Vol. 6 no. 1, pp. 1-9 (March 2009)

http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Pharmacognostic profile and *in vitro* cytotoxic activity of *Adenema hyssopifolium* G. Don.

Aiyalu Rajasekaran[1*] and Ramasamy Arivukkarasu[2]

[1]*Department of Pharmaceutical Chemistry, KMCH College of Pharmacy, Coimbatore 641 048. Tamilnadu, India.*
[2]*Department of Pharmacognosy, S.B. College of Pharmacy, Sivakasi, India.*

Received 9th October 2008; Accepted 23rd December 2008

Abstract
Pharmacognostic study of *Adenema hyssopifolium* was carried out to establish standards in agreement with those of WHO for vegetable drugs. Parameters examined include morphology, quantitative micrographic parameters, physical constant values, extractive values, behavior on treatment with different chemical reagents and fluorescence characters under ultraviolet light after treatment with different reagents. Phytochemical screening of the chloroform, ethyl acetate and ethanol extracts revealed the presence of flavones and iridoids. Chloroform, ethyl acetate and ethanolic extracts were investigated for *in vitro* cytotoxic activity against nine different types of human cancer panel of 60 different strains of tumor cell lines using the sulforhodamine-B (SRB) binding assay. The chloroform extract showed potent cytotoxicity against non small cell lung cancer cell, leukemia cell, ovarian cancer cell, prostate cancer cell and breast cancer cell lines with the GI_{50} of 2.66, 10, 10.8, 13 and 10.1 µg/mL in NCI-H223, HL-60(TB), OVCAR-3, PC-3 and MDA-MB-231/ATCC cell lines respectively. Ethyl acetate extract exhibited potent cytotoxicity against non small cell lung cancer cell, colon, melanoma, renal and breast cancer cell lines with the GI_{50} of 12.7, 10.2, 10, 10.2 and 10.6 µg/mL in EKVX, HCT-15, SK-MEL2, VO-31 and MCF7 cell lines respectively. The information from this study can explain the use of this plant in Indian traditional medicine and suggests great potential for further development into new pharmaceuticals.

Keywords: *Adenema hyssopifolium;* Cytotoxic activity, Human cell lines, Iridoid glycosides; Microscopy

Introduction

Adenema hyssopifolium G. Don (Family: Gentianaceae) is a perennial glabrous herb that grows on the inside surface of irrigation channels in cultivated lands and also grows in plain fields as a weed. In India, the entire plant, in powdered form, has been used traditionally to treat tumors. This is cited in the authoritative book of Siddha system of medicine namely "*Sidda Vaidhya*" "*Pathartha Guna Vilakam*". It is widely used as a substitute for *Swertia chirayita* and is also used in folk medicine for treatment of diabetes mellitus in Western and Southern India. Ethnomedical studies reveal the use of hot aqueous extract of *Adenema hyssopifolium* by tribal inhabitants for the treatment of diabetes, fever, stomach ache, dyspepsia and for malaria in interior part of Gujarat. Extracts of the plant are used in Indian medicine to treat cardiac dropsy, rheumatism and certain mental disorders.

* Corresponding author. *E-mail address*: rsekaran2001in@yahoo.co.in Tel: 0091-422-2626485 Fax: 0091-422-2626485
ISSN 0189-8442 © 2009 Faculty of Pharmaceutical sciences, University of Jos, Jos. Nigeria.

Swertiamarin, a new secoiridoid glycoside was isolated from *Adenema hyssopifolium* (Anwar *et al.* 1996) was reportedly used for its hypoglycemic activity (Vishwakarma *et al.* 2004). Swertiamarin was also reported to produce central nervous system depressant activity (Battacharya, 1976). Hypolipidemic and antioxidant effect for the aqueous extract of this plant was also reported (Vasu *et al.* 2005). Comprehensive reviews of the technical and folk literature (Ramakrishna *et al.* 1984) recorded the use of different part of this plant species for the treatment of cancer, warts and tumors. Betuline, a triterpene sapogenin was isolated by earlier workers (Rai, 1966). Seven flavonoids were isolated from alcoholic extract and their structures were identified as apigenin, genkwanin, isovitexin, swertisin, saponarin, 5-*O*-glucosylswertisin and 5-*O*-glucosylisoswertisin (Ghosal, 2006).

Methanolic extract of *Adenema hyssopifolium G.* Don has been evaluated against Dalton's ascitic lymphoma in Swiss albino mice (Kavimani *et al.* 2000). The hepatoprotective action of *Adenema hyssopifolium* was studied in carbon tetrachloride induced acute liver damage in mice (Viswakarma *et al.* 2004). The anti-inflammatory activity of the plant species was assessed by carrageenan-induced inflammation and cotton pellet granuloma method in rats (Sadique *et al.* 1987).

Local practitioners of Madurai district, Tamilnadu, India, traditionally use this plant for treatment prevention of cancerous growth in vital organs. From the available literature no scientific report has been made on cytotoxic activity in human cell lines. Hence the present investigation was carried out to establish the histological diagnostic characteristics and to evaluate for the first time the cytotoxic activity of chloroform, ethyl acetate and ethanolic extracts in human cell lines.

Experimental

Collection, identification and extraction. The entire plant of *Adenema hyssopifolium* G. Don was collected in September 2004 from Madurai, Tamilnadu, India. The plant was identified and authenticated by Dr. D. Stephen, Taxonomist American College, Madurai, India. A voucher specimen has been preserved in our laboratory for future reference (R.A. No.11/04). The entire plant was washed with water, air dried for 10 days under controlled temperature (25 ± 2°C), powdered and passed through a # 40 mesh sieve, and stored in an air tight container for further use. The air dried powdered plant (1.3 kg) was subjected to sequential maceration with 3500 ml of petroleum ether (60-80° C), chloroform, ethyl acetate and ethyl alcohol (90%) at room temperature. After each step, the extracts were filtered and the solvents were evaporated to dryness under reduced pressure in Eyele Rotary evaporator (Japan) at 45°C to give a residue with a yield of 3.62 % $^{w}/_{w}$, 10.36 % $^{w}/_{w}$, and 14.72 % $^{w}/_{w}$ respectively. Preliminary phytochemical screening indicated the presence of iridoid glycosides (Trim, 1952; Wieffering, 1966) and flavones in chloroform and ethyl acetate extracts (Harbone, 1988).

In vitro cytotoxic activity screening. The chloroform, ethyl acetate and ethanol extracts were evaluated for *in vitro* cytotoxic activity in drug screening programme at the National Cancer Institute (NCI, USA). The three extracts were evaluated against a panel of 60 human cancer cell lines derived from nine different panels of cancer cells. Extracts were tested at a minimum of five concentrations at 10 fold dilutions. A 48 h continuous drug exposure protocol was used and a SRB (sulforhodamine B) protein assay to estimate cell viability or growth (Alley, 1988). The GI_{50} (concentration causing 50% growth inhibition) was determined, which corresponds to the IC_{50} value defined

elsewhere. (Boyd *et al.* 1992; Monks *et al.* 1991).

Pharmacognostic study

Plant morphology. Slender of stout herb, 4-angled or winged stem; Leaves whorled or opposite and decussate. Sub sessile; Flowers axillary cyme; bisexual, 5 – Merous; Calyx: 5 sepals, copular, lobes unequal. Corolla: white, salverform, lobe five, unequal, twisted; Stamens 5, equal, all fetile; Gyoecium: ovary unilocular, ovules many on parietal placenta; stigma capitate; Fruit: Septicidal capsule; Seeds circular, reticulate.

Preparation of specimens. The required parts of the plant were cut and fixed in FAA (Formalin: Acetic acid: 70% Ethyl alcohol 1:1:18). After 24 h of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58–60°C) until tertiary-butyl alcohol solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning. The paraffin embedded specimens were sectioned with the help of Rotary Microtome (Erma, Japan). The thickness of the sections was 10-12 µm. Dewaxing of the sections was done by customary procedure. Since toluidine blue is a polychromatic stain, the staining results were remarkably good, and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, darks green to suberin, violet to the mucilage, blue to the protein bodies etc. Sections were also stained with safranin and fast-green and iodine in potassium iodide (for starch). For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5 % $^{w}/_{v}$ sodium hydroxide solution or epidermal peeling by partial maceration employing Jeffrey's maceration fluid were prepared. Glycerin mounted temporary preparations were made for macerated, cleared materials. Powdered materials of different parts were cleared with sodium hydroxide solution and mounted in glycerin medium after staining. Different cell component were studied and measured.

Photomicrographs. Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphot 2 Microscopic Unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

Powder microscopy. The powdered entire parts of *Adenema hyssopifolium* G. Don showed thin walled parenchyma cells, the epidermal cells with highly wavy anti-clinical walls. Xylems vessels were seen as thick walled fibers. The anisocytic stomata were present. Trichomes were absent. Calcium oxalates in spheroid crystals were identified. Fragments of lignified, spiral, reticulated and pitted vessels elements were found.

Quantitative microscopy. The stomatal number, stomatal index, vein-islet number, vein-terminal number and palisade ratio were determined on fresh leaves using standard procedure (Evans, 1989). Determination of physical constants (which include moisture content, total ash, acid insoluble ash, water soluble ash and sulphated ash) was carried out in accordance with standard methods (Indian Pharmacopoeia, 1985). Behavioral pattern of powdered sample with different reagents and fluorescence analysis was also studied. The behavior of the whole powdered plant with different chemical reagents and fluorescence analysis was observed under UV and visible

light (Trease and Evans, 1989; Raghunathan, 1982).

Extractive values. The extractive values with different solvents like petroleum ether (60–80°C), chloroform, ethyl acetate and ethanol (90%) were determined (Indian Pharmacopoeia, 1985).

Phytochemical analysis. The extracts obtained were subjected to qualitative test for the identification of various plant constituents (Harborne, 1988).

Histology of leaf. The leaf has prominent midrib and uniformly thin lamina. The midrib is 750 µm thick in vertical plane and 600 µm thick along the horizontal plane. In cross sectional view the midrib is more or less flat on the ad axial side and semicircular on the abaxial side. The ground tissue of the midrib consists of circular, thin walled, compact parenchyma cells both in the ad axial and abaxial portions. A single, large arc of vascular bundle occurs in the upper part of the midrib. Several parallel rows of xylem elements occur with phloem elements located both on the upper and lower parts of the xylem band.

The lamina is 150 µm thick; in sectional view, the lamina surfaces are even, smooth and glabrous. The ad axial and ab axial epidermal layers are unistratose and the epidermal cells are cylindrical or squarish and the inner tangential walls are projecting as hemispherical body into the mesophyll tissue. The mesophyll tissue consists of an ad axial portion of ill-defined palisade cells and many layers of spherical parenchyma cells forming the parenchymatous spongy tissue. Vascular strands of smaller lateral veins are embedded in the median part of the mesophyll tissue.

Venation of the lamina as seen in para-dermal sections shows incomplete vein islets; when complete and distinct vein-islets are seen, they have one or two vein-terminations, which are simple and unbranched. The epidermal cells in surface view appear much lobed with highly wavy anti clinical walls. Cuticles striations are fairly distinct. Several thin parallel lines deviate from the guard cells. The stomata are predominantly anisocytic with three unequal subsidiary cells.

Histology of Stem. The stem is distinctly four winged and quadrangular in transactional view. The wings are short, blunt and dilated at the end. The stem consists of broad cortex, thin vascular cylinder and wide pith. In the young stem, the cortex consists of circular, thin walled parenchyma cells, more or less compactly arranged. The epidermis is single layered and consists, of thick walled rectangular or barrel shaped cells with thick cuticle. The cortex including the epidermis is 130 µm wide. The vascular cylinder is closed, hollow and compact. It consists of xylem cylinder of radial rows of vessels and thick walled fibers: the xylem cylinder is 70 µm thick at the widest portion; phloem elements occur on both external and internal portions of the xylem cylinder. Pith is wide and parenchymatous. The central cells are larger tending to undergo disintegration.

In fairly old stem, the cortex develops several small air-chambers divided by partition cells. Due to the formation of the air-chambers the cortex becomes 270 µm wide. The vascular is also increased in its thickness. The xylem cylinder is 150 µm thick and consists of many vessels and many xylem fibers. The outer phloem and the medullar phloem are more developed and quite distinct. The pith has a wide central canal surrounded by a thin parenchymatous periphery.

Histology of Root. Both thin and thick roots were studied. A young thin root is typically about 1.5 mm in diameter. The young root has intact rhizodermis, broad, compact homogeneous parenchymatous cortex and solid core of vascular cylinder. The xylem consists of radially arranged vessels and xylem fibers. Phloem is in a broad continuous

zone around the xylem. In old roots, the epidermis remains intact. However, the cortex becomes wider measuring about 1.3 mm. It is differentiated by outer parenchyma zone and inner compact zone. The parenchyma zone has tangentially oblong, narrow air-chambers divided by thin, uniseriate partitions. The out boundary of the secondary xylem cylinder becomes slightly wavy and the secondary phloem is a narrow continuous zone all around the xylem. The elements of secondary xylem include vessels and fibers. The vessels narrow, solitary and thick walled occur in tangential bands at random manner. Xylem rays are very narrow and less conspicuous. Growth rings are absent.

Results

The quantitative micrographic parameters have been studied and described in Table 1. The physical constant values loss on drying, total ash, acid insoluble ash and sulphated ash evaluated as per WHO guidelines for the air dried powder are 5.6 % $^{w}/_{w}$, 21.4 % $^{w}/_{w}$, 3.16 % $^{w}/_{w}$ and 17.3 % $^{w}/_{w}$ respectively.

The extractive values of petroleum ether (60-80°C), chloroform, ethyl acetate and alcoholic extracts are 4.8 % $^{w}/_{v}$ %, 2.3 % $^{w}/_{v}$, 11.7 % $^{w}/_{v}$ and 13.8 % $^{w}/_{v}$ respectively.

The air dried powder behavior on treatment with different chemical reagents (Table 2) and fluorescence characters after UV treatment with different reagents were also examined to establish standards in as stipulated by WHO (Table 3).

The chloroform extract exhibited cytotoxicity against:

Non small cell lung cancer [Hop-92 (GI_{50}=13.3µM) and NCI H226 (GI_{50}=12.3µM)];
CNS Cancer [V251 (GI_{50}=11.41 µM)];
Melanoma [MALME-3M (GI_{50}=14.1µM), SK-MEL 2 (GI_{50}=12.5µM), SK-Mel 28 (GI_{50}=14.7µM) and VACC-62 (GI_{50}=11.5µM)];
Ovarian Cancer [OVCAR -3 (GI_{50}=10.8µM), OVCAR-8 (GI_{50} = 12.7 µM)];
Renal Cancer [ACHN. (GI_{50}=12.4µM), RXF-393 (GI_{50}=15.3µM), VO-31 (GI_{50}=15.9µM)]
Prostate Cancer [PC-3. (GI_{50}=13.0µM), DV-145 (GI_{50}=15.0µM)];
Breast Cancer [MDA-MBA35 (GI_{50}=12.3 µM), MCF7 (GI_{50}=15.9µM)].

The ethyl acetate extract showed cytotoxicity against:

Leukemia [SR. (GI_{50}=14.1µM)];
Non Small cell lung cancer [EKVX. (GI_{50}=12.7µM)];
Colon Cancer [HCT-15 (GI_{50}=10.2µM), HT29 (GI_{50}=12.1µM), KM12 (GI_{50}=15.0µM) and SW620 (GI_{50}=15.4µM)];
CNS Cancer [V251 (GI_{50}=12.3µM), SF-539(GI_{50}=14.6µM)];
Melanoma [SK-MEL 2 (GI_{50}=10.0µM), MALME 3M (GI_{50}=13.1µM)];
Ovarian Cancer [OVCAR-3 GI_{50}=12.7µM)];
Renal Cancer [VO-31(GI_{50}=10.2µM), 786-O (GI_{50}=15.9µM)];
Prostate cancer [DV-145(GI_{50}=14.9µM)]; and
Breast cancer [MDA-MD-435 (GI_{50}=14.5µM)].

Table 1: *Adenema hyssopifolium* G.Don – Quantitative micrographic parameters

Parameters		Maximum	Average	Minimum
Stomatal Number	UE	15.0	13.2	12.0
	LE	14.0	12.6	11.0
Stomatal index	UE	11.6	13.3	15.0
	LE	11.7	12.6	14.0
Vein islet number		6.0	9.0	13.0
Vein terminal number		13.0	18.0	22.00
Palisade ratio	UE	4.0	7.5	12.0
	LE	2.5	5.1	10.5

UE - Upper epidermis LE – Lower epidermis

Table 2: *Adenema hyssopifolium* G. Don– Behavioral pattern of powdered samples with reagents

Chemical Reagents	Colour of Powder
Powder as such	Dark green
Picric acid	No change
Nitric acid (Conc.)	Reddish brown
Hydrochloric acid (Conc.)	Greenish black
Sulphuric acid (Conc.)	Greenish black
Acetic acid (Glacial)	Yellowish brown
Iodine	Brown
Ferric chloride	Brownish yellow
Sodium hydroxide	Greenish black
Nitric acid (dilute)	Light brown
Hydrochloric acid (dilute)	Dark green

Table 3: Fluorescence analysis *Adenema hyssopifolium* G.Don powder

Treatment	Short UV	Fluorescent study	Visible
Powder as such	No colour change	No fluorescence	No colour change
Powder treated with dilute nitric acid	Green fluorescence	Green fluorescence	No colour change
Powder treated with sodium hydroxide in methanol	Greenish yellow fluorescence	Yellowish green fluorescence	No colour change
Powder treated with sodium hydroxide in water	Greenish yellow fluorescence	Green fluorescence	No colour change
Powder treated with hydrochloric acid	Green fluorescence	Green fluorescence	No colour change
Powder treated with nitric acid in water	Green fluorescence	No fluorescence	No colour change
Powder treated with dilute sulfuric acid	Green fluorescence	Green fluorescence	No colour change

Table 4: *In vitro* cytotoxic activity of the extracts of *Adenema hyssopifolium G.Don*

Type of Cancer	Cell line	GI_{50} (μM) $CHCl_3$ extract	GI_{50} (μM) Ethyl acetate extract	GI_{50} (μM) Ethanol extract
Leukemia	HL-60(TB)	10.0	24.2	97.5
	K-562	30.5	17.7	92.1
	MOLT-4	23.4	32.5	66.5
	RPMI-8226	97.2	75.6	97.8
	SR	68.0	14.1	>100
Non small Cell Lung Cancer	A549/ATCC	27.6	17.0	59.7
	EKVX	21.8	12.7	>100
	HOP-62	28.8	22.9	>100
	HOP-92	13.3	45.3	41.3
	NCI-H226	12.3	90.8	>100
	NCI-H23	2.66	22.5	>100
	NCI-H322M	18.4	18.2	80.5
	NCI-H460	20.6	16.4	76.4
	NCI-H522	67.8	34.6	58.5

Colon Cancer	COLO 205	17.6	22.9	>100
	HCC-2998	20.2	18.6	>100
	HCT-116	13.8	45.8	>100
	HCT-15	51.3	10.2	>100
	HT29	68.3	12.1	>100
	KM12	14.7	15.9	44.1
	SW-620	46.7	15.4	>100
CNS Cancer	SF-268	20.1	21.2	>100
	SF-295	19.1	21.6	>100
	SF-539	19.4	14.6	>100
	SNB-19	24.4	45.7	>100
	SNB-75	17.7	20.4	>100
	U 251	11.4	12.3	79.8
Melanoma	LOX IMVI	56.2	53.6	>100
	MALME-3M	14.1	13.1	56.9
	M14	18.3	18.2	>100
	SK-MEL-2	12.5	10.0	>100
	SK-MEL-28	14.7	18.4	>100
	SK-MEL-5	37.0	48.5	>100
	UACC-257	14.6	74.8	>100
	UACC-62	11.5	-	39.9
Ovarian Cancer	OVCAR-3	10.8	12.7	60.5
	OVCAR-4	15.6	16.0	41.9
	OVCAR-5	21.8	41.8	>100
	OVCAR-8	12.7	99.1	>100
	SK-OV-3	39.1	>100	>100
Renal Cancer	786-0	20.1	15.9	>100
	A498	16.4	52.3	25.5
	ACHN	12.4	12.4	>100
	CAKI-1	23.3	19.6	>100
	RXF 393	15.3	48.4	>100
	SN 12C	71.7	63.7	>100
	TK-10	20.5	26.8	>100
	UO-31	15.9	10.2	>100
Prostate Cancer	PC-3	13.0	62.0	>100
	DU-145	15.0	14.9	>100
Breast Cancer	MCF7	15.9	10.6	>100
	NCI/ADR-RES	30.8	35.5	>100
	MDA-MB-231/ATCC	10.1	69.4	27.4
	HS 578 T	42.9	38.7	>100
	MDA-MB-435	12.3	14.5	>100
	T-47D	45.9	>100	>100

GI_{50}=Concentration required for 50% growth inhibition at micro molar concentration

Discussion

The above data point to the possibility of developing the chloroform (CEAH) and ethyl acetate (EAAH) extracts of *Adenema hyssopifolium* G. Don as potential agents in the area of cancer therapy. Preliminary phytochemical screening indicated the presence of iridoid glycosides and flavones in ethyl acetate and chloroform extracts respectively. Flavones have been shown to possess anti mutagenic and anticarcinogenic activity (Nijveldt, 2001; Middleton, 1996) and may be responsible for the antitumor effect of CEAH and EAAH. Iridoids have been reported to possess cytotoxic activity (Isiguro *et al.* 1986; Sticher, 1969; Sticher and

Salama, 1981). Hence, the anti-cancer activity observed in the present study may be due to the presence of iridoid glycosides in chloroform extract and flavone's in ethyl acetate extract of the aerial parts of *Adenema hyssopifolium* G. Don. The study confirms interface of traditional therapy and scientific proof. In conclusion, both the chloroform and ethyl acetate extracts merit further investigation in identifying the active iridoids responsible for the observed activity.

Acknowledgement

We are grateful to National Cancer Institute (NCI), USA for screening cytotoxic studies.

References

Alley, M. C,; Scudiero, D. A,; Monks, A,; Hursey, M. L,; Czerwinski, M. J,; Fine, D. L,; Abbott, B. J,; Mayo, J. G,; Shoemaker, R. H. and Boyd, M. R. (1988): Feasibility of drug screening with panels of human tumor cell lines using a microculture tetrazolium assay. *Cancer Res*. ***48***, 589-601.

Anwar, M,; Ahmad, M,; Aslam, M. and Aftab, K. (1996): Enicostema littorale: a new source of swertiamarin. *Pak. J. Pharm. Sci.* ***9***, 29-35.

Bhattacharya, S. K,; Reddy, P. K,; Ghosal, S,; Singh, A. K. and Sharma, P. V. (1976): Chemical constituents of Gentianaceae XIX: CNS-depressant effects of swertiamarin. *J. Pharm. Sci.* ***65***, 1547-1549.

Boyd, M. R,; Paull, K, D. and Rubinstein LR (1995): Molecular targets in the National Cancer Institute drug screen. *J. Canc. Res. Clin. Oncol.* ***121***, 495-500.

Evans, W.C. (1989): Trease and Evans' Pharmacognosy. 13th Ed., Bailliere Tindal, London, pp.799-803.

Ghosal, S.; Singh, A. K.; Sharma, P. V. and Chaudhuri, R. K. (2004): Chemical constituents of Gentianaceae IX: Natural occurrence of Erythrocentaurin in *Enicostemma hyssopifolium* and *Swerta lawii*. *J. Pharm. Sci.* ***63***, 944 – 945.

Ghosal, S. and Jaiswal, D. K. (2006): Chemical constituents of Gentianaceae XXVIII: Flavanoids of *Enicostemma hyssopifolium* (Wild) Verd. *J. Pharm. Sci.* ***69***, 53-56.

