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In vitro Anti Cancer Activity of Ethanol Extract Fractions of *Aerva lanata* L.

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Abstract: To explore *in vitro* anticancer potential of *Aerva lanata* L. (flowering aerial part). The study was performed with 5 different human cell lines for the study of lung, leukaemia, prostate, colon and cervix cancer by using Sulphorhodamine B (SRB) assay. There were three doses of 10, 30 and 100 μg mL^{-1} of each *Aerva lanata* L. Chloroform fraction (ALCF) and *Aerva lanata* L. Ethyl Acetate Fraction (ALEAF) used in this study. ALCF showed significant % inhibitory effect for leukaemia, lung and colon cancer at maximum concentration of 100 μg mL^{-1} as compared to standard drug mitomycin. On the other hand ALEAF showed the significant % inhibitory effect for lung and cervix cancer at maximum concentration of 100 μg mL^{-1} as compared to standard drug 5-fluoro Uracil (5-FU). From the above studies it is concluded that, the ethyl acetate fraction and chloroform fraction of *Aerva lanata* L. provide enough experimental evidence for anticancer activity and these fractions could be useful in medical care.

Key words: anticancer, *Aerva lanata* L., sulphorhodamine B assay, human cell lines, 5-fluoro uracil, mitomycin

INTRODUCTION

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this disease (Hail, 2005). There are some ways by which cancer may be caused of one is incorrect diet, other is genetic factors and last the environment hazards. The most important cause of all cancers is life style but it will take long 20-30 years to develop. American Cancer Society along with International Union Against Cancer point out that 12 million cases were reported last year having 7 million deaths worldwide; these statistics are estimated to twice over by 2030 (Aggarwal *et al*., 2009). Around 80% of the total population were depends upon herbal medicine a report given by World Health Organization (Duraipandiyan *et al*., 2006). Natural products have a foremost role in primary health care need of human for the management of various diseases with special reference of cancer. Nature always remains the significant role in new drugs, as lead compound and new drug entities. There are approximately 50% of marketed drugs were originate from natural products and their derivatives (Liu *et al*., 2000). The discovery and development of anticancer agents by using natural leads gives the drugs like vincristine, vinblastine, podophyllotoxin (etoposide and tinoposide), paclitaxel, camptothecin, topotecan and irinotecan from plants; aplidine, dolastatine and citarabine from marines; doxorubicin, dactinomycin and bleomycin from microorganisms; diallyl sulfide, S-allyl cysteine, lycopene, capsaicin, curcumin, resverasrol, eugenol, limonene, ursolic acid, beta carotene, catechins from fruits and vegetables (Bhanot *et al*., 2011). More importantly, there is still enormous scope for the development of natural products as, despite their long history of medicinal use, the potential for most plant species remains unexplored. In recent years, natural products are believed to have an effect on the prevention of disease. Phytoconstituens possessing anti-oxidant properties are believed to prevent or slow down the occurrence of disease such as cancer (Lee *et al*., 2004). The present study was evaluating the *in vitro* anticancer potential of *Aerva lanata* L. by using chloroform as well as ethyl acetate fraction with standard drugs.

MATERIALS AND METHODS

Plant material: The plant consists of dried and flowering aerial part of *Aerva lanata* L. belonging to family Amaranthaceae was collected from district "Una"

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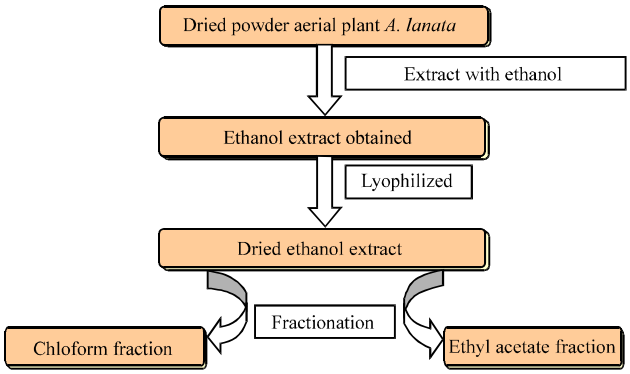


Fig. 1: Schematic presentation of extraction of *Aerva lanata* L.

