WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 8, Issue 2, 1649-1663. Research Article ISSN 2277– 7105

DETERMINATION OF PHYTOCONSTITUENTS & EVALUATION OF ANTIOXIDANT, ANGIOGENIC AND ANTIMICROBIAL ACTIVITY OF *ELYTRARIA ACAULIS (L. FIL.) LINDAU.*

Vinoth M.* and Vijaya C.

Department of Pharmacology, Ultra College of Pharmacy, Madurai, Tamil Nadu, India-625020.

Article Received on
20 Dec. 2018,
Revised on 10 Jan. 2019,
Accepted on 31 Jan. 2019
DOI: 10.20959/wjpr20192-14233

***Corresponding Author**
Vinoth M.
Department of Pharmacology, Ultra College of Pharmacy, Madurai, Tamil Nadu, India-625020.

ABSTRACT

Objective: *Elytraria acaulis* easily available plant and used to treat various diseases in traditional medicine, such as wound healing, leucorrhoea, abscesses etc... Antioxidant, Angiogenic and Antimicrobial activity is essential for wound healing with this background this study presently focused to specifically determining the phytochemical, Antioxidant, angiogenic activity and antimicrobial activity of *Elytraria acaulis*. **Materials & Methods:** Alcoholic Extract of *Elytraria acaulis (l. fil.) Lindau.* (AEEA) subjected to Phytochemical Screening Test, HPTLC analysis, Determination of Total Phenolic & Flavonoid contents. Anti-oxidant Activity by Nitric oxide Scavenging Assay, Reducing Power assay, Lipid Peroxidation assay. Angiogenesis Activity Screened by CAM Models. Anti-microbial studies performed by Contact Bioautography. **Results:** Total Flavonoid & Phenolic content of AEEA was found to be 57.2 ± 0.26 mg Quercetin Equivalents / g 217.5 ± 0.5 mg Gallic acid equivalents /g plant extract respectively. In Nitric oxide Scavenging Assay. IC50 value of Standard was found to be 12.58 µg/ ml and in that of AEEA is 294.18± 11.25 µg/ ml. Reducing Power assay the observation shows reducing power of our drug (AEEA) is 75% compared to standard drug (100%). In Lipid Peroxidation assay IC50 value of Standard found to be 23.71± 2.12 µg/ ml and AEEA was 226.18± 4.04 µg/ ml. Angiogenic effect AEEA treated (500µg) group showed higher number of conspicuous blood vessels with greater thickness compared to control group. Contact Bioautographic antimicrobial study zone of inhibition correlated into HPTLC Analysis. **Conclusion:** The results of the present study conform AEEA have antioxidant, angiogenic and antimicrobial activity.

KEYWORDS: Antioxidant, Angiogenic, Bioautography-Antimicrobial Activity, *Elytraria acaulis.*

INTRODUCTION

Elytraria Acaulis (L.Fil.) Lindau is easily available; Important plant Used to treat various disease in traditional medicine. Such as cuts and wound healing, otolgia, leucorrhoea, venereal diseases, in veterinary practice Broken horns (mixture of plant powder, powdered seeds of *Panicum sumatrense* and some red soil, applied as plaster) etc…[1] Medicinal plants produce several compounds having one or more medicinal properties such as antioxidant, antimicrobials, anti-cancer etc. Free radicals are responsible for causing pathogenesis of healthy cells to lose their structures and functions to develop various degenerative diseases that caused due to aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction.[2] Phenolic compounds are major group of secondary metabolite which include simple phenolic compounds, phenyl propanoids, lignans, lignins, flavonoids and polyphenols include tannins. Phenolics have potential to promote the antioxidants, lower inflammatory markers and facilitate wound healing, antibacterial, antiulcer, anti-inflammatory, antitumor, and antidepressant activities.[3] Angiogenesis is essential for regeneration of tissue in wound healing, myocardial ischemia, peripheral ischemia, cerebral ischemia, Reconstructive surgery.[4] A single extract have different Phytoconstituents, each compounds have one or more different biological activity, especially in antimicrobial screening one isolate may have sensitive to one or more specific organisms in this view Bioautographic method is used evaluate antimicrobial study.[5][6] *Elytraria acaulis* easily available plant and used to treat various diseases in traditional medicine, Antioxidant, Angiogenic and Antimicrobial activities are promoting, essential for wound healing, with this background this study presently focused to specifically determining the phytochemical, Antioxidant, angiogenic activity and antimicrobial activity of *Elytraria acaulis.*