Gopal, R.; Gnanamani, A.; Udayakumar, R. and Sadulla, S. (2004): *Enicostemma littorale* Blume - a potential hypolipidemic plant. *Nat. Prod. Rad.* ***3***, 401-405.

Harborne, J. B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. New York, Chapman and Hall, pp. 1-302.

Isiguro, K.; Yamaki, M.; Takagi, S.; Ikeda, Y.; Kawakami, K,; Ito, K. and Nose, T. (1986): Studies on iridoid-related compounds. IV. Antitumor activity of iridoid aglycones. Chem Pharm Bull 34, 2375-2379.

Kavimani, S. and Manisenthilkumar, K. T. (2000): Effect of methanolic extract of *Enicostemma littorale* on Dalton's ascetic lymphoma. *J. Ethnopharmacol.* ***71***, 349-352.

Middleton, E. (1996): Biological Properties of Plant Flavonoids: An Overview. *Pharmaceut. Biol.* ***34***, 344-348.

Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Gomise, P.; Vaigro-wolfe, A.; Gray – Goodrich, M,; Campbell, H,; Boyd, R. (1991): Feasibility of a high – flux anticancer drug screen utilizing a diverse panel of human tumor cell lines in culture. *J. Natl. Cancer Inst.* ***83***, 757-766.

Natarajan, P. N. and Prasad, S. (1972): Chemical investigations of *Enicostemma littorale. Planta Med.* ***22***, 42-46.

Nijveldt, R. J.; Nood, E.; Hoorn, D. E. C.; Boelens, P. G.; Norren, K. and Leeuwen, P. A. M. (2001): Flavonoids: a review of probable mechanisms of action and potential applications. Am. *J. Clin. Nutr.* ***74***, 418-425.

Pharmacopoeia of India (1985): Govt. of India, New Delhi, Ministry of Health and Family Welfare, New Delhi, Vol. I, pp. 264.

Raghunathan, K and Mitra, R. (1982): *Pharmacognosy of Indigenous drugs*, New Delhi, Central Council for Research in Ayurveda and Siddha, pp. 752–754.

Rai, J. and Thakar, K. A. (1966): Chemical Investigation of *E. Littrale* Blume, *Current Sci.* ***35***,148-149.

Ramakrishna, Y. A.; Manohar, A. I. and Mamata, P. (1984): Plants and novel anti-tumor agents; A review, *Indian Drugs.* ***21,***173-185.

Sadique, J.; Chandra, T.; Thenmozhi, V. and Elango, V. (1987): The anti-inflammatory activity of *Enicostemma littorale* and *Mollugo cerviana. Biochem. Med. and Metabol. Biol.* ***37***, 167-176.

Sticher, O. (1969): Iridoides. *Pharm. Acta Helv.* ***44***, 453-463.

Sticher, O. and Salama, O. (1981): Iridoid Glucosides from Euphrasia rostkoviana. *Planta Med.* ***42***, 122-123.

Trease, G.W. and Evans, W.C. (1989): *Pharmacognosy, Commerce and Quality Control*. London, Bailliere Tindal, pp. 137 – 138.

Trim, A. R. and Hill, R. (1952): The preparation and properties of aucubin, asperuloside and some related glucosides. *Biochem. J.* ***50***, 310-315.

Vasu, V. T.; Modi, H.; Jyoti, V. T. and Sarita, G. (2005): Hypolipidaemic and antioxidant effect of *Enicostemma littorale* Blume aqueous extract in cholesterol fed rats. *J. Ethnopharmacol.* ***101***, 277-282.

Vishwakarma, S.; Rajani, M.; Bagul, M. and Goyal, R. (2004): Rapid Method for the Isolation of Swertiamarin from *Enicostemma littorale*. *Pharmaceut. Biol.* ***42***, 400-403.

Vishwakarma, S. L. and Goyal, R. K. (2004): Hepatoprotective activity of *Enicostemma littorale* in CCl_4-induced liver damage. *J. Nat. Rem.* ***4***, 120-126.

Wieffering, J. H. (1966): Aucubinartige Glucoside (Pseu-doindikane) und verwandte Heteroside als systema-. tische Merkmale. *Phytochem.* ***5***, 1053-1054.