BJP

Bangladesh Journal of Pharmacology

Research Article

Phytochemical characterization, anti–cancer and antimicrobial activity of isolated fractions of *Alysicarpus vaginalis*

A Journal of the Bangladesh Pharmacological Society (BDPS) **Bangladesh J Pharmacol 2017; 12: 77-83**
Journal homepage: www.banglajol.info
Abstracted/indexed in Academic Search Complete, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index; **ISSN:** 1991-0088

Phytochemical characterization, anti-cancer and antimicrobial activity of isolated fractions of *Alysicarpus vaginalis*

Ganesh Tapadiya[1], Mayura A. Kale[2] and Shweta Saboo[2]

[1]Shreeyash Institute of Pharmacy, Aurangabad, Maharashtra 431-010, India; [2]Government College of Pharmacy, Aurangabad, Maharashtra 431-005, India.

Article Info

Received: 10 October 2016
Accepted: 23 January 2017
Available Online: 3 March 2017

DOI: 10.3329/bjp.v12i1.29995

Cite this article:

Tapadiya G, Kale MA, Saboo S. Phytochemical characterization, anti-cancer and antimicrobial activity of isolated fractions of *Alysicarpus vaginalis*. Bangladesh J Pharmacol. 2017; 12: 77-83.

Abstract

The methanolic extract of *Alysicarpus vaginalis* was selected for fractionation due to its known reported biological activity. The four fractions were separated and subjected for *in vitro* antimitotic and anti-proliferative assays along with anti-cancer activity on two human cancers cell lines (SK-MEL-2 and Hep-G2). The antimicrobial potential of fractions had been evaluated against bacteria and fungi. From the all fractions, acetone and *n*-butanol fractions were effective against the cell lines. They show strong inhibitory action with mitotic index 6.2 and 8.4 mg/mL and IC_{50} values of anti-proliferative assay in between 19.7 to 14.2 mg/mL respectively, which was found to be comparable to the standard methothrexate 5.9 mg/mL and 13.2 mg/mL respectively. In antimicrobial activity, the zone of inhibition had been observed in the range of 12-27 mm and MIC value was found in the range of 0.2-0.1 mg/mL. The acetone fraction was found to be most active against fungi, and *E. coli* whereas chloroform and *n*-butanol fractions were more effective against *S. aureus* and *B. subtilis*. The phytochemical characterization by HPLC analysis indicated the presence of important polyphenolic and steroidal compounds.

Introduction

The searching of safe chemotherapeutic agent and healing powers in plants is an ancient idea. Plant metabolites have been applied toward in the treatment and/or prevention of cancer. Important progress has been made in cancer chemotherapy, a considerable portion of which can be attributed to plant-derived drugs (Conforti et al., 2008). The choice of clinical microbiologists to use plant extract as antimicrobial drug because these phytoconstituents will find their way into the arsenal of anti-infective agents prescribed by physicians; several are already being tested in humans. It is reported that, on average every year two or three antibiotics derived from microorganisms are launched (Clark, 1996). After a slowdown in that pace in recent years, again there has been an increase in the demand of plant-based substances or antibiotics as scientists realize that the effective life span of any antibiotic is limited (Hancock, 2005).

Thus, the initial attempts of discovering plant-based effective molecules have been found to be very helpful in reducing the toxic and unwanted effects of several agents because the prime benefits of using herbal medicines are that they are cheaper and relatively safer as compared to commercially available synthetic drugs (Sofowora, 1982).

Alysicarpus vaginalis (L.) DC belongs to the botanical family Fabaceae. Traditionally whole plant has been used for the treatment of cytotoxicity, renal calculi and sepsis (Ediriweera, 2007; Kirtikar and Basu, 1935). The leaf extract has been used for the improvement of eye sight and earache (Tirkey, 2006). The roots of this plant

are generally employed as diuretic; in kidney disorders, skin related problems, leprosy and pulmonary troubles (Burkill, 1985).