MATERIALS AND METHODS

Plant Material

The whole plant of *Elytraria acaulis (L.fil.)* Lindau is collected from Errampatti & Alanganallur area in Madurai, Tamil Nadu, India. Then the plant is authenticated by Dr. Stephen Ph.D., Professor, Dept. of Botany, American College, Madurai. The collected fresh plant is washed and shade dried, made into coarse powder then extracted with 250 ml of

methanol by hot percolation method using soxhlet apparatus. The extraction was carried out for 72 hours. After extraction, the solvents were distilled out the concentrated residues Subjected to qualitative preliminary phytochemical screening.

HPTLC Finger Print Profile of AEEA[7]

HPTLC analysis was carried out CAMAG HPTLC system equipped with Linomat 5 Applicator, TLC scanner 3 and reprostar 3 controlled by software WinCATS-1.4.3. A total of 10 mg extract was dissolved in 10 ml of methanol (95%) and the solution was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis as test solution. Pre-coated silica gel glass plate 60 F 254(3 x 10 cm) (E. Merck) of uniform thickness 0.2mm with aluminium sheet used for study. The Toluene: Ethyl Acetate: Methanol (7:2:1) was employed as mobile phase. Linear ascending development was carried out in 20 cm x 10cm twin trough glass chamber saturated with the mobile phase and the chromatogram plate development for two times with the same mobile phase to get good resolution of phytochemical contents. The plate was kept in photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images under White light at 520, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3, Camag). For specific identification of Phytoconstituents visualization done with Formaldehyde in Sulfuric acid Reagent for Flavonoids & Dragendorff Reagent for alkaloids.

Determination of Total Flavonoid Content[8]

The total flavonoid content of the AEEA was determined by using Aluminium chloride colorimetric method. To an aliquot of 1ml of extract (1mg/ml) or standard solutions of Quercetin (10, 20, 40, 60, 80, 100µg/ml) methanol was added separately to make up the solution upto 2ml. The resulting mixture was treated with 0.1ml of 10% aluminium chloride, 0.1ml of 1M potassium acetate and 2.8ml of distilled water. Shaken well and incubated at room temperature for 30 minutes. The absorbance was measured at 415nm against blank, where a solution of 2ml ethanol, 0.1ml potassium acetate, 2.8 ml distilled water and 0.1ml of aluminium chloride serve as blank solution. The total flavonoid content was determined from the standard Quercetin calibration curve. And it was expressed as milligrams of Quercetin equivalents (QE) per gram of extract.

Determination of Total Phenolic Content[8]

The total phenolic content of the AEEA determined by Folin- Ciocalteau assay method. To an aliquot 100µg of AEEA or standard solution of Gallic acid (10, 20, 40, 60, 80, 100µg/ml)

added 0.5 ml of Folin-ciocalteau reagent and made into 2ml with distilled water and the mixture is incubated for 5min at room temperature. 0.1 ml of 20% sodium carbonate and 0.9 ml of distilled water were added to make the final solution to 3 ml. It was incubated for 30min in dark to complete the reaction. After that absorbance of the mixture was measured at 725 nm against blank. Distilled water was used as reagent blank. The tests were performed in triplicate to get mean the values. The total phenolic content was found out from the calibration curve of Gallic acid. And it was expressed as milligrams of Gallic acid equivalents (GAE) per gram of extract.

Vitro Antioxidant Studies of Extracts

Determination of Nitric Oxide Scavenging Assay[9]

The activity was measured according to the modified method of Sreejayan and Rao, To 4ml of the extract having different concentrations (100-500 µg/ml), 1ml of sodium nitroprusside (SNP) solution (5mM) was added and incubated for 2hr at 27oC. An aliquot (2ml) of the incubation solution was removed and diluted with 1.2ml of Griess reagent (1% sulfanilamide in 5% H3PO4 and 0.1% naphthylethylene diamine dihydrochloride). The absorbance of the chromophore was read immediately at 550nm and compared with standard, Ascorbic Acid.

