protein configuration, alterations in cell morphology, cell death and DNA fragmentation may be the possible factors responsible for cytotoxicity or mitotic depression caused by SSE at biochemical level. This activity may originate from the SSE anti-oxidant and free radical scavenging property. Thus indicates the chemopreventive role of the extracts. Cancer chemopreventive agents are effective as blocking agents that prevent the mutagenic initiation of the carcinogenic process and suppressing agents that prevent the further progression of lesions.[21] Therefore, SSE could act as a blocking agent by scavenging the reactive forms of carcinogens or even as a suppressor against tumor cells. Plants provide us with rich sources of natural products such as flavonoids, terpenoids, steroids and phenols which leads us to anticancer drug discovery used in chemopreventive effects. Phytochemicals such as polyphenols induces the formation of reactive oxygen species (ROS) which creates an intolerable level of high oxidative stress in some cancer cells.[22] Many medicinal plants have been studied in various *in vivo* and *in vitro* experimental models and have shown significant inhibition of cancer cell proliferation. For eg. *Ocimum gratissimum* and *Plinia edulis* (aqueous extract) plant in breast cancer; *Tephrosia purpurea* plant in oral carcinoma.[23-24,11] These medicinal plants possess good antioxidant properties which leads to anticancer activity. The decoction of *Phyllanthus niruri* has been found effective against Human Liver Carcinoma (HepG2) cells.[25] Another species of same genus, *Phyllanthus watsonii*, reported to have cytotoxic property when extracted with methanol, hexane and ethyl acetate solvents at an early event of cell cycle disruption and a later event of apoptosis.[26] While the Progallin A isolated from the acetic ether part of the leaves of *Phyllanthus emblica* has shown a strong inhibition to the Human Hepatocellular Carcinoma (BEL-7404) in a time and dose-dependent manner and even the characteristics of the apoptosis were observed.[27] Further experimental analysis of the plant extracts are needed. So as to isolate active anti-cancer compounds and obtain more detailed mode of action for the development of new drug which may be useful in the treatment or prevention of cancer. This may be boon for the society.

CONCLUSION

Present work has highlighted the significant difference in chemical composition between different solvent extracts of *P. acidus* and its significant influence on biological activities. Phytochemical analysis of the leaf extract of *P. acidus* L. showed the presences of secondary metabolites which are responsible for the therapeutic effects. When the levels of polyphenolic compounds was estimated in each fractions, the methanol and chloroform extracts was noted to have higher contents with comparison to quercetin and gallic acid. The leaf extract of *P. acidus*in chloroform solvent showed more promising anti-cancer activity than other successive solvent extracts by inducing loss of cell viability, changes in cell morphology and ROS generation in MCF-7 and SSC-40 cell lines. The results obtained provides support for chemopreventive properties of *P. acidus* due to its phenols and flavonoid constituents. Thus, it can be a promising candidate for anti-neoplastic drug development. A huge biological potential of *P. acidus* is suggested by various scientific research.We strongly believe that with the detailed information on the phytochemistry and biological properties, this plant may be substantially used to cure different health disorders.

