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IN-VIVO DETERMINATION OF WOUND HEALING AND ANTITUMOR PROPERTIES OF HPLC ELUTED FRACTIONS OF WATER WEEDS (*EICHHORNIA CRASSIPES* AND *PISTIA STRATIOTES*)

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ABSTRACT: The water weeds *Eichhornia crassipes* belonging to family Pontederiaceae and *Pistia stratiotes* belonging to family Araceae were selected for the study to evaluate the wound healing and antitumor property. The wound healing property was investigated on the basis of physical evaluation of wound by vernier calliper and re-epithelization property. 24 adult male mice (F1 hybrid from Swiss albino and C57BL male) were treated with 2 different doses of extracts 200mg/kg body weight and 500mg/kg body weight of mice respectively administered orally for 20 days dissolved in double distilled water. For antitumor activity the mice were irradiated with 4 Gray radiation in 3 fractions by Co-60 ϒ radiation source. It was revealed that crude extract of *Pistia stratiotes* has shown better re-epithelization and wound repair between 15-20 days. In the tumor test groups and radiation test groups, the tumor size decreased significantly as compared with the control groups. The crude and alcoholic fractions of *Eichhornia crassipes* and aqueous and methanolic fractions of *Pistia stratiotes* proved as potential tumor inhibitor.

KEYWORDS: antitumor, re-epithelization, radiation, wound

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1. INTRODUCTION

Medicinal plants have been used for therapeutic and preventive health since ages all over the world. Herbal medicine may be used supportively, prophylactically, symptomatically, or correctively. Till today many herbal medicines and their bioactive constituents have been identified as potential agents against cancer [1]. Research indicated that herbal medicines may act alone or in combination to reduce the risk of cancer through their anti-oxidant, anti-tumorigenic properties and their direct suppressive effect on carcinogen bioactivities [2]. The secondary metabolites in the plant kingdom such as polyphenols, flavonoids and brassinosteroids are being explored as potent anticancer agents. These secondary metabolites have been shown to possess anticancer activities which include antioxidant activity, inhibition of cancer cell growth, induction of apoptosis, target specificity and cancer cell cytotoxicity [3-6]. The menace of aquatic weeds is reaching alarming problems in many parts of the world, particularly severe in tropical countries where abundant sunlight, favorable water temperature, increasing number of dams, barrage and irrigation channels foster aquatic growth [7]. Water weeds are highly populated in both fresh and saline water. It is a menace for the zooplankton and phytoplankton and known as water pollutant. *Eichhornia crassipes* (Pontederiaceae) is commonly known as Water Hyacinth. It contains tannins, flavonoids, alkaloids, terpenoids, steroids, phenolic contents, anthraquinones, quinine & cardiac glycosides [8]. The plant has been reported to show antimicrobial activity [9], antioxidant activity [10], wound healing activity [11] and anti-tumour activity [12].

2. MATERIALSAND METHODS

Crude extract of *Eichhornia crassipes*, *Eichhornia azurea* and *Pistia stratiotes* was prepared in 50% methanol [13]. Extraction of the plant material was done by maceration and percolation technique [14-16]. 50% hydro alcoholic extraction by maceration and percolation was done and successive solvent system was performed for column chromatography. Finally, two fractions of Eichhornia and three fractions of Pistia were obtained. The crude extracts and the fractions obtained were screened through HPLC and the retention time was evaluated. The HPLC analysis was performed with a Jasco UV 2075 Plus HPLC system. Chromatographic separation was achieved on C_{18} column (4.5mm x 25cm, 5µm) [17]. The identified constituents were then investigated for wound healing and tumor inhibition properties. The wound healing property was investigated on the basis of physical evaluation of wound by vernier calliper and re-epithelization property. Total 44 fractions were applied topically with the dose 1.5% and the final wound measured on day 15^{th}. Two animals were subjected for each constituent of all the active constituents obtained by respective solvent fractions. The antitumor activity was recorded from day 1 when the palpable tumor developed till day 20. Total 44 constituents collected through different crude extracts subjected for tumor inhibition assay and in each group 2 animals were taken because the different constituents collected

through HPLC were in fewer amounts. The tumor inhibitory property was scored by tumor volume measured by verniercalliper. The mice were irradiated with 4 Gray dose in 3 fractions by Co-60 ϒ radiation source. The tumor volume measured between day1st and day 5th, day 11th to 15th and day 16th to 20th. Two different doses of 200 mg/kg body weight and 500 mg/kg body weight were administered orally after the palpable tumor developed for 20 days alternatively.

