ejpmr, 2023, 10(8), 382-390

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CHROMATOGRAPHIC ISOLATION AND ESTIMATION OF BIOACTIVE COMPOUNDS EXTRACTED FROM *CHROMOLAENA ODORATA* (L), AND ANTICANCER ACTIVITY AGAINST HELA CELL LINES

K. Charu Nanthini and Dr. N. Kannikaparameswari*

Dr. N.G.P Arts and Science College, Kalapatti, Coimbatore-641 048.

***Corresponding Author: Dr. N. Kannikaparameswari**

Dr. N.G.P Arts and Science College, Kalapatti, Coimbatore-641 048.

Article Received on 07/06/2023 **Article Revised on 27/06/2023** **Article Accepted on 17/07/2023**

ABSTRACT

Herbal medicine has been the main source of primary health care all over the world. From ancient times, plants have been catering as a rich source of effective and safe medicine. Awareness of medicinal plant usage is a result of the many years of struggles against illness due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants (Prakash et al., 2015). The present study explains the phytochemical study of both aqueous and methanolic leaf extract of *Chromolaena odorata*, followed by the Antioxidant activity of both the aqueous and methanolic extract with DPPH and FRAP. Further to extract and purify the bioactive compound, Chromatographic techniques are performed. Followed by the Anticancer activity of the aqueous extract of *Chromolaena odorata* was carried out in the HeLa cell line.

KEYWORDS: Awareness, Antioxidant activity, Bioactive compounds, Purify, Chromatographic.

INTRODUCTION

Medicinal plants play a major role in the life of people and cause innumerable forms. They provide medicine and prevent disease, maintain health, or cure ailments. There are a host of non-prescription plant preparations which have medicinal uses. These are often stated as herbal medicine (Raina *et al.,* 2008). *Chromolaena odorata* (siam weed) is one of the world's worst weeds and is regarded as one of the most harmful weeds present on earth (Vishak and Anima Pandey 2004). But is used by traditional medicine practitioners for the treatment of burns, wound healing, skin infection, post-natal wound healing, and antimalarial. The young leaves are crushed, and the subsequent liquid can be used to treat skin wounds. Also used for rashes and diabetes, and as an insect repellent (Guanasekara 2009). It is a tropical and subtropical species of flowering shrub in the sunflower family and a rapidly growing perennial herb. The plant is considered an invasive weed of field crops (Pierre, 1997). The phytoprostane compound chromophoric acid C-1 has been identified from *chromolaena odorata* as a strong inducer of the activity of the transcriptional factor NFE2L2 (Nrf2), a master regulator of a range of genes with defensive, anti-inflammatory, and detoxifying functions (Heiss *et al*., 2014). Chromatography is a handy separation technique broadly used to acquire pure compounds from mixtures. This technique is both quantitative and qualitative method. Qualitative chromatographic techniques are generally sensitive but suffer from being expensive in manpower requirements (Harryson *et al*., 2003). The method used to separate colored chemicals or substances by an analytical method is called Paper chromatography. It is also useful for separating complex mixtures of compounds having similar polarity (Chu *et al*., 2004). Plant-derived compounds have played a significant role in the growth of several clinically beneficial anticancer agents. These include Vinblastine, Vincristine, Camptothecin derivatives, Paclitaxel (taxol,) and Etoposide (Sirinthipaporn *et al*., 2017). Cervical cancer is the fourth most common cancer among women worldwide. It continues to be listed among the top gynecologic cancers worldwide. According to current data, it is ranked fourteenth among all cancers and fourth-ranked cancer among women worldwide. Cervical cancer intervention focuses on primary and secondary prevention (Fowler JR *et al*., 2023). However, side effects associated with cervical cancer treatments include urinary and sexual dysfunction (Frumovitz et al., 2005; Korfage et al., 2009). Therefore, cancer research in the last decades has focused on natural compounds to develop effective and selective cytotoxicity agents for the treatment of many cancers, including cervical cancer. C. odorata is known to have numerous compounds that are cytotoxic and can boost apoptosis (Kouame et al., 2013). Kaempferide, one of C. odorata compounds was proven to be pharmacologically safe for normal cells while inducing apoptosis in the cervical cancer cell line (HeLa). They are meant to serve as a compliment to conventional approaches. Thus the present study is focused on the

identification of compounds of *Chromolaena odorata* using chromatographic techniques and its phytochemical activity, antioxidant activity was analyzed with both methanolic and aqueous leaf extract of *Chromolaena odorata* undergoes an anticancerous activity against HeLa cell lines.