Nitric oxide scavenging activity (%) = (Abs control − Abs sample) / (Abs control) X 100

Where, Abs (control): Absorbance of the control and Abs (sample): Absorbance of the extracts/standard.

Determination of Reducing Power Assay[10]

Various concentrations of the plant extracts in corresponding solvents were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 min whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. Control was prepared in similar manner excluding samples. Ascorbic acid at various concentrations was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

Determination of Lipid Peroxidation Assay[11]

Egg homogenate (10% in 1.15% potassium chloride, v/v) 0.1 ml and 1.0 ml of extract/standard (100-500µg) were mixed in a test tube and the volume was made up to 2ml, by adding distilled water. Finally, 0.5 ml FeSO4 (0.07 M) was added to the above mixture and incubated for 30min, to induce lipid peroxidation. Thereafter, 0.5 ml of 20% acetic acid (pH 3.5) and 0.5 ml of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl Sulphate) and 0.5ml 20% TCA were added, vortexed, and then heated in a boiling water bath for 60min. After cooling, 5.0mL of 1-butanol was added to each tube and centrifuged at 3000 rpm for 10min. The absorbance of the organic upper layer was measured at 532 nm. For the blank 1.0 ml of distilled water was used in place of the extract.

AI = (1-T/C) × 100

Where T = absorbance of Test, C = absorbance of fully oxidized control.

Screening of Angiogenesis Activity by Chorio Allantoic Membrane- CAM Model[12],[13]

Vascular network formed 7 days of incubated chicken eggs taken for the on day 8 of incubation the outer shell was wiped with 75% ethanol to sterilize the surface. Under aseptic conditions a tiny hole was made carefully in the egg shell with a needle and through the hole the test drugs are applied directly into CAM layer was then placed carefully on the CAM vasculature of the embryos labeled with the corresponding groups and the egg shell window closed with wax before incubation. Then the eggs again incubated same condition for another 72 Hrs, after 72 Hrs drug administered incubation the eggs was creaked the size, thickness, appearance of blood vessels is compared with control and test (n=3) group each group.

Anti-Microbial Studies (Bioautography)[14],[15],[16]

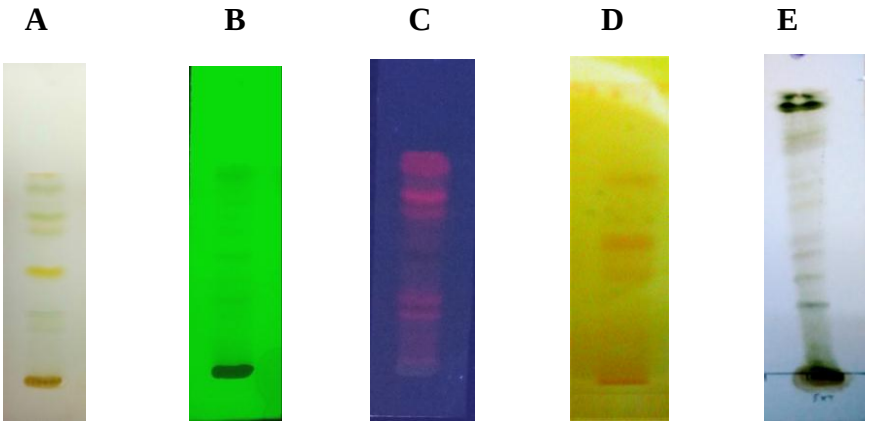
TLC silica gel 60 F254 is activated in hot air oven at 105o c for 30 min. the sample is applied on the activated TLC plate, allowed to dry then the plate is dried. Then plates are developed with solvent system Toluene: Ethyl acetate: Methanol (7:2:1). Then TLC plates allow dry to complete removal of solvents from the chromatogram. The chromatogram is used for next step. Then the developed chromatogram in inverted in placed on the surface media, allowed to diffuse for 5 Hrs and then chromatogram is removed from the plates without damaging the agar layer. Then inoculum is applied on the plates then incubated for 24 Hrs at 37^{o}c finally zone of inhibition is visualized.

