IN VITRO ANTIBACTERIAL AND ANTICANCEROUS ACTIVITY OF FLOWER AND LEAVES OF CALLISTEPHUS CHINENSIS

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Abstract: Aster (*Callistephus chinenesis*) (Asteraceae) is an aromatic annual herb, which is used in traditional system of medicine to treat various diseases like anti-inflammatory, antibacterial and antitumor activity. In the present study antibacterial activity of cold and hot extracts of flower and leaves of Aster are determined. The cold extracts are further selected for its anticancerous activity on Human Melanoma Cell line (SKMEL2). The skin pathogens were isolated from clinical sample of various types of skin aliments. The antibiotic sensitivity pattern was determined. The antibacterial activity was assessed using modified agar cup method. Although all the extracts showed significant antibacterial activity, the highest antibacterial activity was observed with cold water and ethanol extract of flower with MIC of 4-6 mg/ml. The anticancerous activity was done using SRB assay. %GI was observed at concentration 80mcg/ml of Aster flower water and ethanol extract. Thus these extracts would be an ideal alternative that could be used in therapeutic preparation against skin infections.

Key Words: Aster, Antibacterial, Anticancerous, and Agar cup method, SRB assay.

Introduction: Ayurveda, a traditional medicinal practice using plant drugs has been successful in the treatment of various infections and for suppressing tumours [1]. The pharmacological screening of plants is important for the discovery of new, safe, and effective drugs in classical pharmacology [2]. In India bacterial infections of skin constitute a large proportion of skin disease; this skin infection can lead to several topical and systemic complications [3]. *Aster* genus has been used traditionally for a long time for curing diseases. It has expectorant, diuretic, antitumor, antibacterial, antiviral and antiulcer activities [4]. In addition, aqueous and ethanolic extracts of stalks, leaves, and roots of *Aster* plant have low toxicity. Its mouth infusions induced only minor changes in some serum biochemistry [5]. Aster is a perennial ornamental herb used as expectorant, stimulant with antifungal and antibacterial activity [6]. Aster is among the 112 Chinese medicines associated with anticancerous activity [7]. Poly phenols present in *Callistephus chinensis* have anticancerous activity. The present study aims to screen the antibacterial efficacy of *Callistephus chinensis* flower and leaves which is considered as waste material not commonly used as therapeutic agents. The extracts used are cold and hot, extracts of water, alcohol and petroleum ether. The extracts showing best antibacterial activity was further used for studying anticancerous activity using SRB assay.

Materials and Method:

Collection of plant material: Fresh plant parts were collected from More Nursery (Vangani). The taxanomic identification of these plants was done by Dr. Pravin, Blatter Hebarium, St. Xaviers College, Mumbai. The voucher specimens were preserved.

Method of extraction: The flowers and leaves from plant of *Callistephus chinensis* were collected, cleaned and dried in oven at 50°c. The dried leaves and flowers were pulverized by mechanical grinder and passed through mesh sieve. Powdered material were mixed with respective solvent petroleum ether, ethanol, and water and kept on shaker for 24 hours at room temperature. The extracts were filtered and evaporated and concentrated at 45°c (cold extract) [8, 9]. Hot extracts were prepared using soxhlet apparatus using water, ethanol and petroleum ether [10].

Microorganisms used: *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae. Escherichia coli,* Micrococcus spp and *Pseudomonas aeruginosa* were collected from patients of wound infections from hospitals and pathological laboratories. The isolates were identified by studying morphological, cultural and biochemical characteristics [11]. Their antibacterial susceptibility pattern was done by Kirby Bauer method [12].

Antibacterial screening of extracts: The antimicrobial potential of the above plant extracts was done by determining MIC using plate dilution method [13, 14]. MIC was interpreted as the lowest concentration of the extracts showing inhibition of growth of cultures. A series of dilution ranging from 1.0mg/ml to 20mg/ml was prepared by adding different volume of stock to molten nutrient agar. Each plate was then spot inoculated with test organism. After incubation of 24 hrs at 37°C the plates were observed for growth. Appropriate solvent controls were maintained to eliminate inhibition due to solvent [15].

Anticancerous Activity: The sulforhodamine B (SRB) assay is used for cell density determination based on the measurement of cellular protein content. The human cancer cell line SKMEL-2 was cultured in RPMI media supplemented with 10% heat inactivated fetal bovine serum in incubator at 37°C with 5% CO_2. The cells were allowed to adhere and

form monolayer and subcultured once every four days using trypsin EDTA buffer. Adriamycin was used as standard drug. Growth inhibition of SKMEL-2 cells was determined using modified SRB assay. The cells were seeded at a density of 10^6 cells/ml in 96 well micro titre plates. After 24 hrs, the cells were exposed to drugs for continuous 3 days. The culture medium was removed and trichloroacetic acid (50%) was added for fixation. Then the plates were air dried at room temperature, the bound SRB dye is solubilised with 10mM tris base and plates were analysed on a micro titre plate reader at 595 nm [16, 17].