3. RESULTS AND DISCUSSION

In *Eichhornia crassipes* phenolic and flavonoids were isolated. The phenolic group revealed Gallic acid, Caffeic acid, Protocatechuic acid and Ellagic acid, rest of the components were flavonoids. In Eichhornia, Gallic acid, Rutin, Protocatechuic acid, Unknown2, Naringinin were found to be more in their area percentage respectively. However, Kaempferol, Quercetin, Myricetin, Astragalin and Caffeic acid presented with less percentage area. The methanolic fractions of Eichhornia mainly contained Gallic acid followed by Catechin, Epicatechin, Epicatechingallate and Apigenin. The aqueous extract of Eichhornia contains Protocatechuic acid, Naringinin, Epigallocatechin, Rutin in maximum amount followed by Gallic acid and Quercetin. The crude extract of Eichhornia contained Rutin in high amount followed by others. The acetone fraction of Pistia contained Gallic acid in higher amount and Rutin. The methanolic extract of Pistia was found to have Gallic acid, Rutin, Catechin and Kaempferol where the area percentage was more in Gallic acid followed by Rutin and Catechin. An unknown peak has been observed at 11.88 retention time. Aqueous extract of Pistia revealed higher area percentage of Gallic acid, 1-O-galloyl-β-D-Glucose, Rutin, Catechin, Epicatechin, Epicatechingallate and Unknown where unknown peak was observed at 45.49 retention time, a steroid or flavonoid may be present in this peak.

Three unknown peaks were revealed in Eichhornia which have shown fairly good pharmacological evidence.

Wound Healing Activity: Significant reduction of wound was observed in Gallic acid of Methanolic fraction of *Eichhornia crassipes* (0.83mm^3/15th day) followed by Rutin (0.82mm^3/15th day) and Vallinic acid (0.88mm^3/15th day) found in acetone fraction of *Pistia stratiotes* (Table 2). It was also revealed that of all the fractions, Methanolic fraction of Eichhornia and Acetone fraction of Pistia has shown appreciable amount of wound healing and re-epithelization. This is to submit that in all the constituents the rate of re-epithelization was significantly better than vehicle, povidone iodine and placentrex. No doubt, Methanolic fractions of Eichhornia& acetone fractions of Pistia have signatured for faster re-epithelization than other groups. Betadine and Povidine iodine revealed better re-epithelization than normal control, but could not able to prove better than Pistia and Eichhornia. This may be due to the presence of phenolic and flavonoids.

Antitumor Activity: The tumor inhibition by different constituents eluted through HPLC found in different crude extracts was analyzed. The LD50 did not produce any animal death at 500 mg/kg

body weight of the drug. The antitumor activity of 50% methanolic extract of *Eichhornia crassipes* revealed that there was a slow and steady growth in tumor volume but a drastic fluctuation in tumor size was observed after 6^{th} -10^{th} day. It was revealed that the tumor volume was reduced maximum by Gallic Acid isolated from Methanolic fraction of *Eichhornia crassipes* (tumor volume 0.14cm^3) and Kaempferol, Quercetin, Myricetin and Gallic acid isolated from aqueous extract of *Eichhornia crassipes* followed by Rutin isolated from methanolic and aqueous fraction of *Pistia stratiotes* (tumor volume 0.024 cm^3). However all the other constituents of the respective fractions have shown better result than radiation alone group as listed in Table **Error! Reference source not found.**1.