RESULTS

The percentage yield of alcoholic extract of was *Elytraria acaulis* (L.fil) Lindau. Was found to be 6.24 % w/w. Preliminary phytochemical screening of AEEA, revealed the presence of following Phytoconstituents Alkaloids, Carbohydrates, Proteins & amino acids, Phenols, Tannins and Flavonoids. HPTLC Analysis of AEEA shows 9 compounds @ 520nm, 8 compounds @ 366 nm, 6 compounds @ 254 nm, (Fig No: 1) and their Densitometry Data (3D-Display) (Fig No: 2), AREA (AU) & Rf Value, visualization done for Flavonoids & alkaloids. (Table No: 1).

Table No: 1 HPTLC Densitometry Analysis Data of AEEA at various nm.

@520nm	**R_f Value**	0.19	0.3	0.37	0.46	0.55	0.66	0.73	0.8	0.92
	Area (AU)	267	233	1248	1506	2135	2900	2825	3385.9	14507
@366nm	**R_f Value**	0.18	0.3	0.32	0.36	0.55	0.73	0.8	0.92	
	Area (AU)	264	1001	733.6	1126	4191	2880	4490	11453	
@254nm	**R_f Value**	0.3	0.36	0.55	0.73	0.8	0.92			
	Area (AU)	215	318	635.9	661.4	1206	2624			
HCOH in H_2SO_4 Reagent		0.18	0.26	0.31	0.35	0.45	0.51	0.55	0.65	
Dragendorff Reagent		0.36	0.47	0.67						



A- HPTLC Chromatograms @ 520nm

B- HPTLC Chromatograms @ 366nm

C- HPTLC Chromatograms @ 254nm

D- Visualizations With Dragendorff Reagent

E- Visualizations With Formaldehyde In Sulfuric Acid Reagent

Fig. 1 Visualizations of HPTLC Chromatograms.

@520 nm **@366 nm** **@254 nm**

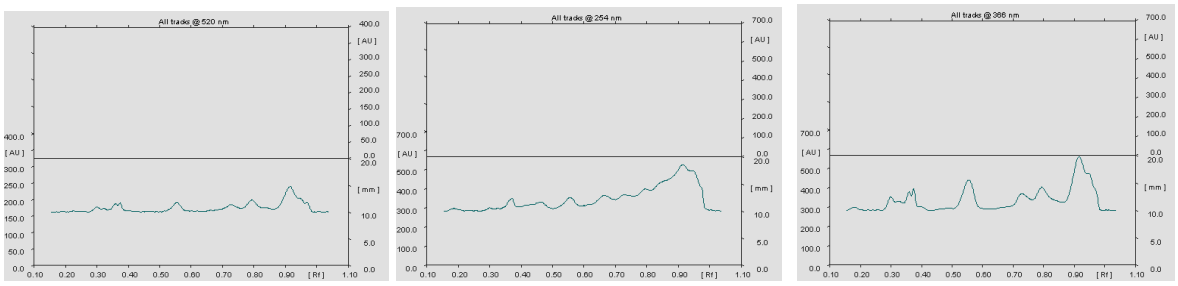


Fig. 2: 3D Display of Chromatogram @ various nm.

The Total Flavonoid content of the AEEA was determined by using Aluminium chloride colorimetric method & found to be 57.2 ± 0.26 mg Quercetin Equivalents / g Plant Extract. (Table No: 2 Fig No: 3).

Table 2: Determination of Total Flavonoid Content of AEEA.

Sample	Concentration µg/ ml	OD value	Amount Found
Standard (Quercetin) 1mg/ml	10	0.03	**57.2 ± 0.26** mg Quercetin Equivalents / g Plant Extract.
	20	0.09	
	40	0.26	
	60	0.5	
	80	0.78	
	100	1.05	
Test (AEEA) 1mg/ml	100	0.04	
	100	0.04	
	100	0.05	