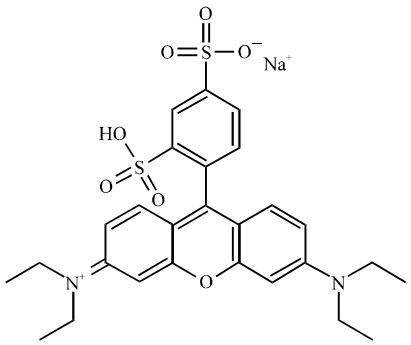
Himachal Pradesh in the month of July was shade dried and stored in a cool place. It was authenticated and identified as *Aerva lanata* L. Juss. ex Schult. Syn. *Achyranthes lanata* L. by Dr. H.B.Singh, Scientist F and Head, Raw Materials Herbarium and Meuseum, National institute of science communication and information Resources (NISCAIR), New Delhi (NISCAIR/RHMD/ Consult/-2010-11/1450/48).

Preparation of extract and fractions: The dried aerial part of plant *Aerva lanata* L. was extracted with ethanol and after lyophilized, the dried ethanol extract was further fractioned with chloroform and ethyl acetate to get *Aerva lanata* L. Chloroform Fraction (ALCF) and *Aerva lanata* L. Ethyl Acetate Fraction (ALEAF) (Scheme shown in Fig. 1).

Phytochemical Screening of *Aerva lanata* L.: The ALCF and ALEAF were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, steroids, flavonoids, glycosides, tannins, phenolic compounds, carbohydrates, proteins, amino acids and fats (Harborne, 1998).

TLC profiling of *Aerva lanata* L.: TLC profiles of the all extracts of onion and garlic were prepared (Stahl, 2005).

***In vitro* anti cancer activity by sulphorhodamine B (SRB) assay:** The Sulphorhodamine B (SRB) assay as first described by Skehan and colleagues was developed for use in the disease-orientated, large-scale anticancer drug discovery program of the National Cancer Institute (NCI) that was launched in 1985 (Skehan *et al*., 1990). Under mild acidic conditions, SRB binds to basic amino acid residues of Trichloroacetic Acid (TCA)-fixed cells. It can be quantitatively extracted from cells and solubilized for Optical Densitiy (OD) measurement by weak bases such as Tris base. The SRB assay is sensitive, simple, reproducible and more rapid than the formazan-based assays and gives better linearity, a good signal-to-noise ratio and has a stable end-point that does not require a time-sensitive measurement, as do the 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) or 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) assays. A higher OD value indicates higher dye uptake by cells which means more cell viability and less cytotoxic or anticancer potential of the sample tested.



Sulphorhodamine B

Statistical analysis: The data of *in vitro* anti cancer activity was statistically analyzed by one way ANOVA followed by Tukey's multiple range tests (compare all pairs of column) p-value <0.05 was considered to be statistically significant.

RESULTS

Yield of ethanol extract and its fractions: The yields of ethanol extract and its fraction ALCF and ALEAE are shown in following Table 1.

Table 1: Yield of ethanol extract and its fractions of *Aerva lanata* L.

Solvent system	Extractive value (%w/w)
Ethanol	3.67
ALCF	2.42
ALEAF	0.043

ALCF: *Aerva lanata* L. chloroform fraction, ALEAF: *Aerva lanata* L. ethyl acetate fraction

Table 2: Phytochemical screening of *Aerva lanata* L.