Table 1: Antitumor Activity (B16F10) of HPLC Eluted Fractions

Sample Name	Fraction Isolated	Tumor Volume (cm^3) after			
			Day 1-5	Day 11-15	Day 16-20
Methanolic fraction of *Eichhornia crassipes*	Gallic Acid	A	0.139 ± 0.008	0.115 ± 0.035	0.14 ± 0.02
		B	0.129 ± 0.005	0.15 ± 0.027	0.10 ± 0.01
	Catechin	A	0.241 ± 0.0175	0.28 ± 0.035	0.28 ± 0.05
		B	0.230 ± 0.0150	0.210 ± 0.030	0.17 ± 0.03
	Epicatechin	A	0.989 ± 0.096	0.251 ± 0.090	0.210 ± 0.024
		B	0.785 ± 0.085	0.249 ± 0.76	0.15 ± 0.023
	Epicatechingallate	A	0.827 ± 0.079	0.276 ± 0.64	0.18 ± 0.028
		B	0.818 ± 0.071	0.269 ± 0.70	0.19 ± 0.030
	Apigenin	A	0.848 ± 0.083	0.289 ± 0.72	0.22 ± 0.034
		B	0.850 ± 0.091	0.270 ± 0.75	0.25 ± 0.039
Aqueous fraction of *Eichhornia crassipes*	Gallic Acid	A	0.137 ± 0.005	0.245 ± 0.029	0.16 ± 0.04
		B	0.135 ± 0.008	0.239 ± 0.025	0.13 ± 0.02
	Epigallocatechin	A	0.254 ± 0.19	0.212 ± 0.098	0.16 ±0.005
		B	0.243 ± 0.21	0.209 ± 0.087	0.18 ± 0.010
	Protocatechuic Acid	A	0.261 ± 0.32	0.219 ± 0.90	0.20 ± 0.014
		B	0.258 ± 0.45	0.215 ± 0.85	0.20 ± 0.017
	Unknown 1	A	0.272 ± 0.51	0.221 ± 0.78	0.21 ± 0.020
		B	0.270 ± 0.55	0.223 ± 0.70	0.19 ± 0.018
	Unknown 2	A	0.691 ± 0.59	0.217 ± 0.65	0.15 ± 0.010
		B	0.659 ± 0.48	0.230 ± 0.60	0.25 ± 0.010
	Rutin 1	A	0.592 ± 0.45	0.209 ± 0.58	0.14 ± 0.009

		B	0.587 ± 0.40	0.205 ± 0.55	0.18 ± 0.007
	Rutin 2	A	0.582 ± 0.39	0.242 ± 0.50	0.22 ± 0.005
		B	0.578 ± 0.35	0.199 ± 0.45	0.16 ± 0.008
	Ellagic Acid	A	0.601 ± 0.62	0.327 ± 0.91	0.37 ± 0.027
		B	0.545 ± 0.59	0.349 ± 0.85	0.32 ± 0.021
	Vallinic Acid	A	0.620 ± 0.71	0.264 ± 0.97	0.23 ± 0.013
		B	0.615 ± 0.52	0.260 ± 0.93	0.27 ± 0.011
	Kaempferol	A	0.29 ± 0.45	0.264 ± 0.099	0.104 ± 0.0247
		B	0.352 ± 0.62	0.255 ± 0.110	0.112 ± 0.0260
	Quercetin	A	0.391 ± 0.71	0.245 ± 0.010	0.117 ± 0.008
		B	0.370 ± 0.65	0.241 ± 0.009	0.115 ± 0.008
	Myricetin	A	0.692 ± 0.54	0.423 ± 0.017	0.25 ± 0.018
		B	0.650 ± 0.59	0.429 ± 0.024	0.20 ± 0.016
	Naringinin	A	0.687 ± 0.55	0.525 ± 0.020	0.22 ± 0.015
		B	0.679 ± 0.60	0.510 ± 0.018	0.24 ± 0.012
Acetone fraction of *Pistia stratiotes*	Gallic Acid	A	0.140 ± 0.070	0.116 ± 0.048	0.131 ± 0.021
		B	0.142 ± 0.045	0.112 ± 0.033	0.106 ± 0.019
	Rutin	A	0.621 ± 0.075	0.428 ± 0.050	0.325 ± 0.025
		B	0.589 ± 0.090	0.405 ± 0.039	0.282 ± 0.022
	Vallinic Acid	A	0.752 ± 0.072	0.543 ± 0.052	0.346 ± 0.032
		B	0.749 ± 0.069	0.549 ± 0.045	0.324 ± 0.028
Aqueous fraction of *Pistia stratiotes*	Gallic Acid	A	0.142 ± 0.078	0.132 ± 0.069	0.128 ± 0.021
		B	0.144 ± 0.080	0.127 ± 0.062	0.112 ± 0.010
	1-O-Galloyl-β-D-Glucose	A	0.989 ± 0.124	0.736 ± 0.112	0.542 ± 0.057
		B	0.980 ± 0.127	0.729 ± 0.120	0.539 ± 0.085
	Rutin	A	0.494 ± 0.065	0.218 ± 0.043	0.024 ± 0.016
		B	0.490 ± 0.060	0.209 ± 0.021	0.020 ± 0.010
	Catechin	A	0.868 ± 0.013	0.334 ± 0.027	0.213 ± 0.0106
		B	0.710 ± 0.095	0.366 ± 0.058	0.251 ± 0.012
	Epicatechin	A	0.850 ± 0.078	0.263 ± 0.045	0.182 ± 0.018
		B	0.649 ± 0.065	0.258 ± 0.040	0.150 ± 0.014
	Epicatechingallate	A	0.898 ± 0.070	0.260 ± 0.039	0.176 ± 0.012