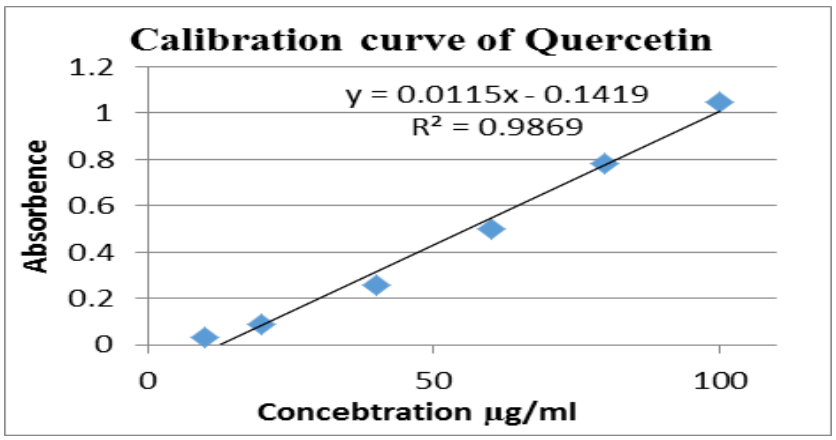


Fig. 3 Calibration curve of Quercetin.

The total phenolic content of the AEEA determined by Folin- Ciocalteau assay method & found to be 217.5 ± 0.5 mg Gallic acid equivalents /g plant extract. (Table No: 3, Fig No: 4).

Table 3: Determination of Total Phenolic Content of AEEA.

Sample	concentration µg/ ml	OD value	Amount Found
Standard (Gallic Acid) 1mg/ml	10	0.09	**217.5 ± 0.5** mg Gallic acid Equivalents /g Plant Extract.
	20	0.12	
	40	0.21	
	60	0.34	
	80	0.40	
	100	0.50	
Test (AEEA) 1mg/ml	100	0.13	
	100	0.13	
	100	0.11	

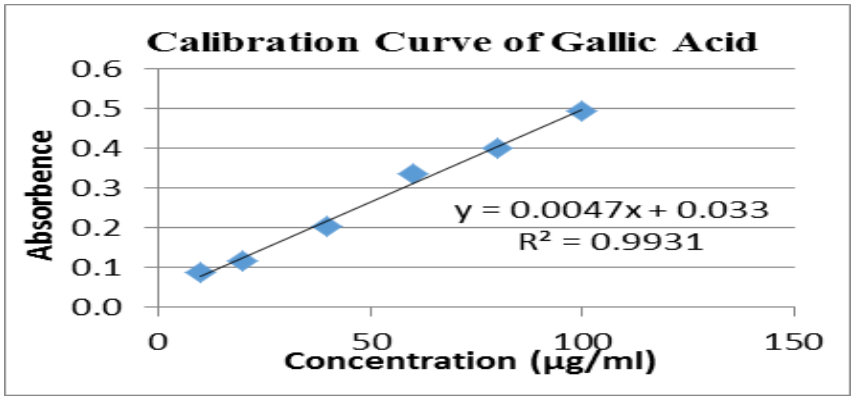


Fig. 4: Calibration Curve of Gallic Acid.

In-vitro antioxidant activity of AEEA was screened by three methods. In Nitric oxide Scavenging Assay. IC50 value of Standard Ascorbic Acid was found to be 12.58 µg/ ml and in that of AEEA is 294.18± 11.25 µg/ ml. (Table No: 4 Fig No:5).

Table 4: Determination of Nitric oxide Scavenging Assay.

S No	Concentration µg/ ml	% of Inhibition Ascorbic Acid	% of Inhibition AEEA	IC 50 Value of Ascorbic Acid µg/ ml	IC 50 Value of AEEA µg/ ml
1	100	88.38 ± 1.34	32.79± 1.22	12.58± 4.25	294.18± 11.25
2	200	90.40 ± 1.56	44.76± 1.38		
3	300	91.41 ± 1.52	49.99± 1.72		
4	400	92.93 ± 1.76	74.00± 2.24		
5	500	93.94 ± 1.49	90.00± 2.24		