Tests	ALCF	ALEAF
Alkaloids	+	-
Sterols	+	+
Carbohydrates	+	-
Tannins	+	+
Flavonoids	-	+
Triterpenoids	-	+

ALCF: *Aerva lanata* L. chloroform fraction, ALEAF: *Aerva lanata* L. ethyl acetate fraction

Table 3: TLC profile of ALCF

Solvent system	Ratio	No. of Spots	R_f values
Chloroform:	3:2	3	0.25,0.19, 0.14
Methanol	7:3	4	0.81, 0.49,0.29, 0.09
	4:1	6	0.50, 0.49 0.46, 0.41, 0.18, 0.10, 0.05
Hexane:	4:1	4	0.39, 0.31, 0.17, 0.11
Chloroform	9:1	5	0.48, 0.44, 0.37, 0.30, 0.24

Table 4: TLC profile of ALEAE

Solvent system	Ratio	No. of Spots	R_f values
Chloroform:	8:2	5	0.46, 0.41, 0.18, 0.10, 0.05
Methanol	9:1	5	0.76, 0.50, 0.41, 0.19,0.07
Hexane:Chloroform	4:1	2	0.28, 0.35

Phytochemical studies: Result of phytochemical screening was given in following Table 2.

TLC profile: Thin layer chromatography of *Aerva lanata* L. chloroform fraction showed different R_f values which are shown below Table 3.

Thin layer chromatography of *Aerva lanata* L. ethyl acetate fraction showed different R_f values which are shown in Table 4.

***In vitro* anticancer studies by sulphorhodamine B assay:** The study was performed on 5 different human cell lines for the study of lung, leukaemia, prostate, colon and cervix cancer. Three doses of 10, 30 and 100 μg mL^{-1} of each ALCF and ALEAF were used. ALCF showed significant % inhibitory effect of 83, 88 and 77 at maximum concentration of 100 μg mL^{-1} for leukaemia, lung and colon cancer as compared to Mitomycin 61 and 62 (Fig. 2-5). On the other hand ALEAF showed the significant % inhibitory effect of 91 and 100 at maximum concentration of 100 μg mL^{-1} for lung and cervix cancer as compared to 5-FU 50

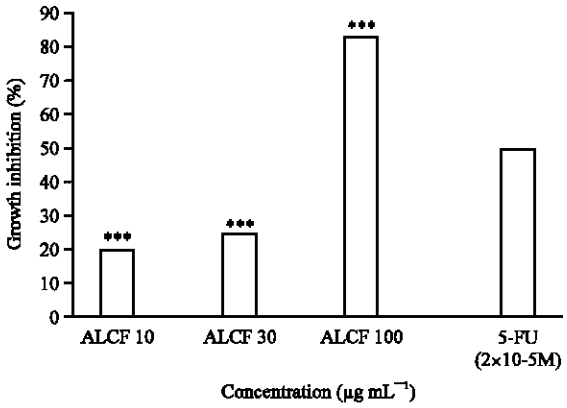


Fig. 2: Percentage growth inhibition of *Aerva lanata* L. chloroform fractions (ALCF) on A549 (Lung) human cell lines. *** = p<0.05 vs. 5-FU

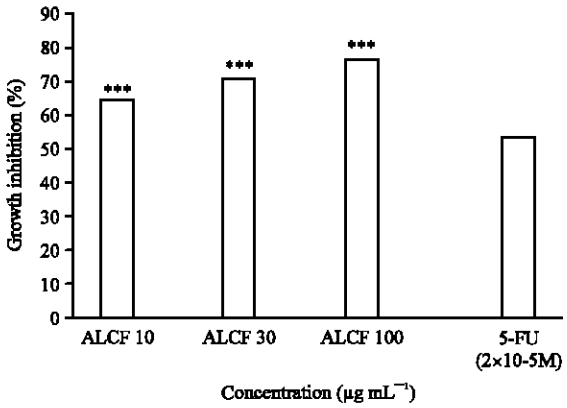


Fig. 3: Percentage growth inhibition of *Aerva lanata* L. chloroform fractions (ALCF) on Caco (Colon) human cell lines. *** = p<0.05 vs. 5-FU