		B	0.878 ± 0.076	0.254 ± 0.035	0.169 ± 0.009
	Unknown	A	0.650 ± 0.059	0.152 ± 0.029	0.158 ± 0.007
		B	0.639 ± 0.047	0.147 ± 0.021	0.150 ± 0.006
Methanolic fraction of *Pistia stratiotes*	Gallic Acid	A	0.521 ± 0.09	0.320 ± 0.05	0.152 ± 0.023
		B	0.540 ± 0.0095	0.357 ± 0.059	0.109 ± 0.017
	Rutin	A	0.589 ± 0.042	0.261 ± 0.02	0.0243 ± 0.02
		B	0.549 ± 0.044	0.264 ± 0.020	0.212 ± 0.017
	Catechin	A	0.666 ± 0.051	0.352 ± 0.042	0.254 ± 0.015
		B	0.590 ± 0.049	0.312 ± 0.0.38	0.215 ± 0.009
	Unknown	A	0.525 ± 0.052	0.325 ± 0.059	0.210 ± 0.020
		B	0.520 ± 0.050	0.322 ± 0.054	0.205 ± 0.018
	Kaempferol	A	0.624 ± 0.049	0.269 ± 0.032	0.198 ± 0.015
		B	0.620 ± 0.040	0.264 ± 0.029	0.190 ± 0.010
Crude extract of *Eichhornia crassipes*	Rutin	A	0.427 ± 0.085	0.312 ± 0.029	0.102 ± 0.005
		B	0.420 ± 0.080	0.309 ± 0.025	0.104 ± 0.007
	Ellagic Acid	A	0.520 ± 0.082	0.543 ± 0.030	0.213 ± 0.012
		B	0.535 ± 0.087	0.535 ± 0.032	0.210 ± 0.014
	Vallinic Acid	A	0.550 ± 0.090	0.529 ± 0.028	0.217 ± 0.016
		B	0.549 ± 0.083	0.525 ± 0.024	0.215 ± 0.009
	Kaempferol	A	0.383 ± 0.868	0.259 ± 0.029	0.013 ± 0.010
		B	0.451 ± 0.114	0.210 ± 0.037	0.010 ± 0.041
	Unknown	A	0.423 ± 0.151	0.322 ± 0.034	0.106 ± 0.035
		B	0.429 ± 0.149	0.319 ± 0.031	0.101 ± 0.029
	Myricetin	A	0.572 ± 0.61	0.424 ± 0.015	0.30 ± 0.020
		B	0.570 ± 0.59	0.435 ± 0.019	0.29 ± 0.015
	Quercetin	A	0.258 ± 0.012	0.190 ± 0.031	0.008 ± 0.012
		B	0.281 ± 0.016	0.251 ± 0.038	0.010 ± 0.050
	Astragalin	A	0.589 ± 0.82	0.352 ± 0.040	0.210 ± 0.010
		B	0.580 ± 0.79	0.349 ± 0.033	0.208 ± 0.007
	Caffeic Acid	A	0.550 ± 0.75	0.361 ± 0.037	0.215 ± .0112
		B	0.575 ± 0.70	0.359 ± 0.030	0.211 ± 0.011
	Apigenin	A	0.439 ± 0.62	0.263 ± 0.045	0.126 ± 0.017