ACKNOWLEDGEMENT

We acknowledge Officer In-charge of In-vitro SRB assay for anticancer activity evaluation of drugs, Anti-Cancer Drug Screening Facility (ACDSF), ACTREC, Tata Memorial Centre, Kharghar, Navi Mumbai, MS, India and The Institute of Science, Mumbai for providing facilities. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. Science. 1995 Mar 10;267(5203):1456-62.
2. Schmitt CA. Senescence, apoptosis and therapy—cutting the lifelines of cancer. Nat Rev Cancer 2003 Apr 1;3(4):286-95.
3. Nandkumar A, Prabhakaran PS, Dinshaw KA. Consolidated report of the population based cancer registries 1990–1996. New Delhi: National Cancer Registry Program. Indian J Med Res. 2001.
4. Mahdi H, Moulton L, Nutter B, Cherian S, Rose P. The Impact of Combined Radiation and Chemotherapy on Outcome in Uterine Clear Cell Carcinoma Compared with Chemotherapy Alone. Clin Oncol. 2016 Dec 31;28(12):776-82.
5. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J Biotechnol. 2008;7(12).
6. Rowinsky EK, Donehower RC. The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. Pharmacol Ther. 1991 Oct 1;52(1):35-84.
7. Lv JJ, Yu S, Xin Y, Cheng RR, Zhu HT, Wang D, Yang CR, Xu M, Zhang YJ. Anti-viral and cytotoxic norbisabolane sesquiterpenoid glycosides from *Phyllanthus emblica* and their absolute configurations. Phytochem. 2015 Sep 30;117:123-34.
8. Chakraborty R, Biplab D, Devanna N, Sen S. Antiinflammatory, antinociceptive and antioxidant activities of *Phyllanthus acidus* L. extracts. Asian Pac J Trop Biomed. 2012 Feb 1;2(2):S953-61.
9. Marimuthu J, Aparna JS, Jeeva S, Sukumaran S, Anantham B. Preliminary phytochemical studies on the methanolic flower extracts of some selected medicinal plants from India. Asian Pac J Trop Biomed. 2012 Jan 1;2(1):S79-82.
10. Harborne B. A guide to Modern techniques of plant analysis. 3rd ed. Chapman and Hall, London, England;. Phytochemical methods; 1998 pp. 63-89.
11. Carvalho AJ, Ishikawa T, Gouvea CM. Aqueous extract of *Plinia edulis* leaves: Antioxidant activity and cytotoxicity to human breast cancer MCF-7 cell line. S Afr J Bot. 2012 Jul 31;81:1-7.
12. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat. Protoc. 2006 Aug 1;1(3):1112-6.
13. Jain NK, Singhai AK. Protective effects of *Phyllanthus acidus* (L.) Skeels leaf extracts on acetaminophen and thioacetamide induced hepatic injuries in Wistar rats. Asian Pac J Trop Med. 2011 Jun 1;4(6):470-4.
14. Mahmoudi S, Khali M, Benkhaled A, Benamirouche K, Baiti I. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. Asian Pac J Trop Med. 2016 Mar 31;6(3):239-45.
15. Shilali K, Ramachandra YL, Rajesh KP, Swamy BE. Assessing the antioxidant potential of *Phyllanthus acidus* bark extracts. Int J Pharm Pharm Sci. 2014;6(6):522.
16. Sreelatha S, Jeyachitra A, Padma PR. Antiproliferation and induction of apoptosis by *Moringa oleifera* leaf extract on human cancer cells. Food Chem Toxicol. 2011 Jun 30;49(6):1270-5.
17. Iqbal E, Salim KA, Lim LB. Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. J King Saud Univ Sci. 2015 Jul 31;27(3):224-32.
18. Huang WY, Cai YZ, Zhang Y. Natural phenolic compounds from medicinal herbs and dietary plants: potential use for cancer prevention. Nutr Cancer. 2009 Dec 31;62(1):1-20.
19. Brown JM, Attardi LD. The role of apoptosis in cancer development and treatment response. Nat Rev Cancer. 2005 Mar 1;5(3):231-7.
20. Ekwall B, Silano V, Paganuzzi-Stammati A, Zucco F. Toxicity tests with mammalian cell cultures. Short-term toxicity tests for non-genotoxic effects. 1990:75-99.
21. Fresco P, Borges F, Marques MP, Diniz C. The anticancer properties of dietary polyphenols and its relation with apoptosis. Curr Pharm Des. 2010 Jan 1;16(1):114-34.
22. Loo G. Redox-sensitive mechanisms of phytochemical-mediated inhibition of cancer cell proliferation. J Nutr Biochem. 2003 Feb 28;14(2):64-73.
23. Nangia-Makker P, Tait L, Shekhar MP, Palomino E, Hogan V, Piechocki MP, Funasaka T, Raz A. Inhibition of breast tumor growth and angiogenesis by a medicinal herb: *Ocimum gratissimum*. Int J Cancer. 2007 Aug 15;121(4):884-94.
24. Kavitha K, Manoharan S. Anticarcinogenic and antilipidperoxidative effects of *Tephrosia purpurea* (Linn.) Pers. in 7, 12-dimethylbenz (a) anthracene (DMBA) induced hamster buccal pouch carcinoma. Indian J Pharmacol. 2006 May 1;38(3):185.
25. Ooi KL, Loh SI, Sattar MA, Muhammad TS, Sulaiman SF. Cytotoxic, caspase-3 induction and in vivo hepatoprotective effects of phyllanthin, a major constituent of *Phyllanthus niruri*. J Funct Foods. 2015 Apr 30;14:236-43.
26. Ramasamy S, Wahab NA, Abidin NZ, Manickam S. Effect of extracts from *Phyllanthus watsonii* Airy Shaw on cell apoptosis in cultured human breast cancer MCF-7 cells. Exp Toxicol Pathol. 2013 Mar 31;65(3):341-9.
27. Zhong ZG, Wu DP, Huang JL, Liang H, Pan ZH, Zhang WY, Lu HM. Progallin A isolated from the acetic ether part of the leaves of *Phyllanthus emblica* L. induces apoptosis of human hepatocellular carcinoma BEL-7404 cells by up-regulation of Bax expression and down-regulation of Bcl-2 expression. J Ethnopharmacol. 2011 Jan 27;133(2):765-72.

Reviewers of this article

Prof.Dr.R.A.Satpute

Professor of Botany, Dept of Botany, SRT Nagpur, University, Nagpur

Prof. Srawan Kumar G.Y

Associate Professor,Nalanda Institute of Pharmaceutical Sciences, Sattenapalli, Guntur, Andrapradesh, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript