INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

EVALUATION OF PHYTOCHEMICAL AND ANTI-MITOTIC POTENTIAL OF POLY-HERBAL EXTRACT BY USING ONION ROOT MODEL

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Article Received on: 27/02/17 Approved for publication: 15/04/17

DOI: 10.7897/2230-8407.080447

ABSTRACT

Cancer is one of the main causes of mortality around the globe. Majority of the plants are believed to have anticancer properties. The present review was done to assess the antitumor action of various plants in combination, which was an attempt to prove the poly-herbal combination for in-vitro anticancer property. In present study, different plant extracts such as *Withania somnifera, Asparagus gonoclados, Azadirachta indica* and *Streblus asper* were used to prepare the combined extract which helps to determine cytotoxicity.

Anti-mitotic assay by onion root tip method was selected due to comprehensive results. Phytochemical investigation was carried out by standard procedures for investigation of phytoconstituents. Significant reduction in mitotic index was observed in Poly-herbal extract in high concentration in comparison to standard Vincristine as well as in comparison to individual plant extract with P value < 0.01.

Keywords: Cancer, Anti-Mitotic, Poly-Herbal, Vincristine, Anticancer

INTRODUCTION

Cancer is one of the most lethal diseases in human populace. Both synthetic and natural sources are used either alone or in combination for the prevention of cancer. Natural sources of plants are being flavored nowadays to fight against cancer compared to the use of allopathic drugs. The plants showing different chemical moiety such as flavonoids, terpenoids and steroids have pharmacological properties like antiulcer, antihyperlipidemic, antioxidant and antimitotic activity.

In Ayurveda, the drug formulation is based on two principles: Firstly, the usage as a single drug and usage of more than one drugs. The latter is known as PHF (Poly-herbal formulation). This key traditional therapeutic herbal strategy exploits the combination of several medicinal herbs to achieve an additional therapeutic effectiveness usually known as polypharmacy or polyherbalism[1].

Withania somnifera is a plant belonging to the family Solanaceae. The latter is referred as the Nightshade family. It is commonly known as winter cherry, poison gooseberry, ashwagandha, Indian ginseng. It mainly contains withaferin A which is mainly used in the therapy for glioblastomas[2]. The plant's long, brown, tuberous roots are used in traditional medicine[3]. In traditional medicine, the berries and leaves are applied externally to tumours, tubercular glands, carbuncles and ulcers. The roots are used to prepare the herbal remedy ashwagandha[4].

Azadirachta indica is commonly known as Neem, Indian Lilac and Nim tree. It consists of the dried whole plant of *Azadirachta indica* belonging to the family Meliaceae. It is a fast-growing tropical evergreen tree with a highly branched and stout and solid stem. Due to its tremendous therapeutic, agricultural, ethnomedicinal significance and its proximity with human culture and civilization, neem is called as 'the wonder tree' and 'nature's drug store'[5]. All parts of this tree, that is the leaves, bark, seed-oil and their purified products are widely used for treatment of cancer[6]. There are over 60 different types of biochemical including terpenoids and steroids have been purified from this plant[7]. Pre-clinical research work performed during the last 10 years has fine-tuned our understanding of the anticancer properties of the crude and purified products from this plant. The anticancer properties of the neem plant have been studied widely in terms of its preventive, protective, tumour-suppressive, immunomodulatory and apoptosis effects against various types of cancer and their molecular mechanisms[8].

Streblus asper is a tree known as the toothbrush tree, Siamese rough bush, khoi and serut. belonging to the family *Streblus asper* Lour. It is a small tree found in tropical countries such as India, Sri Lanka, Malaysia, Philippines and Thailand. Several parts of this plant have been used in Ayurveda and other folk medicines for the treatment of different ailments such as filariasis, leprosy, toothache, diarrhea, dysentery and cancer[9,10,11,12].

Asparagus gonoclados is a medicinal plant commonly known as Shatavari belonging to the family Liliaceae[13]. Root tubers of shatavari possesses adaptogenic, antioxidant, cooling, emollient, diuretic, galactagogue, nervine tonic, rejuvenating and stomachic properties. They are useful in the treatment of epilepsy, fatigue, inflammation, tuberculosis, tumours. They possess anti-cancer properties as well[14].

An estimate of 12.5% of the world population suffers from cancer. Plant sources are used as drugs for the treatment of cancer. Most of them are potent in action individually. Hence,

the present study is an attempt to screen Poly-herbal combination for *in vitro* anti cancer activity by using the different drugs such as *Withania somniferum*, *Asparagus gonoclodos*, *Azardirachta indica* and *Strebulus asper* extracts were used in the ratio 1:1

MATERIALS AND METHODS[15, 16, 17]

The powders were obtained in Dept. of Pharmacognosy, T. John College of Pharmacy.

Reagents and chemicals

Ethanol, 45% Acetic acid, HCl (1N), aceto-carmine, of n-Hexane, Chloroform, Dichloromethane, Ethyl acetate, n-Butanol, Methanol, Chloroform and water

Evaluation of the powders
Withania somnifera

The following tests were performed to confirm the identity of the powder of *Withania somnifera:*

Table 1: Test analysis of *Withania somnifera*

S. N	Phytochemical	Test	Result
1	Test for starch	Iodine test	Present
2	Test for Steroids	Salkowski test	Present
		Liebermann-Burchard test	Present
3	Test for Flavonoids	Shinoda test	Present
		Lead acetate test	Present
		Alkaline reagent test/NaOH test	Present
4	Test for Alkaloids	Hager's test	Present
		Wagner's test	Present
		Mayer's test	Present
		Dragendorff's reagent	Present
5	Test for Tannins	Gelatin test	Present
		Ferric chloride test	Present
6	Test for Saponins	Foam test	Absent
		Froth test	Absent
7	Test for carbohydrates	Molisch's test	Present
		Benedicts test	Present
		Fehlings test	Present
8	Test for Phenolics	Lead acetate test	Present
9	Test for Glycosides	Foam test	Absent

The results revealed presence of starch, steroids, flavonoids, alkaloids, tannins, carbohydrates, phenols and the absence of saponins and glycosides.

HPLC analysis was performed by reversed phase column subjected to binary gradient elution. The two solvents used for the analysis consisted of water containing 0.1% acetic acid (solvent A) and methanol containing 0.1% acetic acid (solvent B). Gradient programming of the solvent system was carried out at 27°C and was: initially at 60% A, changed to 40% A at 30.0 min, maintained for the next 2.0 min, changed to 25% A at 45 min, and then to 5% A at 54.0 min at a flow-rate of 0.6 ml/min and then at a flow rate of 1.0 ml/min the mobile phase was changed to 0% A at 55 min and this solvent composition was maintained until the run time reached 60 min. The chromatograms were recorded at 227 nm.

The test set of withanolides consisted of

- 27-hydroxy withanone (1),
- 17-hydroxy withaferin A (2),
- 17-hydroxy-27-deoxy withaferin A (3),
- withaferin A (4),
- withanolide D (5),
- 27-hydroxy withanolide B (6),
- withanolide A (7),
- withanone (8),
- 27-deoxywithaferin A (9)

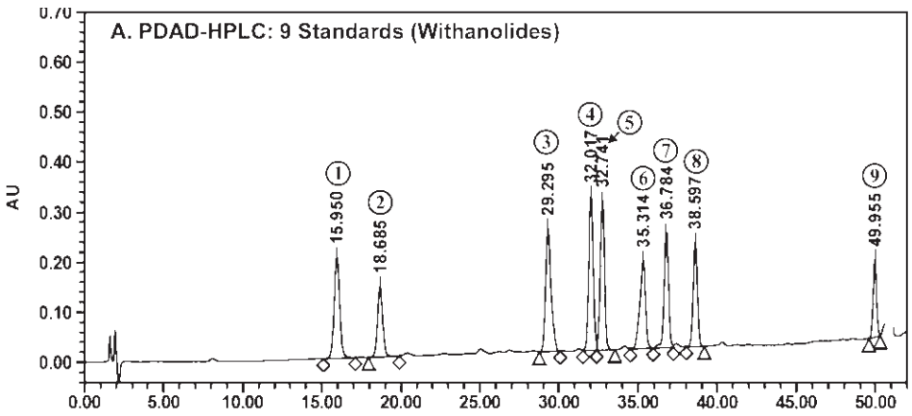

Figure 1: Test set of Withanolides by HPLC

Asparagus gonoclados

The hydro-alcoholic and aqueous extracts of *Asparagus racemosus* were subjected to qualitative phytochemical screening for the detection of phytoconstituents like carbohydrates, glycosides, alkaloids, proteins, amino acids, tannins, phenolics, saponins, flavonoids, triterpenoids, steroids, fixed oils, gums and mucilages. The results (Table 2) revealed the presence of saponins, carbohydrates, glycosides and mucilages which confirms the identity of *Asparagus gonoclados*

Table 2: Qualitative Phytochemical analysis of *Asparagus racemosus* root extracts

S. No	Phytoconstituents	Hydro-alcoholic extract	Aqueous extract
1.	Carbohydrates	+	+
2.	Alkaloids	-	-
3.	Proteins & Amino acids	-	-
4.	Tannins & Phenolics	-	-
5.	Saponins	+	+
6.	Flavonoids	-	-
7.	Triterpenoids	-	-
8.	Steroids	-	-
9.	Glycosides	+	-
10.	Fixed oils	-	-
11.	Gums	-	-
12.	Mucilages	+	+

Azardiracta indica

The analysis of *Azardiracta indica* was performed with different tests as follows:

Table 3: Phytochemical Screening of leaves of *Azadirachta indica*

Extract	Chemical Constituents	Ethanol extract	Aqueous
1	Alkaloids	++	+
2	Glycosides	+	++
3	Saponins	_	+
4	Tannins	+	+
5	Reducing compounds	++	+++
6	Polyphenol	++	+++
7	Phylobatanin	_	_
8	Anthraquinones	_	_
9	Hydroxymethyl Anthraquinones	_	_
10	Flavonoids	+++	++

+present
-absent

The result obtained in Table 3 confirmed the identity of *Azadirachta indica*.

Streblus asper

Histochemical tests of root bark of ***Streblus asper*** (Table 4) and Ultraviolet tests analysis of root bark powder of *Streblus asper* (Table 5) were performed as follows:

Table 4: Histochemical tests of root bark of *Streblus asper*

Treatment	Visible light	UV light	
		short wave (254 nm)	long wave (365 nm)
Powder as such	Light brown	Light brown	Light brown
In methanol	Dark brown	Dark green	Brown
In methanol NaOH	Brown	Green	Light brown
In ethanol	Dark brown	Green	Brown
In ethanol NaOH	Dark brown	Dark green	Light brown
In dil HCl	Light brown	Light green	Brown

Table 5: Ultraviolet tests analysis of root bark powder of *Streblus asper*

Material	Reagent	Test for	Colour change	Result
Section	Iodine	Starch	Blue	++
Section	Ferric chloride solution (10%)	Tannin	Black	++
Section	Sudan III solution	Oil globules	No change	– –
Section	dil Hcl + pinch of phloroglucinol	Lignin	Majenta colour	++
Section	con Hcl	Calcium oxalate crystals	Little effervescence	++

+: present; – : absent

The results confirm the identity of *Streblus asper* powder.

Preparation of extracts[18]

Dry powder (1:1) was used for carrying out successive Soxhlet extraction with 2 liters each of n-Hexane, Chloroform, Dichloromethane, Ethyl acetate, n-Butanol, Methanol, Chloroform and water for 72h at room temperature by maceration. Each time after completion of cycle the extract was removed and powder was dried at 45^0C for 24h. All the extracts were filtered and filtrates were evaporated using Hot air oven.

Phytochemical screening

The extracts obtained were subjected for phytochemical screening using standard procedure. The dried extracts (few mg) were dissolved in sufficient amount of respective solvents and tested for various constituents. The Anti-mitotic activity was screened by Onion Root Inhibition assay.

Antimitotic assay[19]

Onions were left in water for 2 days. Germinated root tips were excised and incubated at 3 hours in the presence of test and control solutions.
Root tips were macerated and placed in ethanol and 45% Acetic acid (3:1) for 12 Hours. Root tips were hydrolysed with HCl (1N) for 15 min at 60^0C. Root tips were taken in a slide and a drop of aceto-carmine stain was added and gently heated. Red discoloured roots were observed under 100x for different stages of cell division. Approximately 100 cells were counted per slide. Average mitotic index of 3 root tips for each extract were determined and repeated thrice.

Percentage inhibition in mitotic index was calculated by using the formula:

% Mitotic Index =
(Total No. of dividing cells / Total Number of cells examined) × 100 [20]

RESULTS AND DISCUSSION

Onion Root tip method is an effective method for evaluation of the different plant extracts for *in vitro* anticancer activity. In present study attempt was made to compare the cytotoxicity of the Poly-herbal extract with standard vincristine and individual extracts by using Onion Root tip cells. In this study the Poly-herbal extract of *Withania somnifera*, root tubers of *Asparagus gonocladus*, leaves of *Azardirachta indica* and root barks of *Strebulus asper* were prepared.

Poly-herbal extract showed the comprehensive results in high concentration. Poly-herbal extract of 100µg/ml showed 96% of significant reduction in mitotic index in comparison to standard vincristine (100%) which is more significant than the individual extracts shown in Table 6 and Fig 2. Whereas low concentration of Poly-herbal extract has shown significant reduction of 93% in mitotic index in comparison to Vincristine that is 97 %, which is shown in Table 6 and Fig 3. The present study proved significant reduction in mitotic index which may be due to disturbance of microtubules which are the structures that pulls the cells apart at the time of cell divisions. The drugs which contain alkaloids, flavonoids and saponins commonly show these effects which are the common chemical constituents of selected drugs.

Table 6: Percentage inhibition of Mitotic index by different drugs and poly herbal extract

Drug name	Concentration (µg/ ml)	Mitotic Index	% Inhibition
Control	-	100	-
Vincristine	50	3	97
	100	0	100
Withania somnifera	50	20	80
	100	10	90
Asparagus gonocladus	50	60	40
	100	50	50
Strebulus asper	50	35	65
	100	25	75
Azardirachta indica	50	60	40
	100	40	60
Poly-herbal extract (1:1)	50	7	93
	100	4	96

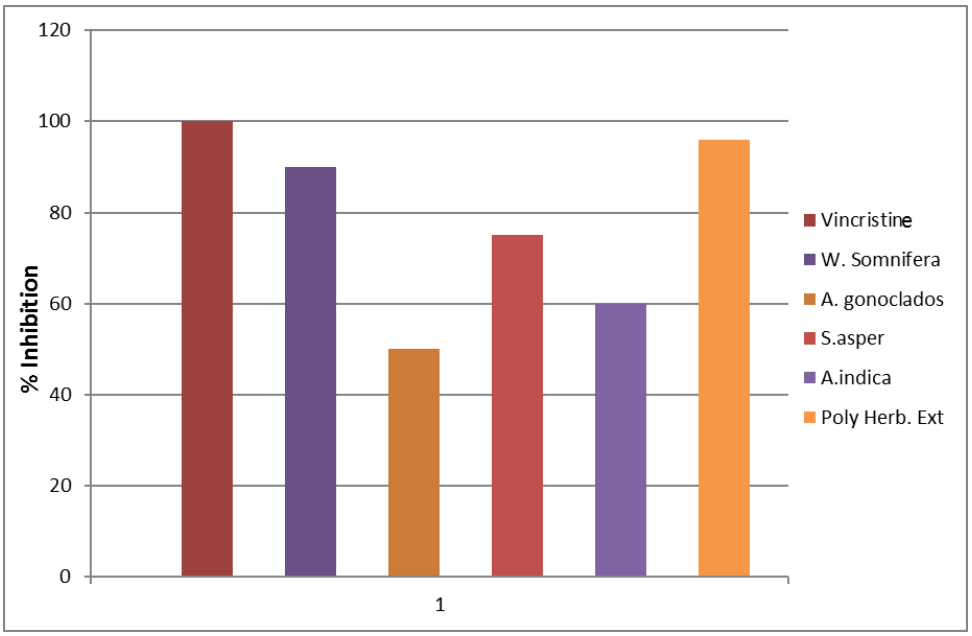

Figure 2: Percentage inhibition in Mitotic index by Poly herbal extract in comparison to other plants and standard in High concentration

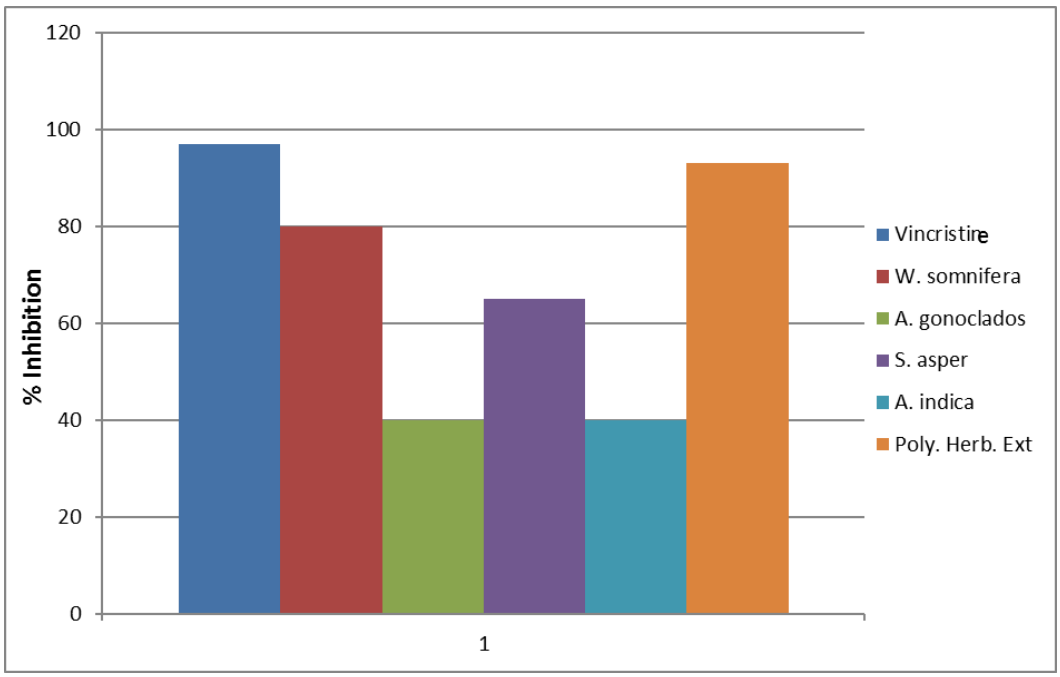

Figure 3: Percentage inhibition in Mitotic index by Poly herbal extract in comparison to other plants and standard in Low concentration

CONCLUSION

Poly-herbal Formulations has set up an example since ages by using the Ayurvedic concept, The various diseases that has been treated by Poly-herbal Formulations provide a holistic approach. The study of different phytoconstituents and discovery of useful medicinal herbs combinations has proved that Poly-herbal Formulations can work in a synergistic way to produce desirable effect. Today, the "renaissance" of Ayurvedic Poly-herbal Formulations has conquered the world over, owing to its comparable efficacy, fewer side effects and better acceptability than allopathic drugs.

Present study proved the synergistic effect of Poly-herbal extract against cell division of Onion Root tips which may be an alternative option to evaluate the Poly-herbal formulation for cancer therapy by using wide flora of the nature.

Ayurvedic Poly-herbal Formulations can exert the best effect in human health.