Result and Discussion:

Antibacterial Activity: The organisms in the study are pathogens isolated from skin infections like burn wounds, impetigo, furuncle, sepsis etc. The six most resistant isolates were selected for study. The antibacterial assay showed that water, ethanol and petroleum ether extracts of both flower and leaves of *Callistephus chinensis* exhibited in vitro antibacterial activity against both Gram positive and Gram negative bacteria. Minimum inhibitory concentration of the active extracts of both flowers and leaves of Aster against the clinical isolates is shown in Table1 and Table 2 respectively. The lowest MIC values were observed for aqueous flower extract of *Callistephus chinensis* i.e 4 mg/ml against *S. aureus, S. epidermidis and Micrococcus,* 5mg/ml for *E.coli* and 6 mg/ml for *K. pneumoniae, P. aeruginosa.* In soxhlet hot extracts, ethanol extracts was most effective with the MIC of 4 to 10 mg/ml [18]. Cold and hot extracts of leaves of *Callistephus chinensis* also showed antibacterial activity but with higher MIC concentrations as shown in table 2. The most effective was cold ethanol extract of leaves having MIC 18 mg/ml for *S. aureus, S. epidermidis and Micrococcus* whereas 20mg/ml for *E. coli*, *K. Pneumoniae* and *P. aeruginosa.* Asteraceae family display strong biological activity, such as anti-diabetic, antioxidant effects and inhibitory effects against bacteria and viruses [19].

Anticancerous Activity: In cold extracts, crude aqueous and ethanol extracts of flower were selected for screening their anticancerous activity using concentrations of 10, 20, 40 and 80mcg/ml against Human Melanoma cell line SKMEL-2. Whereas in leaves only hot ethanol extract was selected for anticancerous activity. In SRB assay percentage growth inhibition (%GI) was calculated by comparing inhibition with the standard drugs used. Standard drug Adriamycin showed100% inhibition of cells at all concentrations except at concentration 80mcg/ml. All 4 extracts of *Callistephus chinensis* showed anticancerous activity, most effective extract was ethanol extract of flower at concentration of 40 and 80 mcg/ml as shown in table 3, followed with water extracts. Both Aster ethanol and water extracts of flower showed inhibition of cancer cell proliferation. The results are in accordance with *Aster thomsonii* having highest anti-tumour and anticancerous activity on human cancer cell line H157 exhibited by CME at100 µg/ml [16]. Asteraceae members are known to have anticancerous activity due to the rich phytoconstituents [17].

Conclusion: *Callistephus chinensis* extracts (flower and leaf) both showed broad spectrum antibacterial activity against skin pathogens. Flower extracts are more effective than leaves extract in antibacterial as well as anticancerous activity. Cold water and ethanol extracts of flowers found to be the best as compared to petroleum ether extracts with minimum MIC of 4 mg/ml. Significant inhibition of cancerous cell growth was found in water and alcohol extracts of aster as compared to leaf at concentration of 80 µg/ml. These results open up new avenues for finding new antibacterial and antitumor compounds in *Callistephus chinensis* to prove its therapeutic efficacy.

Acknowledgements: We are thankful to our principal for providing us with facilities and encouragement for carrying out this project at Smt. C.H.M College, Ulhasnagar. We are also thankful to Department of Anticancer Drug screening facility, ACTREC, Navi Mumbai for providing us the technical support to carry out the project.

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Table1: Minimum Inhibitory Concentration of *Callistephus chinensis* Flower extracts

Bacteria	MIC of extracts mg/ml					
	Cold extracts			Hot extracts		
	Aqueous extract	Ethanol extract	Petroleum ether extract	Aqueous extract	Ethanol extract	Petroleum ether extract
S.aureus	4	5	12	14	4	15
S.epidermidis	4	4	12	14	4	15
K.Pneumoniae	6	6	18	16	8	20
P. aeruginosa	6	6	18	17	7	20
E. coli	5	5	15	15	6	16
Micrococcus	4	5	15	15	10	20

Table2: Minimum Inhibitory Concentration of *Callistephus chinensis* Leaves extracts

Bacteria	MIC of extracts mg/ml					
	Cold extracts			Hot extracts		
	Aqueous extract	Ethanol extract	Petroleum ether extract	Aqueous extract	Ethanol extract	Petroleum ether extract
S.aureus	20	18	22	30	30	33
S.epidermidis	20	18	22	30	30	33
K.Pneumoniae	22	20	23	32	35	36
P. aeruginosa	22	20	23	33	35	37
E. coli	21	20	22	32	35	37
Micrococcus	20	18	18	30	30	33

Table3: Anticancerous activity of *Callistephus chinensis Flower* and Leaves extracts on Human Melanoma Cell line SKMEL-2

Extracts	% Control growth (Concentration mcg/ml)			
	10	20	40	80
ACW1	103.1	103.2	68.5	51.4
AAH2	98.0	76.5	57.3	54.3
AL5	108.5	105.8	108.1	117.3
ADR	-11.03	-19.84	-22.91	47

Note: ACW1 – Cold water flower extract, AAH2 – Hot alcohol flower extract, AL5- Aster cold leaf extract. ADR – Adriamycin (standard anticancerous drug)

Figure 1: Photoplates for Anticancerous activity

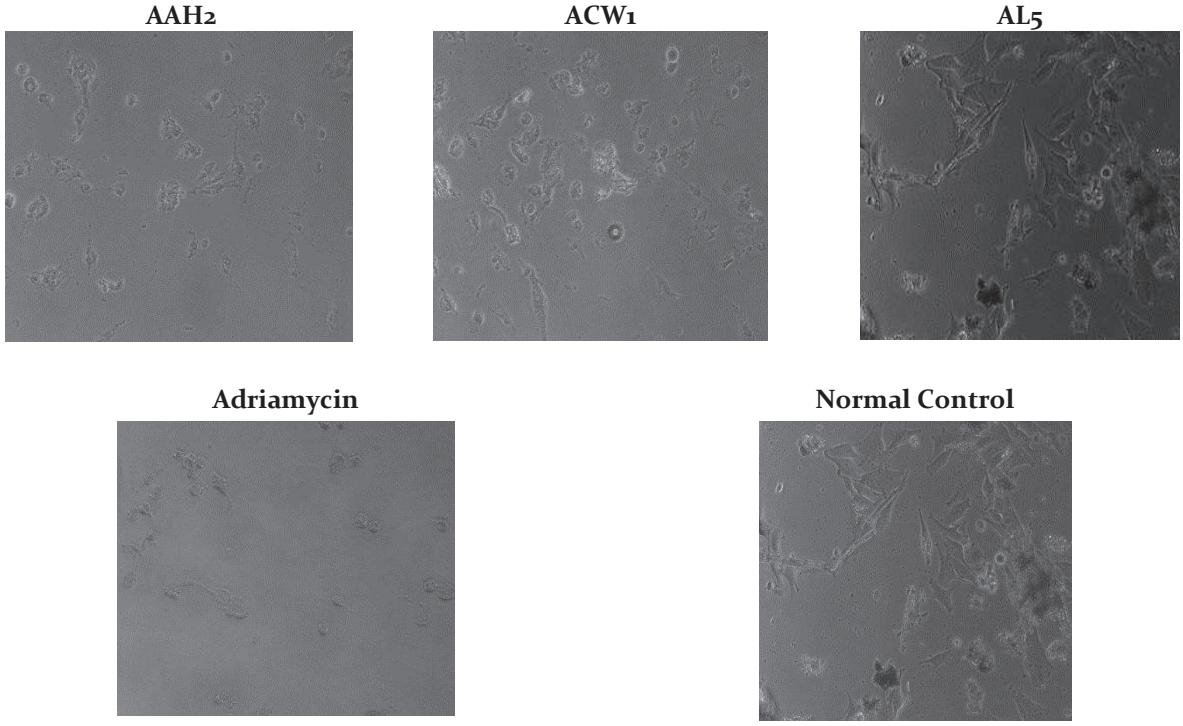
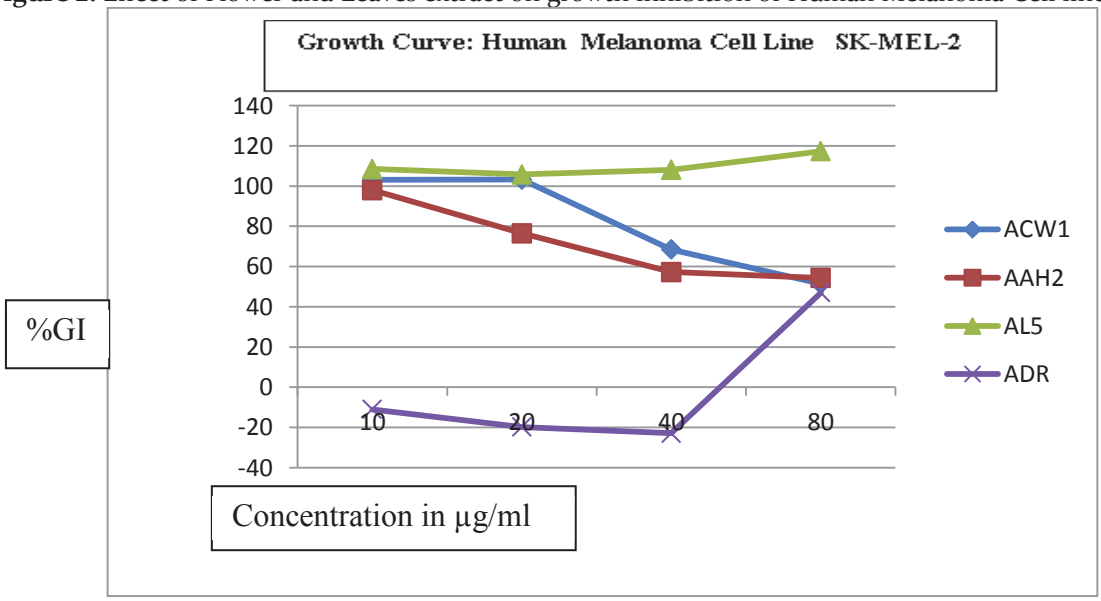


Figure 2: Effect of Flower and Leaves extract on growth inhibition of Human Melanoma Cell line SKMEL-2



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