MATERIALS AND METHOD

Plant collection and extract preparation

The fresh leaves of *Chromolaena odorata* were collected from the Pekkadam, Trikkaripur, Kasaragod, Kerala. The collected leaves of *C. odorata* were weighed each 2 g and crushed using a mortar and pestle. The crushed plant material (2g) was dissolved in 20 ml of each in aqueous and methanol, and this was incubated in an orbital shaker for 400C and 60-70 rpm for 24 hrs. After incubation, it was filtered through Whatman No 1 filter paper and the extract was stored in an air-tight container for further study.

Phytochemical study

The Chemical tests were carried out on the aqueous and methanolic extracts to qualitatively determine phytochemical constituents.

Antioxidant activity

DPPH (1-Diphenyl 1-2 picrylhydrazyl)

The free radical scavenging activity of aqueous and methanolic extracts of *C.odorata* was measured by using 1,1-diphenyl—picryl-hydrazine (DPPH). The method used was almost the same as the one used by other authors (Ibanez *et al.*,2003; Dorman *et al*.,2004), but was modified in detail, 2 ml of methanol solution of DPPH radical in the concentration of 0.05 mg/ml and 1 ml of extract were placed in cuvettes. The mixture was shaken vigorously and left to stay at room temperature for 30 min. after that, the absorbance was measured at 517 nm in a spectrophotometer ("Jenway" UK). ascorbic acid, butylated hydroxyanisole (BHA), and α-tocopherol were used as positive control.

FRAP

Add 1 ml of sample add 1 ml of phosphate buffer solution, and 1 ml of potassium ferric cyanide (0.1%). Incubate 50oC for 20 minutes. Add 1 ml of 10% trichloroacetic acid. Mix it well incubate for 5 minutes and add 1ml of Distilled water and 300 µl of 0.1 % ferric chloride solution. Mix it well and take the OD at 700nm.

Compound identification

Column chromatography

The sample to be analyzed is dissolved in a very small amount of solvent (4g of silica pellet dissolved in 10 ml of distilled water which is solidified by keeping 50-20 minutes incubation) and is added to the top of the column. The pinch clamp is opened and the solvent is allowable to drain just to the top of the column. A small amount of eluting solvent ***(C.odorata)*** is added and allowed to drain in until the mixture is a little way into the absorbent, then the column is filled to the top with eluting solvent. The column is now ready to run – continue adding solvent at the top and collecting six fractions at the bottom until the compound elutes at the bottom. If applicable, change the eluting solvent to a more polar solvent during the eluting process. Never rent the solvent level drop below the top of the adsorbent. The process is continued when the compounds looked for are off the column.

Paper chromatography

It is proven that Paper Chromatography is very successful in the analysis of chemical compounds. Measured the filter paper of 0.5cm from the bottom of the plate. Using a pencil draw a line across the plate at the 0.5cm mark. Under the line, the name of the samples was marked. Dipped a toothpick into the sample and then gently touch the plate. After drying ascorbic acid and gallic acid were added separately and allowed for drying. The addition of extract was continued when it reached 20 times. The edge of the paper is immersed in a solvent (Mixture of 1ml Methanol, 1ml Acetic acid,1ml Water, and 1ml chloroform), and the solvent moves up the paper by capillary action.

Cell culture

HeLa Cells were grown in RPMI-1640 medium (Hi Media, Mumbai) supplemented with 10% fetal bovine serum (FBS) (Hi Media, Mumbai), 100 U/ml penicillin and 100 µg/m*l* streptomycin (Hi Media, Mumbai) Cells were incubated in a humidified incubator containing 5% CO2 at 37 ºC. After 24hrs the cells were seeded into 96 well The cell culture suspension was washed with 1 X PBS (Phosphate Buffered Saline) and then added with 20 µl MTT [3-(4, 5-Dimethyl thiazole-2yl)-2, 5-diphenyl tetrazolium Bromide] solution to the culture flask. It is then incubated at 37°C for 3 hours, removed all MTT solution, was washed with 1 X PBS and added with 30 µl DMSO to each culture flask and incubated at room temperature for 30 minutes, till all cells get lysed and homogenous color was attained. The solution was then transferred and centrifuged for 2 minutes to precipitate cell debris. Debris was dissolved using DMSO. OD was measured at 570 nm using a DMSO blank. Then by using the formulated percentage viability, the percentage of viability was calculated.

Percentage of viability; At/Ac 100
T: OD of test
C: OD of Control
(Mon *et al*, 2011)

RESULT

Table 1 represents the qualitative assay of phytochemicals study on leaf extract of *chromolaena odorata* in the aqueous and methanolic extract which revealed the presence of active phytochemical constituents. The phytochemical active compounds of *Chromolaena odorata* were qualitatively analyzed from leaves and the results are mentioned in Figure 2 & 3. Tests for Terpenoids, Phenol, Tannins, and quinine

were positive in both methanolic and aqueous leaf extracts. Saponins and Protein were detected only in the aqueous extract. Sugar, flavonoids, and Steroids were absent in both methanolic and aqueous leaf extracts. The phytochemical test result shows that the aqueous leaf extract of *Chromolaena odorata* has more phytochemical constituents than the methanolic extract.

The scavenging activity of DPPH radicals of the studied extracts is shown in Table 4. Methanolic leaf extract from *Chromalaena odorata* showed weaker DPPH radical scavenging activities than Aqueous extract. 32 mg/g of these extracts were determined with the help of standard ascorbic acid. This value was tabulated in table 2 compared with the value of ascorbic acid(mg/g). The aqueous extract has more phytochemical and DPPH scavenging activity than the methanolic extract so we have chosen the aqueous extract of *chromolaena odorata* for further studies. The ferric ability of the *C.odorata* extracts 1 ml was in the absorbance of 0.038. Table 3 shows the FRAP activity of *C.odorata* in an aqueous extract. The obtained results are shown in Graph 1, as absorbance increases at 700 nm. The fraction in the Eppendorf tube is collected every five minutes interval. The concentration was confirmed by OD value with different nm in a UV-Visible spectrometer. The sample which has high optical density was selected for the chromatographic technique. From Table 4 it is observed that the third fraction has a high OD value in 400nm with the value of 1.121. So the third fraction was selected for further study (Graph 2). From Paper chromatography figures 6 & 7 it is concluded by saying the third fraction of *Chromolaena odorata* shows a high Rf value of 0.95 in table 5. The Paper chromatography analysis also detected the phytochemical present in the extract**.** The Rf value of *Chromolaena odorata* shows the presents of Alkaloids. The anticancer activity of the extract was confirmed by MTT assay. Anticancer activity of aqueous extract of *Chromolaena odorata* leaves showed cytotoxicity against the HeLa cell line in figure 8 and was analyzed by MTT assay. The percentage viability was measured for three concentrations (10,20,30 µg/ml) of sample and it was shown in table 6. From Graph 3, it was observed that the viability of the cell was decreased when the concentration of *C.odorata* aqueous extract was increased. 68.3% viability was observed at the concentration of 30µl/ml. The observation of cell morphology after treatment was done which has shown, as a control in Figure 9. Figure 10, represents the percentage of viability in 10µl concentration, Whereas Figure 11 & 12 represents the percentage of viability in concentrations of 20µl & 30µl.

DISCUSSION

In the present study, aqueous and methanolic leaf extracts were prepared from *the Chromolaena odorata* plant. The bioactive compounds were extracted and studied and the phytochemical compounds were screened. The compounds extracted from both the extract are Alkaloids, Terpenoids, Phenol, Tannin and Quinine extract. The investigation reveals that there is significant variation in the contents like Sugar, Saponins, Proteins, and Steroids. These variations are due to several environmental factors such as climate, altitude, rainfall, etc as mentioned (Kokate *et al.*, 2004). Antioxidant activity cannot be unrushed directly but rather by the properties of the antioxidant in controlling the extent of oxidation. Methods show extreme diversity. Some approaches involve a different oxidation step followed by measurement of the consequence, for example, linoleic acid oxidation followed by diene conjugation determination. On other occasions, there is no strong discrepancy between the various steps in the procedure. An antioxidant study was carried out and revealed that DPPH content was high in aqueous extract(32mg/g). Thus, the aqueous extract has more phytochemical and DPPH scavenging activity than the methanolic extract so we have chosen the aqueous extract of *chromolaena odorata* for further studies. Phenolic compounds can donate hydrogen to free radicals and this way stop the chain reaction of lipid oxidation at the initial stage. This ability of phenolic compounds to scavenge radicals comes due to the presence of their phenolic hydroxyl groups (sawa *et al*., 1999). The *Chromolaena odorata* extracts have a strong antioxidant activity against various oxidative systems in-vitro. The differences in the antioxidant activity of various solvents may be the result of different capabilities to extract bioactive substances (Behera *et al*., 2005). FRAP Activity of *Chromolaena odorata* in the aqueous extract was also carried out the result shows that FRAP activity was higher (38 mg/g) than DPPH. Benzie (1999) agrees with Frankel and Meyer (1925) however, the measured reducing capacity does not necessarily reflect the antioxidant activity. It provides instead a very useful 'total' antioxidant concentration, without measurement and summation of the concentration of all antioxidants involved. The method was originally applied to plasma but has been extended to other biological fluids, foods, plant extracts, etc. ORP, also identified as the redox potential, is a measure of the potential for electrons to exchange from one chemical species to another. To quench the damaging effects of oxidants, antioxidants work by donating electrons to the oxidants, thereby reducing the chances of oxidants acquiring electrons from other nearby structures and causing damage. ORP is a measure of this relationship between oxidants and antioxidants, providing an inclusive measure of oxidative stress *(*McCord *et al., 2000).* To extract and purify the bioactive compound, the extract was purified to get the bioactive compounds using a silica column(8+2cm) and various fractions were collected at 5 minutes intervals. Further, the selected fractions were subjected to paper chromatography. The third fraction of *Chromolaena odorata* shows a high Rf value of 0.95**.** The Rf value of *Chromolaena odorata* shows the presents of Alkaloids. These Alkaloids exhibit a promising role as anticancer agents by restraining the enzyme topoisomerase which is associated with the replication of

DNA, instigate apoptosis, and modulate various other intracellular targets and signaling pathways. Specifically, these alkaloids with diverse chemical structures showing varied cytotoxicity against various cancer cell lines (Arijit Mondal *et al., 2019*). The anticancer activity was carried out using HeLa cell lines. The cytotoxic activity was studied using MTT dye. The plant extract reduced the percentage of cell viability. The viability percentage was 84.11 to 64.03% at the plant extract concentration of 10 to 30µg respectively. Thus, traditional practice has long suggested that cancer prevention and therapy may be achievable with native plants. In conclusion, the present study suggested that the *Chromolaena odorata* leaves have the potential to act as a source of useful drugs because of the presence of various phytochemical constituents such as alkaloids, terpenoids, sugar, saponins, Protein, Quinines, Phenol, and Tannins. The aqueous leaf extract of *Chromolaena odorata* had potent cytotoxic activity against HeLa cells. The findings also showed that the mechanism underlying cell death was due to growth inhibition and its antiproliferative activity. Further studies on the active components for proper assessment of their chemotherapeutic properties and feasible development as capable anticancer drugs are defensible.

ILLUSTRATIONS

Table 1: Phytochemical studies on leaves extract of *chromolaena odorata* in Aqueous and Methanolic extract.

Sl. No.	Phytochemical test	Aqueous Sample	Methanol sample
1	Alkaloids	+	+
2	Terpenoids	+	+
3	Phenol and tannins	+	+
4	Sugar	-	-
5	Saponins	+	-
6	Flavonoid	-	-
7	Quinines	+	+
8	Protein	+	-
9	Steroid	-	-

Table 2: DPPH Scavenging Activity of Water and Methanolic extract of *chromolaena odorata.*

DPPH (517 nm)	Water	Methanol
	32 mg/g	18 mg/g

Table 3: FRAP Activity of *Chromoleana odorata* in water extract.

FRAP (700 nm)	Water	Blank
	38 mg/g	0

Table 4: Optical densifractionse first six fraction of *C.odorata.*

	Optical density at various nanometer		
Fraction	400 nm	500nm	600nm
1.	0.423	0.364	0.364
2.	0.489	0.331	0.224
3.	**1.121**	0.684	0.343
4.	0.296	0.181	**0.801**
5.	0.531	**0.708**	0.143
6.	0.629	0.645	0.605

Table 5: Measurement and Identification bioactive compound of C.odorata fractions by paper chromatography.

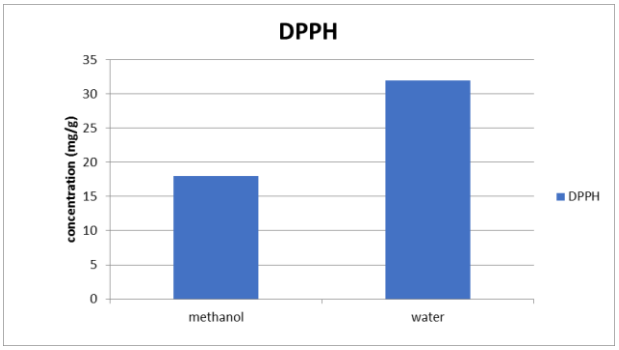
Fraction	R_f Value*	Bioactive compound
Third	0.9571	Alkaloids

$$*R_f \text{ Value} = \frac{\text{Distance moved by Solute}}{\text{Distance moved by Solvent}}$$

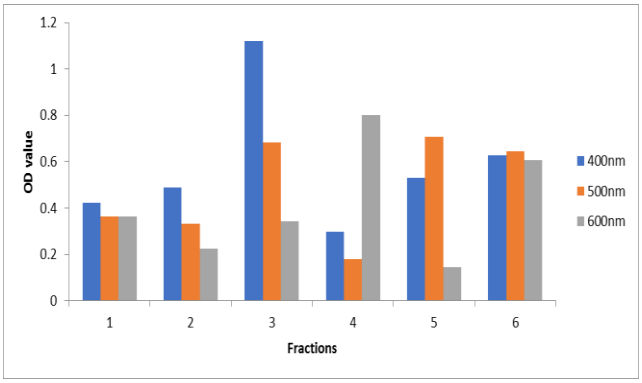
Table 6: Anticancer activity of *Chromolaena odorata in* HeLa cell line.

Concentration(µg/ml)	% of viability
Control	100
10	84.17

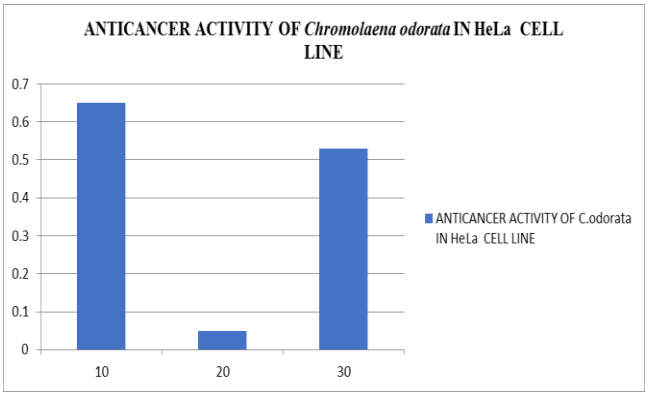
20	79.27
30	68.03



Graph 1: DPPH Scavenging activity of Methanolic and Water extract of *Chromolaena odorata.*



Graph 2: Optical density of the first six fraction of *C.odorata.*



Graph 3: Anticancer activity of *C.odorata* in HeLa cell line.

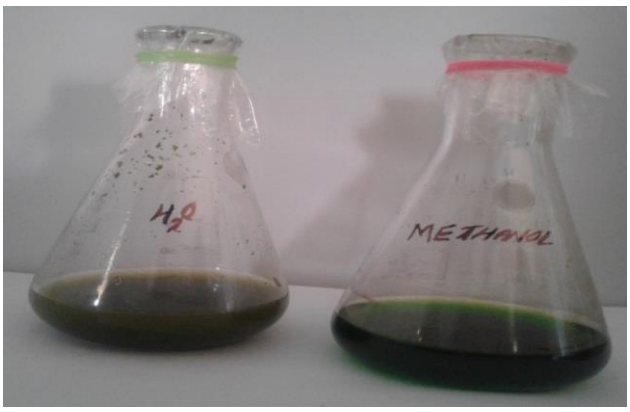


Figure 1: *Chromoleana odarata* extract of Water and Methanol.

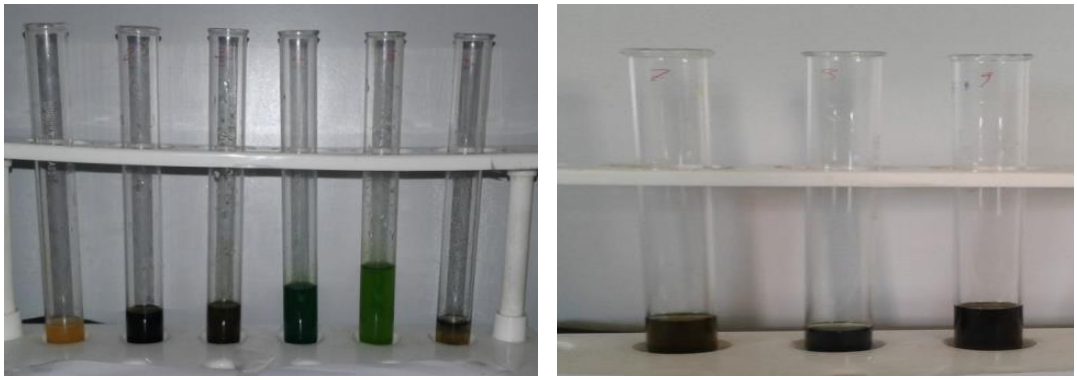


Figure 2: Phytochemical studies on leaves extract of *chromolaena odorata* in methanolic extract.

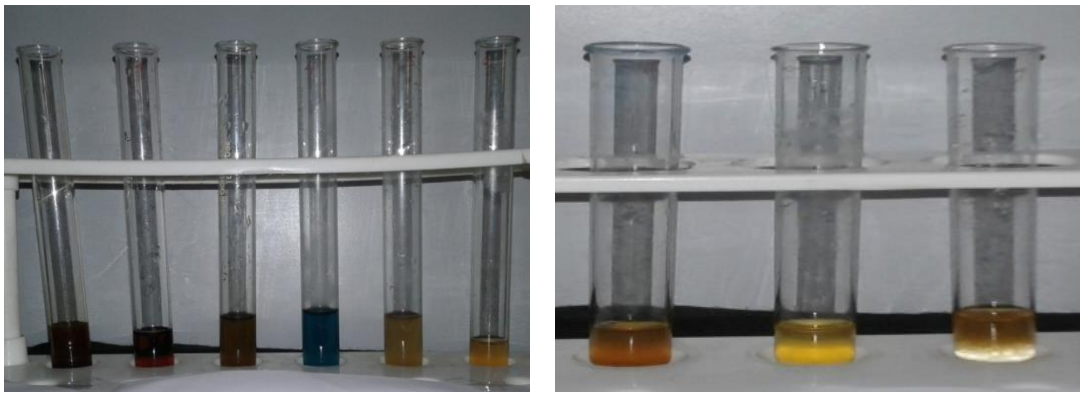


Figure 3: Phytochemical studies on leaves extract of *chromolaena odorata* in water extract.

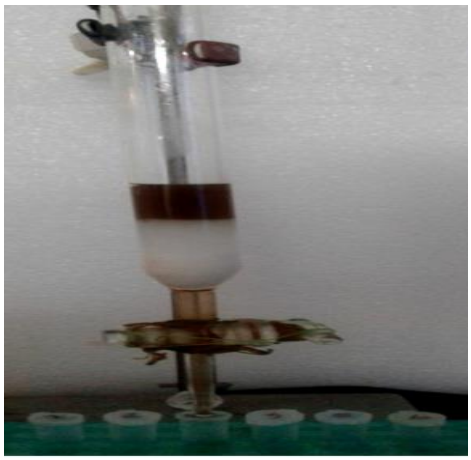


Figure 4: Collection of fraction from *Chromalaena odorata* - Column Chromatography.

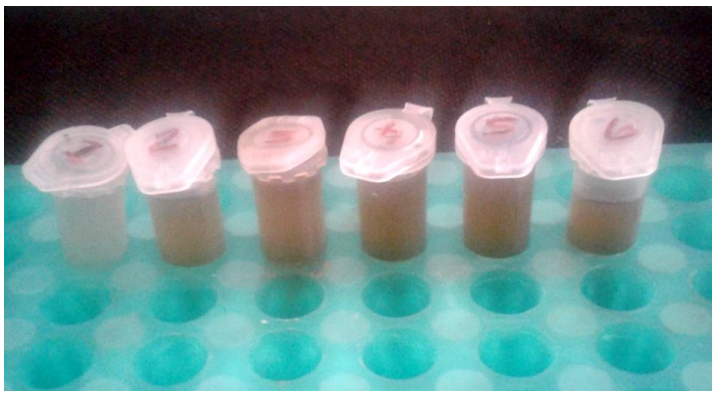


Figure 5: Collections of six fractions of *Chromalaena odorata.*

Figure 6: 3rd fraction of C.odorata water extract

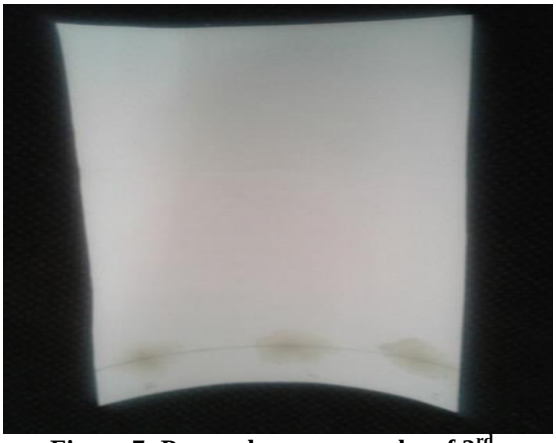


Figure 7: Paper chromatography of 3rd fraction of water extract

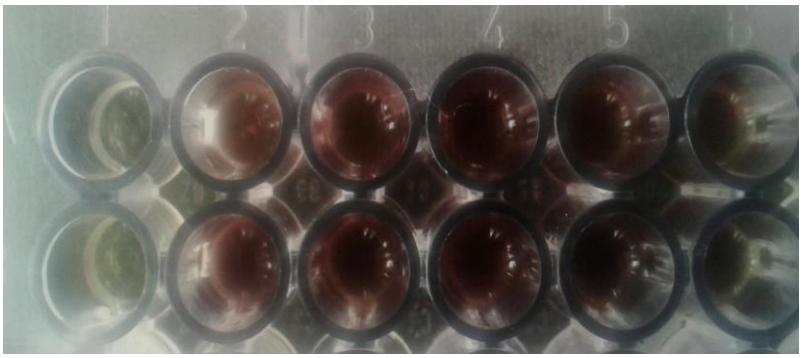


Figure 8: Anticancer activity of *Chromolaena odorata in* HeLa cell line.

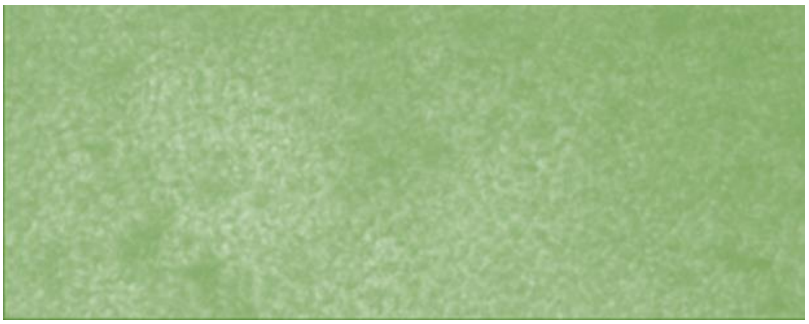


Figure 9: Control of *Chromalaena odorata.*

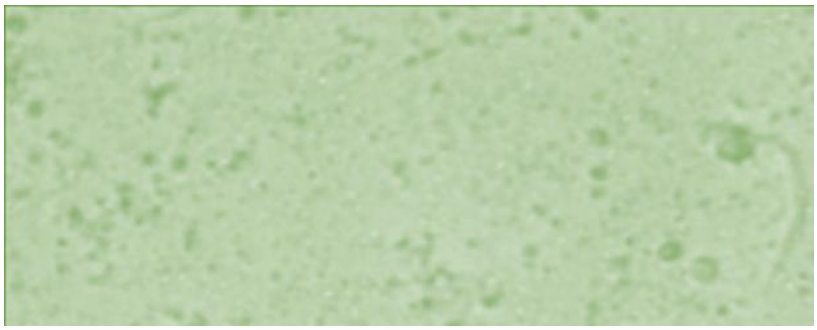


Figure 10: The percentage viability - 10µl concentration.

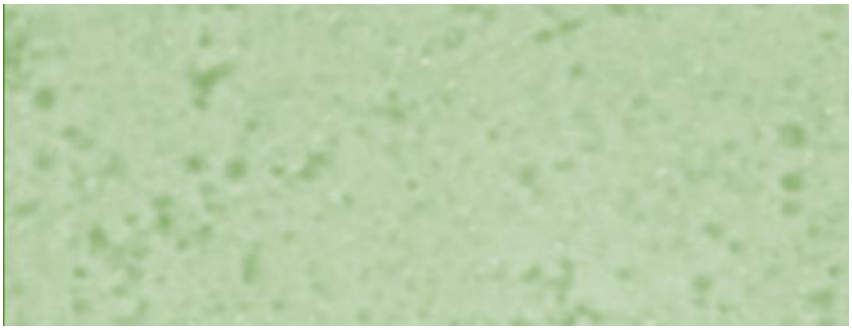


Figure 11: The percentage viability - 20µl concentration.

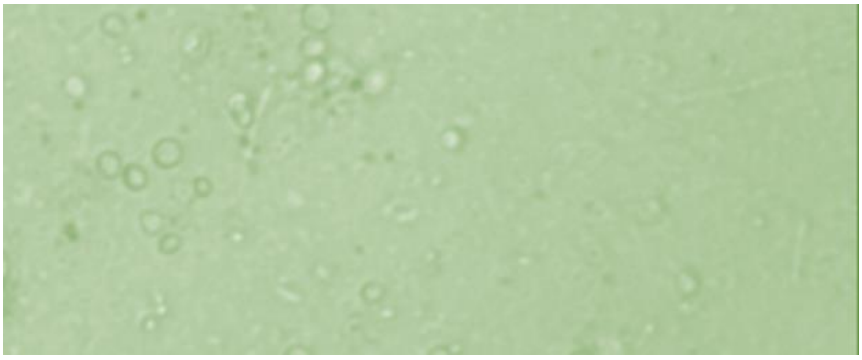


Figure 12: The percentage viability - 30µl concentration.

ACKNOWLEDGEMENT

I am very thankful to my guide Dr. N. Kannikaparameswari, Professor, Dr. N.G.P Arts and Science College and DBT (Department of Biotechnology) Star College Scheme. I am ever indebted to my guide for the timely guidance, excellent ideas, pragmatic criticism and the moral support extended to me.