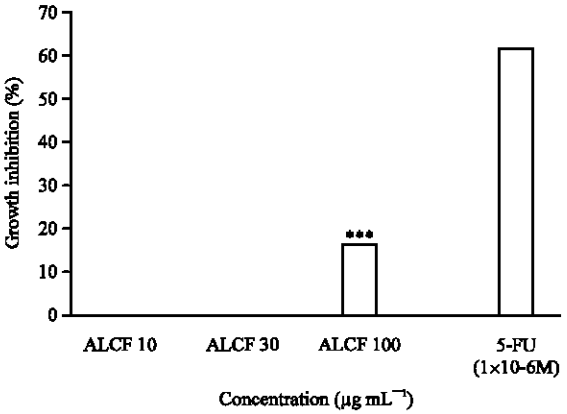


Fig. 4: Percentage growth inhibition of *Aerva lanata* L. chloroform fractions (ALCF) on PC-3 (Prostate) human cell lines. *** = p<0.05 vs. Mitomycin

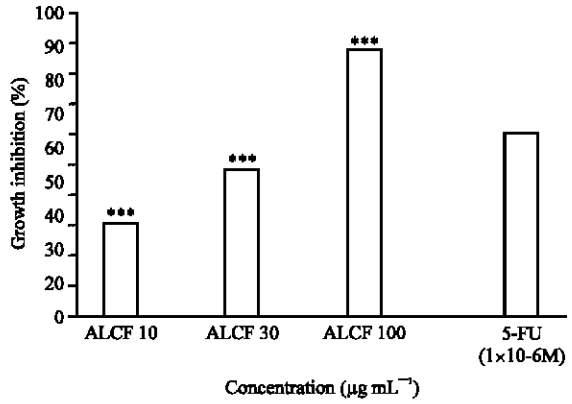


Fig. 5: Percentage growth inhibition of *Aerva lanata* L. chloroform fractions (ALCF) on THP-1 (Leukaemia) human cell lines. *** = $p<0.05$ vs. Mitomycin

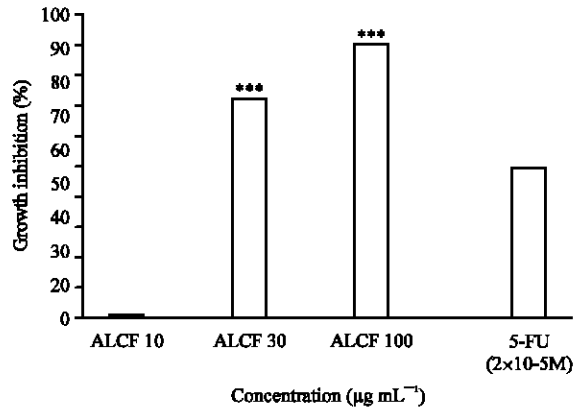


Fig. 6: Percentage growth inhibition of *Aerva lanata* L. ethyl acetate fractions (ALEAF) on A549 (Lung) human cell lines. *** = $p<0.05$ vs. 5-FU

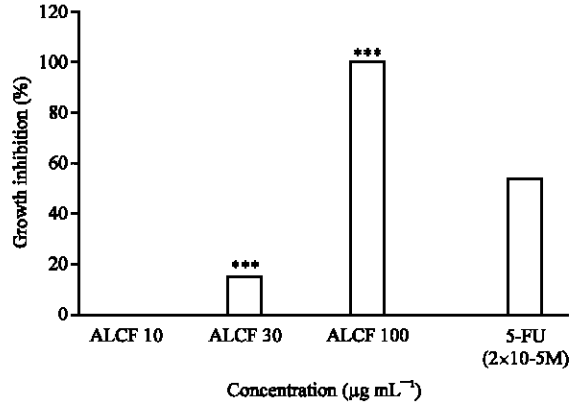


Fig. 7: Percentage growth inhibition of *Aerva lanata* L. ethyl acetate fractions (ALEAF) on Hela (Cervix) human cell lines. *** = $p<0.05$ vs. 5-FU

and 54 (Fig. 6, 7). The most effective anticancer activity was observed at the dose level of 30 and 100 µg mL^{-1} for ALCF and ALEAF on selected human cell lines.