From the literature review, it was observed that the plant was still scientifically less explored, so in the present study we have attempted to evaluate the plant on the basis of its chemical constituents and confirm its potential as a source of natural anti-infective and chemotherapeutic agents.

Materials and Methods

Microorganisms

The bacterial and fungal strains used for evaluation of antimicrobial activity were *Escherichia coli* (ATCC 10535), *Staphylococcus aureus* (ATCC 6538), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (ATCC 13315), *Candida albicans* (ATCC 10231), *Aspergillus niger* (ATCC 16404) and *A. flavons* (ATCC 15517) from the stock cultures of microorganisms (Department of Microbiology, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad). Sabouraud 2% (w/v)-glucose agar, Muller–Hinton agar and Muller–Hinton broth were supplied by the Merck (Germany) and RPMI 1640 broth by the Institute of Immunology.

Herbal material

The aerial parts of *A. vaginalis* was collected in August-September, from the Aurangabad, Maharashtra, India. The plant was identified and authenticated at the Dr. Babasaheb Ambedkar Marathwada University, Aurangabad and plant specimen was submitted to the Botany Department of the University (Accession No. 0607). The crude material was dried in the shade for five days and then powdered with the help waring blender.

Extraction and phytochemical analysis

The dried powdered plant material was successively extracted with various solvents like petroleum ether (40 -60°C), chloroform, methanol and water. The four successive extracts, thus, obtained were labeled as petroleum ether extract (PE), moderately polar chloroform (SCH), polar methanol (MEE) and aqueous extract (SAE). The chemical profile of plant revealed the presence of secondary metabolites such as steroids, flavonoids, alkaloids, triterpenoids and phenolics which were identified with the help of general chemical tests (Khandelwal and Sethi, 2010).

Fractionation

The methanol extract, rich in polyphenolic compounds was selected for fractionation. Fractionation had been achieved by column chromatography using different polarities of solvents such as chloroform, *n*-butanol, and acetone successively to get chloroform, *n*-butanol and acetone fractions respectively. The residue remaining after acetone fractionation was dissolved in methanol to get methanol soluble fraction (MSF). The flowchart of fractionation is shown in Figure 1.

In vitro cell lines study by SRB assay

In vitro anti-cancer activity has been tested by sulforhodamine B, SRB method (Skehan et al., 1990; Vanicha and Kanyawim, 2006). The cell lines were grown in RPMI 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine. For the present screening experiment, cells were inoculated into 96-well microtiter plates in 100 µL at plating densities which depended on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 hours prior to the addition of fractions.

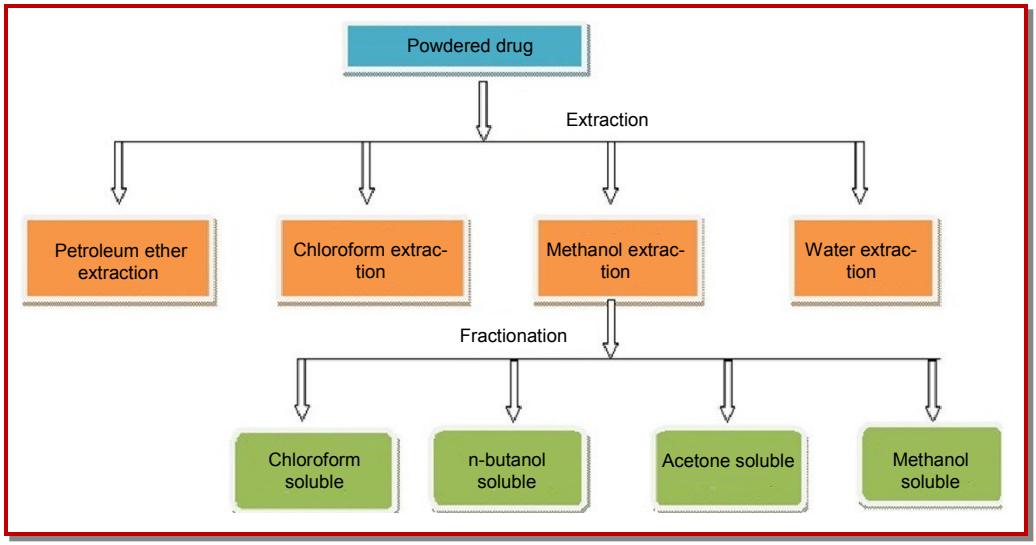

Figure 1: Flowchart of fractionation

Fraction samples were initially solubilized in dimethyl sulfoxide at 100 mg/mL and diluted to 1 mg/mL using water and stored frozen prior to use. At the time of drug addition, an aliquote of frozen concentrate (1 mg/mL) was thawed and diluted to get the required final concentrations i.e.10, 20, 40, and 80 µg/mL.

After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold trichloroacetic acid (TCA). Cells were fixed *in situ* by the gentle addition of 50 µL of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. 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 min at room temperature. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 mM trizma base, and the absorbance was read on a plate reader at a wavelength of 540 nm with 690 nm reference wavelength. The activity results of sample were compared with the reference compound adriamycin.

Percent growth was calculated on a plate by plate basis for test wells relative to control wells. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells.

Using the six absorbance measurements [time zero (Tz), control growth (C), and test growth in the presence of drug at the four concentration levels (Ti)], the percentage growth was calculated at each of the drug concentration levels. Percentage growth inhibition was calculated as:

[Ti/C] x 100%

Antimitotic activity

The antimitotic activity had been evaluated by using *Allium cepa* root (Saboo et al., 2012). *A. cepa* bulbs were sprouted in water for 48 hours at room temperature. The bulbs with uniform root were selected for the study. These roots were dipped in the fraction (1 mg/mL) for 3 hours and distilled water was used for dilution as well as a blank. The methothrexate was used as a reference substance for study. After 3 hours, the root tips were fixed in the fixing solution of acetic acid and alcohol (1:3). The squash preparation was made stain by acetocarmine solution. The mitotic index was calculated by following formula:

Mitotic index = Number of dividing cell X 100/ Total number of cells

Antiproliferative activity

For the antiproliferative assay, yeast *Saccharocymes cerevisiae* was used (Saboo et al., 2012). The yeast was inoculated with sterilized potato dextrose broth and incubated at 37°C for 24 hours and referred to as seeded broth. The seeded broth was diluted with sterilized distilled water, in order to get 25.4 x 10^4 cells (average). For the cell viability count, solution containing 2.5 mL of potato dextrose broth and 0.5 mL of yeast inoculums were prepared in four separate test tubes. In the first four tubes, 1 mL of fractions was mixed while in the fifth tube, standard methothrexate was added. The sixth tube was kept as control. All the tubes were incubated at 37°C. After incubation, 0.1% methylene blue dye was added in all tubes containing cell suspension and observed under microscope. In the microscope, viable cells were observed as non-stained, transparent with oval shape, while dead cells get stained and appeared blue in color. The average number of cells was counted in the hemocytometer.

Antibacterial and antifungal testing

The disk diffusion assay was carried out according to Bauer et al. (1966) for the evaluation of antibacterial activity. The fresh cultures of microbial strains were used for preparation of inoculums, which aseptically cultured at 37°C on Sabouraud 2% (w/v) glucose agar medium for 18 hours and for fungi Sabouraud dextrose agar for 48 hours with saline. The agar plates were prepared approximately 4-5 mm of depth. On the solid media plates, about 100 µL suspension of bacteria was spread and sterile disks of filter paper were impregnated with 50 µL (1 mg/mL) of the fraction and then

Table I

HPLC method for different phytoconstituents

Extracts	Marker phyto-chemical	Mobile phase	Flow rate (mL/min)	Detection wave-length (nm)	Retention time (min)
Chloform fraction	β-Sitosterol	Acetonitrile : Water (90:10)	0.9	220	11.0
	Oleanolic acid	Acetonitrile : Water (90:10)	0.2	215	22.0
	Lupeol	Acetonitrile : Water (90:10)	0.7	230	6.0
n-Butanol fraction	Catechin	Acetonitrile : Water (80:20)	0.3	280	2.5
Acetone fraction	Gallic acid	Methanol : Water (70:30)	0.7	280	3.3

Table II

Phytochemical characterizations of extracts

Tests	PE	SCH	SME	SAE
Carbohydrates	-	-	-	+
Proteins	-	-	-	+
Cardiac glycosides				
Saponin glycosides	-	-	-	-
Steroids	+	+	-	-
Terpenoids	+	+	-	-
Fatty acids	+	-	-	-
Alkaloids	-	+	-	-
Flavonoids	-	-	+	-
Tannins	-	-	-	+
Essential oils	-	-	-	-

Present (+), Absent (-), PE: Petroleum ether extract, SCH: Successive chloroform extract, SME: Successive methanol extract, SAE: Successive aqueous extract

Table III

GI_{50} value of SRB assay on different cell lines

Cell lines	Concentration (µg/ mL)			
	Chloro-form	Butanol	Ace-tone	Adriamy-cin
SK-MEL-2	>80	48.8	49.1	<10
Hep-G2	>80	52.2	53.6	<10

Table IV

Mitotic index and IC_{50} value of antiproliferative assay

Fractions	Mitotic index	IC_{50} (mg/mL)
Chloroform	13.2	23.6
Butanol	6.2	14.2
Acetone	8.4	19.7
Methothreaxate	5.9	13.2

placed on inoculated plates. These plates were incubated for 24 hours at 37°C and 48 hours at 30°C for bacteria and fungi respectively. For positive control, standard tetracycline disks for bacteria and standard ketaconozol disks for fungi, respectively, were used in the same concentration. The zone of inhibition in mm was measured by the antibiotic zone reader.

Minimal inhibitory concentration

The antimicrobial activity of the fractions was determined in Muller–Hinton broth by 2-fold dilution method for the bacterial strains and RPMI 1640 for the yeast and mould (Bauer et al., 1966) and MICs were read in mg/mL after overnight incubation at 37°C. All experiments were made in triplicate.

HPLC analysis of fractions

HPLC is an important tool in qualitative analysis of the complex mixture or plant extracts. The presence of important phytoconstituents in the sample was identified by using marker compounds (Sigma-Aldrich Chemie, Germany). Shimadzu HPLC system, a Luna C18 reverse-phase column (250 x 4.6 mm, i.d. particle size 5 µm) with LC-10AT, UV detector (Spectra System UV1000) was used. The analytical parameters such as mobile phase, wavelength, and flow rate for individual constituent have been given in Table I.

Results

The phytochemical tests confirmed the presence of alkaloids, flavonoids, steroid, triterpenoids and phenolic substances in the different extracts of the plant (Table II). The methanol extract, out of four extracts had been selected for chemical identification, since the presence of various phenolics, flavonoids, steroids and other chemical compounds had been detected by qualitative phytochemical screening results.

It was found that out of the four fractions, acetone and *n*-butanol fractions have very strong cytotoxic effect and their GI_{50} value was very close to the adriamycin (Table III).

A promising activity on cell lines was also supported by antimitotic and anti-proliferative assays. Antimitotic study indicated the inhibition of meristamatic cell at different stages of cell cycle (Figure 2) while in anti-proliferative study; there was an exponential decrease in the number of viable yeast cell numbers which indicates strong inhibitory effect of fraction on the cells. The mitotic index of fractions was found in the range of 6.2 to 13.2 mg/mL and IC_{50} value in anti-proliferative assay was found to be 14.2 to 19.7 mg/mL for *n*-butanol and acetone fractions respectively. This had been found to be close to the standard drug, methothrexate. The other fractions had variable effects on the above activity (Table IV).

The zone of inhibition of different fractions was found to range from 22 to 27 mm. The *n*-butanol fraction was more active against *S. aureus* and *B. subtilis* having zone of inhibition in the range of 22 to 27 mm. While the acetone fraction was effective against *E. coli.*, *P. aeruginosa P. vulgaris* and fungi, *A. flavons, A. niger* and *C. albicans*. Their inhibition was observed in the range of 24 to 27 mm. The remaining fractions chloroform and methanol fractions had varying antimicrobial potencies and the MIC values were observed in the range of 1 to 0.06 mg/mL (Table V).

The HPLC analysis of different fractions revealed the

Table V

Minimum inhibitory concentration of different fractions of methanol extract

Fraction	mg/mL	Bacteria					Fungi		
		S. aureus	*B. subtilis*	*E. coli*	*P. aeruginosa*	*P. vulgaris*	*A. niger*	*C. albicans*	*A. flavon*
Chloro-form	1	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
	0.2	-	-	++	++	+	+	+	-
	0.1	+	+	++	++	++	++	+	+
	0.06	+	+	++	+++	+++	++	++	++
Acetone	1	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	+
	0.1	++	++	+	+	+	+	+	+
	0.06	+++	+++	+	+	+	+	+	+
Butanol	1	-	-	-	-	-	-	-	-
	0.5	-	-	-	-	-	-	-	-
	0.2	-	-	+	+	-	+	-	-
	0.1	+	+	+	++	++	++	+	+
	0.06	+	+	++	+++	+++	++	++	++
Methanol	1	-	-	-	-	-	-	-	-
	0.5	-	-	+	-	-	+	-	-
	0.2	+	-	-	-	-	-	-	+
	0.1	++	++	+	+	+	+	+	+
	0.06	++	+++	+	+	+	+	+	+
Tetracy-cline	1	-	-	-	-	-	*	*	*
	0.5	-	-	-	-	-	*	*	*
	0.2	-	-	-	-	-	*	*	*
	0.1	-	-	-	+	-	*	*	*
	0.06	+	+	+	++	+	*	*	*
Ketocona-zol	1	*	*	*	*	*	-	-	-
	0.5	*	*	*	*	*	-	-	-
	0.2	*	*	*	*	*	-	-	-
	0.1	*	*	*	*	*	-	+	-
	0.06	*	*	*	*	*	+	++	+

- no growth, + less growth, ++ moderate growth, +++ Heavy growth, * not performed

presence of steroidal compounds, olianolic acid, lupeol, β-sitosterol in chloroform fraction while presence of catechin and gallic acid was confirmed in *n*-butanol and acetone fractions.

Discussion

The results of *in vitro* cell line studies indicate that two fractions of extract acetone and *n*-butanol are very effective against both the cell lines. HPLC analysis identified and confirmed presence of phenolic compound, catechin and gallic acid. Polyphenolic compounds are well-known for causing anti-cancer effect by preventing initiation of the carcinogenic process and suppressing carcinogenicity in cells, also inhibiting cancer promotion and progression which is also supported by anti-mitotic and anti-proliferative activities (Russo, 2007; Maurya et al., 2011). These supporting activities might give an insight about the mechanism of action and explain probable pathway of anti-cancer activity.

Further plant extract fractions were explored for their antimicrobial potential to confirm the traditional use of plant as an anti-infective agent (Rattanata et al., 2014; Silva et al., 2015). Previous report shows that *A. vaginalis* could have antibacterial properties (Narintorn et al., 2014). But our results are differed than the research study. All the fractions exhibited strong inhibitory action on different types of bacteria and fungi at very low concentrations of fractions. The effectiveness of these fractions might be due to the presence of