BIBLIOGRAPHY

1. Prakash Ved, Sarika Saxena, Savitha Gupta, Ashish Kumar Saxena, Raj Kumar, Yadav, Sunil Kumar Singh, "Preliminary phytochemical screening and biological activities of Adina Cardifolia" J Microb Biochem Technology, 2015; 7: (1).
2. Raina R,. Prawez S, Verma P K and Pankaj N.K. Medicinal Plants and their Role in Wound Healing. VetScan, 2008; 3(1): 221-224.
3. M. N. Vaisakh and Anima Pandey, The invasive weed with healing properties: a review on chromolaena odorata, IJPSR, 2012; 3: 1. http://dx.doi.org/10.13040/IJPSR.0975-8232.3(1).80-83.
4. Gunasekera SP, Kinghorn AD, Cordell GA, Farnsworth NR. Plant Anticancer Agents. XIX. Constituents of Aquilaria malaccensis. J Nat Prod, 1981; 44(5): 569-72.
5. Pierre Binggeli "Chromolaena odorata (L.) King & Robinson (Asteraceae)", 1997. at http://pages.bangor.ac. uk/~{}afs101/iwpt/web-sp4.htm
6. Heiss EH, Tran TV, Zimmermann K, Schwaiger S, Vouk C, Mayerhofer B, Malainer C, Atanasov AG, Stuppner H, Dirsch VM. Identification of Chromomoric Acid C-I as an Nrf2 Activator in Chromolaena odorata. J Nat Prod, 2014; 29. PMID24476568.
7. Harrison UN. Aqueous Extract of Tridax procumbens Leaves: Effect on Lipid Peroxidative Stress and Antioxidant Status in Chloroquine-Induced Hepatotoxicity In Rats. J Herbs, Spices & Medicinal Plants, 2008; 14: 154-165.
8. Chu, K.O., C.C. Wang, C.Y. Chu, M.S. Rogers, K.W. Choy, and C.P. Pang. "Determination of Catechins and Catechin Gallates in Tissues by Liquid Chromatography with Coulometric Array Detection and Selective Solid Phase Extraction". Journal of Chromatography B, 2004; 810(2): 187-195.
9. Sirinthipaporn A, Jiraungkoorskul W. Wound Healing Property Review of Siam Weed, Chromolaena odorata. Pharmacogn Rev, 2017; 11(21): 35-38. doi: 10.4103/phrev.phrev_53_16. PMID: 28503052; PMCID: PMC5414454.
10. Fowler JR, Maani EV, Dunton CJ, et al. Cervical Cancer. [Updated 2022 Nov 2]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing, 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK431093/
11. Frumovitz, M. Quality of Life and Sexual Functioning in Cervical Cancer Survivors. Journal of Clinical Oncology, 2005; 23(30): 7428–7436. doi:10.1200/jco.2004.00.3996.
12. Kouamé, P.B.-K., Jacques, C., Bedi, G., Silvestre, V., Loquet, D., Barillé-Nion, S., Robins, R.J. and Tea, I., Phytochemicals Isolated from Leaves

of Chromolaena odorata: Impact on Viability and Clonogenicity of Cancer Cell Lines. Phytother. Res, 2013; 27: 835-840. https://doi.org/10.1002/ptr.4787

13. Ibanez E, Kubatova A, Senorans FJ, Cavero S, Reglero G, Hawthorne SB. Subcritical water extraction of antioxidant compounds from rosemary plants. J Agric Food Chem, 2003; 51: 375–382. doi: 10.1021/jf025878j.
14. Dorman HJ, Bachmayer O, Kosar M, Hiltunen R. Antioxidant properties of aqueous extracts from selected Lamiaceae species grown in Turkey. J Agric Food Chem, 2004; 52: 762–770. doi: 10.1021/jf034908v.
15. Kokate, C.K., Purohit, A.P., and Gokhale, S.B. Practical Pharmacognosy, 2nd edition. Vallabh Prakashan, New Delhi, 2004; 466470.
16. Sawa T, Nakao M, Akaike T, Ono K, Maeda H Alkylperoxyl radical-scavenging activity of various flavonoids and other phenolic compounds: implications for the anti-tumor-promoter effect of vegetables. J. Agri. Food Chem, 1999; 47(2): 397-402.
17. Behera, B. C., N. Verma, A. Sonone and U. Makhija, Antioxidant and Antibacterial activities of lichen Usnea ghattensis in vitro. Biotechnology Letters, 2005; 27: 991-995.
18. Edwin N Frankel; Anne S Meyer The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants, 2000; 80(13): 1925–1941. doi:10.1002/1097-0010(200010)80:13<1925::aid-jsfa714>3.0.co;2-4.
19. McCord, J.M., Fridovich, I. 1969. Superoxide dismutase: an enzymatic function of erythrocuprein. J. Biol. Chem. Nagarajan, S., Jain, H.C., Aulakh, G.S. Indigenous Plants Used in the Control of Diabetes. Publication and Information Directorate CSIR, New Delhi, 1987; 586, 244: 6049/6055.
20. Arijit Mondal, Arijit Gandhi, Carmela Fimognari, Atanas G. Atanasov, Anupam Bishayee, Alkaloids for cancer prevention and therapy: Current progress and future perspectives, European Journal of Pharmacology, 2019; 858: 172472, ISSN 0014-2999, https://doi.org/10.1016/j.ejphar.2019.172472.