DISCUSSION

The plant *Aerva lanata* L. belonging to family Amaranthaceae have been used for very long times in traditional system of medicine as diuretic and in lithiasis. Besides the traditional uses the plant were reported numerous pharmacological effects viz. diuretic (Udupihille and Jiffry, 1986), anti inflammatory (Vetrichelvan *et al*., 2000), anti-microbial, cytotoxic (Chowdhury *et al*., 2002), anthelmintic, demulcent (Pullaiah and Naidu, 2003), nephroprotective (Shirwaikar *et al*., 2004), anti-diabetic, anti-hyperglycaemic (Vetrichelvan and Jegadeesan, 2002; Deshmukh *et al*., 2008), expectorant, hepatoprotective (Manokaran *et al*., 2008), hypoglycemic, anti-hyperlipidemic (Krishnan *et al*., 2009), anti-parasitic and anthelmentic activities (Anantha *et al*., 2010), anti cancer activity was carried out against Dalton's Ascitic Lymphoma (DAL) cell lines which show significant cancer control of the same (Rajesh *et al*., 2011). Beside this vast studies were carried out by various researchers to showing importance of *Aerva lanata* L. which includes finger printing chromatographic technique analysis of steroids, terpenoids, flavanoids and glycosides (Yamunadevi *et al*., 2011a, b Mariswamy *et al*., 2011a, b). As per previous study the alkaloidal, phenolic, flavanoidal content with various antioxidant models where studied. In this the maximum antioxidant activity was observed in ALEAF followed by ALCF (Bhanot *et al*., 2012). Phytoconstituens possessing anti-oxidant properties are believed to prevent or slow down the occurrence of disease such as cancer. Qualitative Phytochemical screening concluded that ALCF contains alkaloids, steroid and carbohydrates content while ALEAF was found to be containing only flavonoids and tannins content. The objective of the present study was to investigate the *in vitro* anticancer properties of *Aerva lanata* L. (flowering aerial part). *In vitro* anticancer activity was performed on the ALCF and ALEAE in comparison with known standard drugs that were 5-FU and mitomycin. The MTT reagent assay have wide limitations over SRB assay because formation of colour in MTT relies on the activity of the mitochondria so, if the function of these is inhibited by variations in cellular levels of NADH, glucose and other factors, variable results are obtained and a similar result may be given as if the cells were not alive or not proliferating. The SRB assay was used for testing cytotoxicity of ALCF and ALEAF. *In vitro* anticancer activity was performed on 5 different human cell lines i.e., lung, leukaemia, prostate,

cervix and colon cancer by using Sulphorhodamine B (SRB) assay. This method is based on the principle of uptake of the negatively charged pink aminoxanthine dye, SRB by basic amino acids in the plasma membrane of the cells. From this assay the maximum % growth inhibition is measured in ALEAF which shows the 100 % inhibition for cervix cancer (Hela) and 91% inhibition for lung cancer (A549) while other side of experiment ALCF shows 88% inhibition for leukaemia (THP-1), 83% inhibition for lung (A549), 77% inhibition for colon (Caco) and 17% inhibition for prostate cancer (PC- 3), respectively.

CONCLUSION

From the above studies it is evident that, the ethyl acetate fraction and chloroform fraction of *Aerva lanata* L. is having a promising anticancer activity and these fractions could be useful in medical care. In the same way, the qualitative phytochemical screening and thin layer chromatographic studies shows the presence of many unknown compounds. Further identification and isolation of compounds responsible for biological activity could be used as a prototype to design new substances with anticancer activity.

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