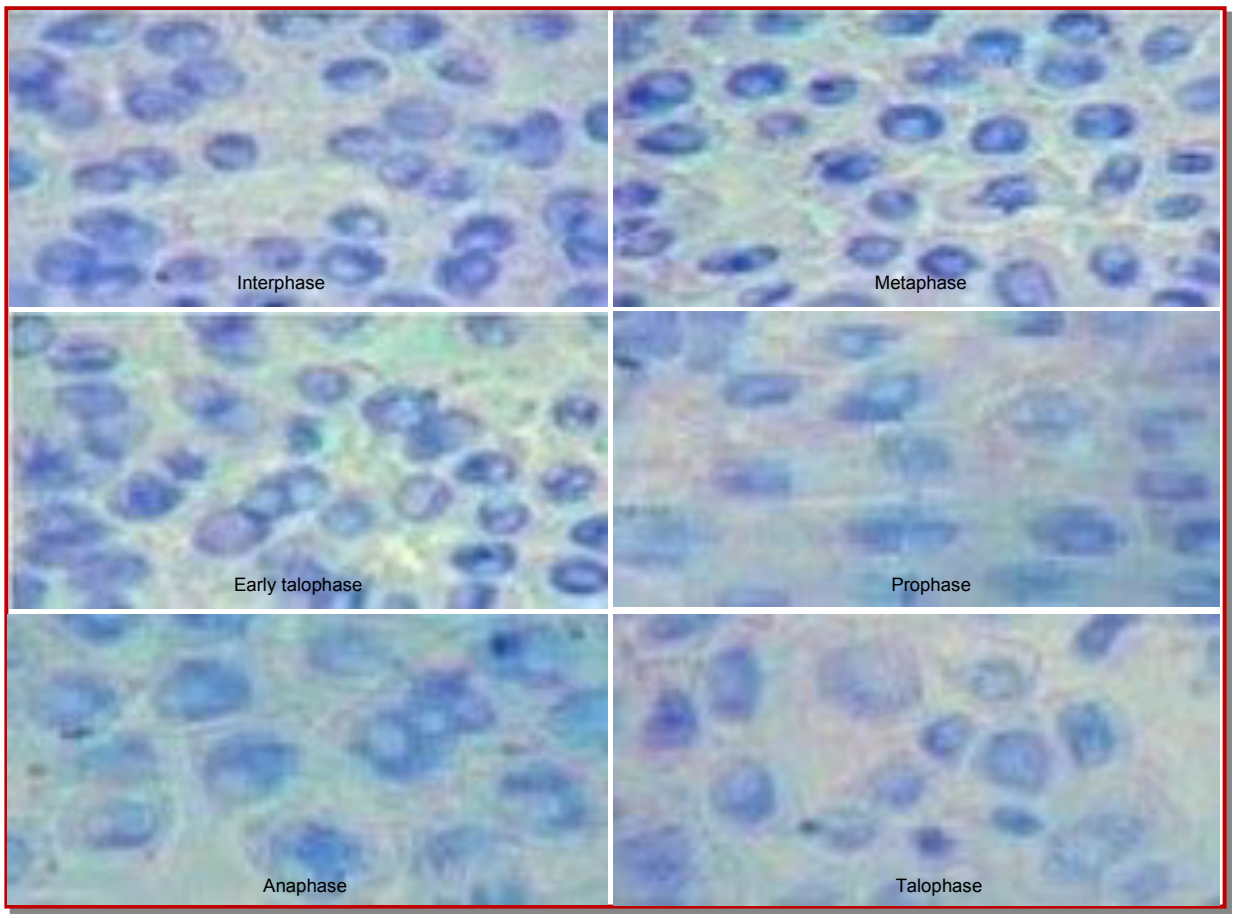

Figure 2: Antimitotic effect on cell cycle

identified steroid, phenolic, and flavonoid compounds (Arora and Kaur, 1999; Taylor et al., 2005).

Conclusion

The biological activity of *A. vaginalis* against the cancer and pathogenic microorganism along with identification of steroid, flavonoid and phenolic substance in the fractions is reported for the first time in this study.

Conflict of Interest

All authors have completed the ICMJE uniform disclosure form and declare no support from any organization for the submitted work.

Acknowledgement

We are very thankful to Prof. Dhabe, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India for plant identification and authentication and also grateful to Advanced Centre for Treatment, Research and Education in Cancer, Mumbai, Maharashtra, India for conducting cell line studies.

References

Arora D, Kaur D. Antimicrobial activity of spices. Int J Antimicrob Agents. 1999; 12: 257-62.

Bauer Aw, Kirby WMM, Sheriss JC, Turck M. Antibiotic susceptibility testing by standardized single disk method. Am J Clin Pathol. 1966; 45: 493–96.