		B	0.430 ± 0.57	0.260 ± 0.041	0.120 ± 0.015
	Naringinin	A	0.442 ± 0.88	0.259 ± 0.047	0.123 ± 0.012
		B	0.435 ± 0.80	0.255 ± 0.043	0.121 ± 0.009

Table 2: Wound Healing Activity- Mean and Standard Error of HPLC Eluted fractions

HPLC Eluted Sample	**Fractions**	**Day 10**	**Mean ±SE of Day 10**	**Day 15**	**Mean ±SE of Day 15**
Methanolic fraction of *Eichhornia crassipes*	Gallic Acid	a) 21.45 b) 22.13	21.79±0.34	a)0.89 b)0.78	0.83±0.05
	Catechin	a) 22.57 b) 21.26	21.91±0.65	a)0.98 b)0.82	0.9±0.08
	Epicatechin	a) 22.18 b) 23.65	22.91±0.73	a)0.92 b)0.90	0.91±0.010
	Epicatechingallate	a) 23.92 b)22.15	23.03±0.88	a)1.01 b)0.89	0.95±0.06
	Apigenin	a)23.95 b)24.10	24.02±0.07	a)1.04 b)0.90	0.97±0.07
Aqueous fraction of *Eichhornia crassipes*	Gallic Acid	a)23.22 b)22.98	23.1±0.12	a)1.20 b)0.98	1.09±0.11
	Epigallocatechin	a)24.53 b)23.86	24.19±0.33	a)1.19 b)1.29	1.24±0.05
	Protocatechuic Acid	a)23.56 b)22.12	22.84±0.72	a)1.25 b)1.95	1.23±0.020
	Unknown 1	a)23.42 b)22.36	22.89±0.53	a)1.22 b)1.20	1.21±0.010

	Unknown 2	a)23.25 b)22.21	22.73±0.52	a)1.19 b)1.12	1.15±0.03
	Rutin 1	a)23.75 b)23.50	23.62±0.12	a)1.98 b)0.75	1.36±0.61
	Rutin 2	a)23.62 b)23.25	23.43±0.18	a)1.85 b)1.60	1.72±0.12
	Ellagic Acid	a)23.98 b)23.78	23.88±0.10	a)1.97 b)1.21	1.59±0.38
	Vallinic Acid	a)23.47 b)23.21	23.34±0.13	a)1.59 b)1.45	1.52±0.07
	Kaempferol	a)23.84 b)22.95	23.39±0.44	a)1.20 b)0.28	1.24±0.04
	Quercetin	a)23.96 b)23.12	23.54±0.42	a)1.29 b)0.32	1.30±0.01
	Myricetin	a)23.93 b)24.94	24.43±0.50	a)1.35 b)0.80	1.57±0.22
	Naringinin	a)23.21 b)23.10	23.15±0.05	a)1.76 b)1.22	1.49±0.27
Acetone fraction of *Pistia stratiotes*	Gallic Acid	a)22.21 b)21.20	21.70±0.50	a)0.91 b)0.79	0.85±0.06
	Rutin	a)22.32 b)21.87	22.09±0.22	a)0.85 b)0.80	0.82±0.02
	Vallinic Acid	a)22.58		a)0.95	

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		b)21.95	22.26±0.31	b)0.82	0.88±0.06
Aqueous fraction of *Pistia stratiotes*	Gallic Acid	a)23.99 b)22.89	23.44±0.55	a)2.25 b)1.98	2.11±0.13
	1-O-Galloyl-β-D-Glucose	a)24.98 b)24.85	24.91±0.06	a)2.93 b)2.10	2.51±0.41
	Rutin	a)22.95 b)21.76	22.35±0.59	a)2.08 b)1.97	2.02±0.05
	Catechin	a)22.54 b)21.43	21.98±0.55	a)2.01 b)1.78	1.89±0.11
	Epicatechin	a)22.32 b)21.16	21.74±0.58	a)2.18 b)1.49	1.83±0.34
	Epicatechingallate	a)22.76 b)22.21	22.48±0.27	a)2.58 b)1.99	2.28±0.29
	Unknown	a)22.98 b)21.94	22.46±0.52	a)2.84 b)1.52	2.18±0.66
Methanolic Fraction of *Pistia stratiotes*	Gallic Acid	a)23.95 b)22.82	23.38±0.56	a)1.18 b)1.34	1.26±0.08
	Rutin	a)22.90 b)21.74	22.32±0.58	a)1.39 b)1.57	1.48±0.09
	Catechin	a)22.65 b)21.87	22.26±0.39	a)1.68 b)1.87	1.77±0.09
	Unknown	a)22.88 b)21.90	22.39±0.49	a)1.77 b)1.62	1.69±0.07

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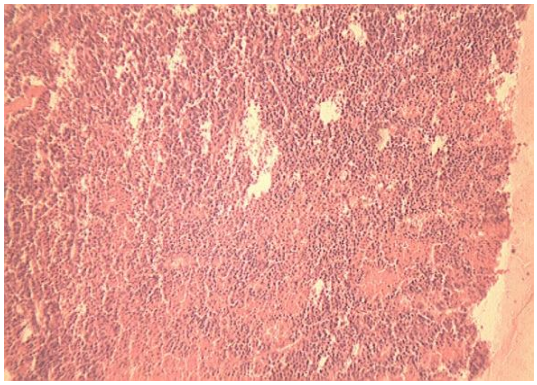
	Kaempferol	a)24.01 b)23.22	23.61±0.39	a)1.45 b)1.22	1.33±0.11
Crude extract of *Eichhornia crassipes*	Rutin	a)22.92 b)22.01	22.46±0.45	a)0.99 b)0.86	0.92±0.06
	Ellagic Acid	a)23.55 b)22.17	22.86±0.69	a)1.00 b)0.89	1.89±0.05
	Vallinic Acid	a)23.01 b)22.85	22.93±0.08	a)2.21 b)2.01	2.11±0.10
	Kaempferol	a)23.96 b)22.01	22.98±0.97	a)1.27 b)1.10	1.18±0.08
	Unknown	a)22.67 b)21.45	22.06±0.61	a)2.09 b)1.87	1.98±0.11
	Myricetin	a)23.01 b)22.09	22.55±0.46	a)1.54 b)0.98	1.26±0.28
	Quercetin	a)22.08 b)21.98	22.03±0.05	a)1.90 b)0.76	1.33±0.57
	Astragalin	a)23.22 b)21.56	22.39±0.83	a)1.65 b)1.39	1.52±0.13
	Caffeic Acid	a)23.10 b)21.31	22.20±0.89	a)1.39 b)1.21	1.3±0.09
	Apigenin	a)23.01 b)21.16	22.08±0.92	a)1.73 b)1.54	1.6±0.09
	Naringinin	a)22.98 b)21.10	22.04±0.94	a)1.84 b)1.28	1.5±0.28



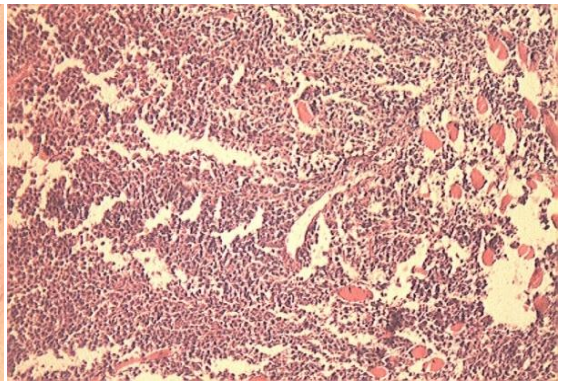
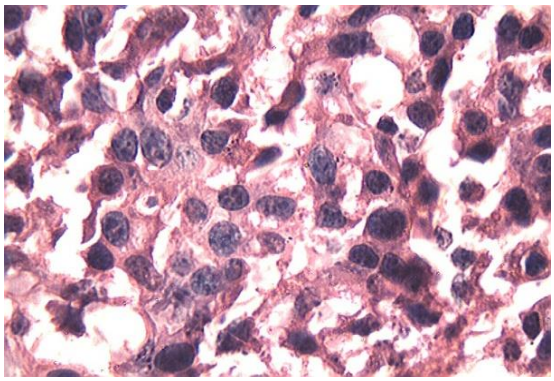


Figure 1: Tumor TS without treatment (10x) **Figure 2: Gallic Acid from Methanolic fraction of *Eichhornia crassipes* (10x)**



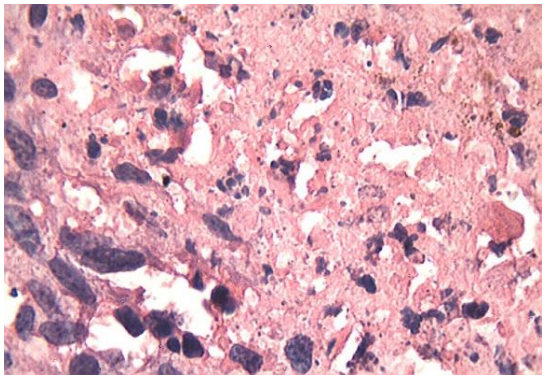


Figure 3: Tumor TS without treatment (100x) **Figure 4: Gallic Acid Treated from Methanolic fraction of *Eichhornia crassipes* (100x)**

Figure 5: Normal Wound Control **Figure 6: Betadine Treated** **Figure 7: Placentrex Treated**

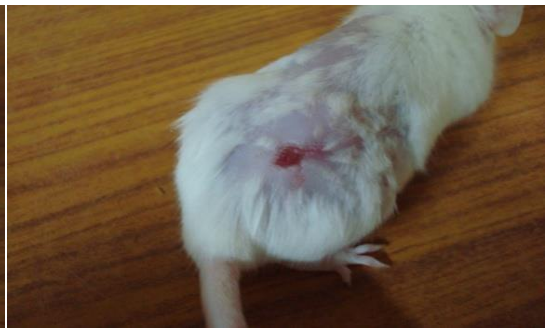


Figure 8: Gallic Acid Treated from Methanolic fraction of *Eichhornia crassipes* **Figure 9: Rutin Treated from Acetone fraction of *Pistia stratiotes***

4. CONCLUSION

Gallic acid and Rutin are well-known antioxidants with their use in treating skin diseases as well as anticancer property. Quercetin, kaempferol and myricetin were suggestive to posses antioxidant, anti-inflammatory & antitumor property. *Eichhornia crassipes* and *Pistia stratiotes* were found to be rich in Gallic acid (Phenolic) and Rutin (Flavonoids). Due to their antioxidant properties, they also posses best wound healing (Re-epithelization) better than Vaseline, Povidone iodine and placentrex. The crude and alcoholic fractions of Eichhornia and aqueous & methanolic fractions of Pistia proved as potential tumor inhibitor. The generic drugs available in the market are more costly and produce number of tissue and organ toxicity. Eichhornia and Pistia may be a drug of choice to reduce the burden of non-healing wound and tumor load. This indigenous aquatic weed will be an asset for cancer treatment to reduce the oxidative stress to the tissue level. The wound healing property of Eichhornia and Pistia may help to cure the wounds of bed ridden patients; radiation induced wounds and injured soldiers at far flung area fighting for the nation with open wounds. No doubt, the formulations obtained by these water weeds will serve poor man as these are cost effective and may be considered as drug of choice for different ailments.

5. CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

6. ACKNOWLEDGEMENT

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