REFERENCES

1. Subramani Parasuraman, Gan Siaw Thing and SAD. Polyherbal formulation Concept of ayurveda. Pharmacognosy Review. 2014;8(16):73–80.
2. Kulkarni SK, Dhir A. Withania somnifera: An Indian ginseng. Vol. 32, Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2008: 1093–105.
3. Archana R, Namasivayam A. Antistressor effect of Withania somnifera. J Ethnopharmacol. 1998;64(1):91–3.
4. Misra L, Mishra P, Pandey A, Sangwan RS, Sangwan NS, Tuli R. Withanolides from Withania somnifera roots. Phytochemistry. 2008;69(4):1000–4.
5. Kumar VS, Navaratnam V. Neem (Azadirachta indica): Prehistory to contemporary medicinal uses to humankind. Asian Pacific Journal Tropical Biomedical. 2013;3(7):505–14.
6. Ragasa CY, Nacpil ZD, Natividad GM, Tada M, Coll JC, Rideout JA. Tetranortriterpenoids from Azadirachta indica. Phytochemistry. 1997;46(3):555–8.
7. Pankaj S, Lokeshwar T, Mukesh B, Vishnu B. Review on Neem (Azadirachta Indica): Thousand Problems One Solution. Int Res J Pharm. 2011;2(12):97–102.
8. Raji Y, Ogunwande IA, Osadebe CA, John G. Effects of Azadirachta indica extract on gastric ulceration and acid secretion in rats. J Ethnopharmacol. 2004;90(1):167–70.
9. Rastogi S, Kulshreshtha DK, Rawat AKS. Streblus asper Lour. (Shakhotaka): A review of its chemical, pharmacological and ethnomedicinal properties. Vol. 3, Evidence-based Complementary and Alternative Medicine. 2006: 217–22.
10. Ibrahim NM, Mat I, Lim V, Ahmad R. Antioxidant Activity and Phenolic Content of Streblus asper Leaves from Various Drying Methods. Antioxidants. 2013;2:156–66.
11. Prakash K, Deepak D, Khare A, Khare MP. A pregnane glycoside from Streblus asper. Phytochemistry. 1992;31(3):1056–7.
12. Vanhaelen M, Fontaine J, De Graef C, Heenen M. Antimicrobial activity of Streblus asper leaf extract. Phyther Res. 2001;15(2):119–21.
13. Sun T, Tang J, Powers JR. Antioxidant activity and quality of asparagus affected by microwave-circulated water combination and conventional sterilization. Food Chem. 2007;100(2):813–9.
14. Laney C, Morris EK, Bernstein DM, Wakefield BM, Loftus EF. Asparagus, a Love Story. Exp Psychol. 2008;55(5):291–300.
15. Mitosis RT. Mitosis in Onion Root Tip Cells. :1–6.
16. Maity P, Bepari M, Choudhury SM. Evaluation of Antimitogenic and Cytotoxic Potential of Anacardiumoccidentale Leaf Extracts in Allumcepa Root Tip Cells and Against Sarcoma-. 2015;4(4):2013–6.
17. Bengal W. Evaluation of Antimitotic and Genotoxic Effects of the Triterpenoid Enriched Extract from Trichosanthes dioica Root Department of Pharmaceutical Technology , Jadavpur University ,. 2012;4(December 2009):20–3.
18. S. S. Mahamuni, S. G. Killedar, H. N. More, A.B. Nale AAP and SVC. Evaluation of Phytochemical and Antimitotic Potential of Lagenaria Siceraria Fruit Using Onion Root Model. Ijpsr. 2012;3(8):2857–61.
19. Murthy GS, Francis TP, Singh CR, Nagendra HG, Naik C. An assay for screening anti-mitotic activity of herbal extracts. Curr Sci. 2011;100(9):1399–404.
20. Raj GG, Varghese HS, Kotagiri S, Vrushabendra Swamy BM, Swamy A, Pathan RK. Anticancer Studies of Aqueous Extract of Roots and Leaves of Pandanus Odoratissimus f. ferreus (Y. Kimura) Hatus: An In Vitro Approach. J Tradit Complement Med. 2014;4(4):279–84.

Cite this article as:

Anntoniya Rintelen. Evaluation of phytochemical and anti-mitotic potential of poly-herbal extract by using onion root model. Int. Res. J. Pharm. 2017;8(4):44-49 http://dx.doi.org/ 10.7897/2230-8407.080447

Source of support: Nil, Conflict of interest: None Declared