Burkill HM. The useful plants of west tropical Africa. USA, Kew Publishing, 1985, p 85.

Clark AM. Natural products as a resource for new drugs. Pharm Res. 1996; 13: 1133-41.

Conforti F, Ioele G, Statti GA, Marrelli M, Ragno G, Menichini F. Anti-proliferative activity against human tumor cell lines and toxicity test on Mediterranean dietary plants. Food Chem Toxicol. 2008; 46: 3325-32.

Ediriweera ERHSS. A review on medicinal uses of weeds in Sri Lanka. Tropical Agric Res Ext. 2007; 10: 11-16.

Hancock E. Mechanisms of action of newer antibiotics for gram positive pathogens. Lancet Infect Dis. 2005; 5: 209-18.

Khandelwal KR, Sethi VK. Practical pharmacognosy, techniques and experiments. 24th edi. India, Nirali Prakashan, 2010, p 29.

Kirtikar KR, Basu BD. Indian medicinal plants. India, Int Book

Distributors, 1935, p. 251.

Maurya DK, Nandakumar N, Devasagayam TP. Anti-cancer property of gallic acid in A549, a human lung adenocarcinoma cell line, and possible mechanisms. J Clin Biochem Nutr. 2011; 48: 85-90.

Narintorn R, Sakda D, Suthep P, Wandee B, Bundit P, Ratree T, Phangthip U, Patcharee B, Jureerut D. Anti-oxidant and antibacterial properties of selected Thai weed extracts. Asian Pacific J Tropical Biomed. 2014; 4: 890-95.

Rathi MA, Meenakshi P, Guru Kumar D, Arul Raj C, Thirumoorthi L, Gopalakrishnan VK. Potential anti-oxidant and anti-proliferative activities of *Alysicarpus vaginalis* (L.) DC. J Pharm Res. 2010; 3: 2375-77.

Rattanata N, Daduang S, Phaetchanla S, Bunyatratchata W, Promraksa B, Tavichakorntrakool R, Uthaiwat P, Boonsiri P, Daduang J. Anti-oxidant and antibacterial properties of selected Thai weed extracts. Asian Pac J Trop Biomed. 2014; 4: 890 -95.

Russo GL. Ins and outs of dietary phytochemicals in cancer chemoprevention. Biochem Pharmacol. 2007; 74: 533-44.

Saboo S, Khadabadi S, Tapadiya G. *In vitro* evaluation of antimitotic, anti-proliferative, DNA fragmentation and anti-cancer activity of chloroform and ethanol extracts of *Revia hypocrateriformis*. Asian Pacific J Tropical Dis. 2012; 1-5.

Silva ARN, Dissanayake DMRK, Ranaweera CB, Pathirana P, Ratnasooriya WD. Evaluation of *in vitro* antibacterial activity of some Sri Lankan medicinal plants. Int J Pharmaceutical Res Allied Sci. 2015; 4: 54-57.

Skehan P, Storeng R, Scudiero A, Monks J, McMohan D, Vistica D, Jonathan TW, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anti-cancer drug screening. J Natl Cancer Inst. 1990; 82: 1107-12.

Sofowora A. Medicinal plants and traditional medicine in Africa, New York, John Wiley and Sons Ltd, 1982, p 168.

Taylor PW, Jeremy MT, Hamilton-Miller, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull. 2005; 2: 71-81.

Tirkey A. Some ethanobotanical plants of family- Fabaceae of Chhattisgarh state Indian. J Tradit Knowl. 2006; 5: 551-53.

Vanicha V, Kanyawim K. Sulforhodamine B colorimetric assay for cytotoxicity screening. Nat Protoc. 2006; 1: 1112-16.

Author Info
Ganesh Tapadiya (Principal contact)
e-mail: ganeshtapadiya@gmail.com

Your feedback about this paper

1. Number of times you have read this paper 0

2. Number of times you have seen the video clip 0

3. Quality of paper Excellent

4. Your comments