Values are mean ± SEM of 3 replicates

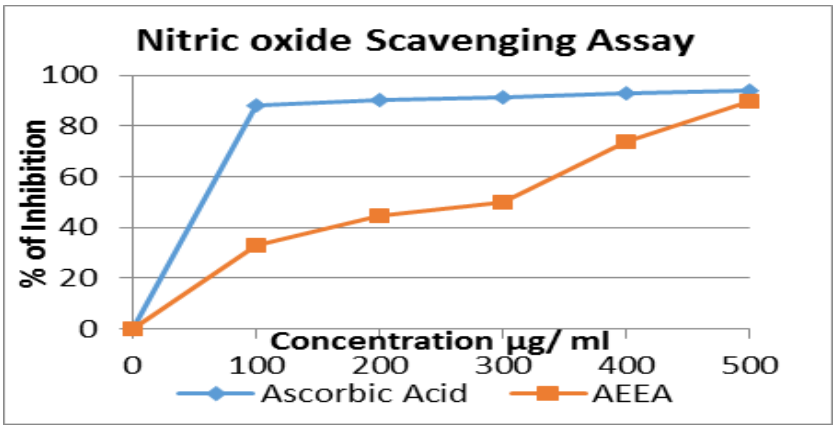


Fig. 5: Nitric oxide Scavenging Assay.

Reducing Power assay was also performed and the concentration Vs OD value are tabulated. Table No: the observation shows reducing power of our drug (AEEA) is 75% compared to standard drug (100%). (Table No: 5 Fig No: 6).

Table 5: Determination of Reducing Power Assay.

S No	Concentration µg/ ml	OD value of Ascorbic Acid	OD value of AEEA
1	100	1.21±0.06	0.44 ± 0.03
2	200	1.63±0.04	0.63 ± 0.06
3	300	1.66±0.09	0.98 ± 0.09
4	400	1.77±0.07	1.24 ± 0.02
5	500	1.89±0.08	1.44 ± 0.03

Values are mean ± SEM of 3 replicates

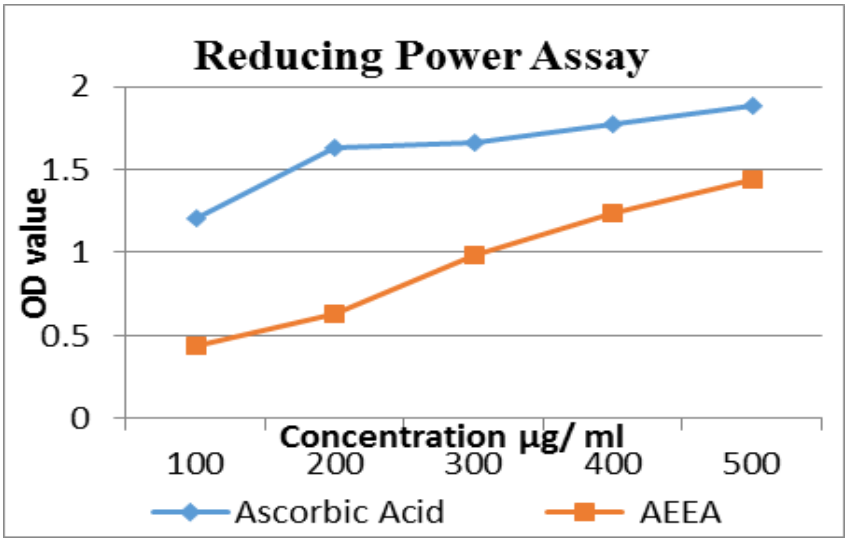


Fig. 6: Reducing Power Assay.

In Lipid Peroxidation assay percentage of inhibition and IC50 value are calculated. IC50 value of Standard Ascorbic Acid found to be 23.71± 2.12 µg/ ml and AEEA was 226.18± 4.04 µg/ ml. (Table No: 6 Fig No:7).

Table 6: Determination of Lipid Peroxidation Assay.

S No	Concentration µg/ ml	% of Inhibition of Ascorbic Acid	% of Inhibition of AEEA	IC 50 Value µg/ ml	IC 50 Value µg/ ml
1	100	70.59 ± 1.32	23.86 ± 2.37	23.71± 2.12	226 ± 4.04
2	200	77.31 ± 1.62	34.15 ±1.25		
3	300	82.35 ± 1.21	40.53 ± 1.61		
4	400	86.55± 1.45	53.08 ± 0.94		
5	500	90.76± 1.68	62.75 ± 0.90		

Values are mean ± SEM of 3 replicates

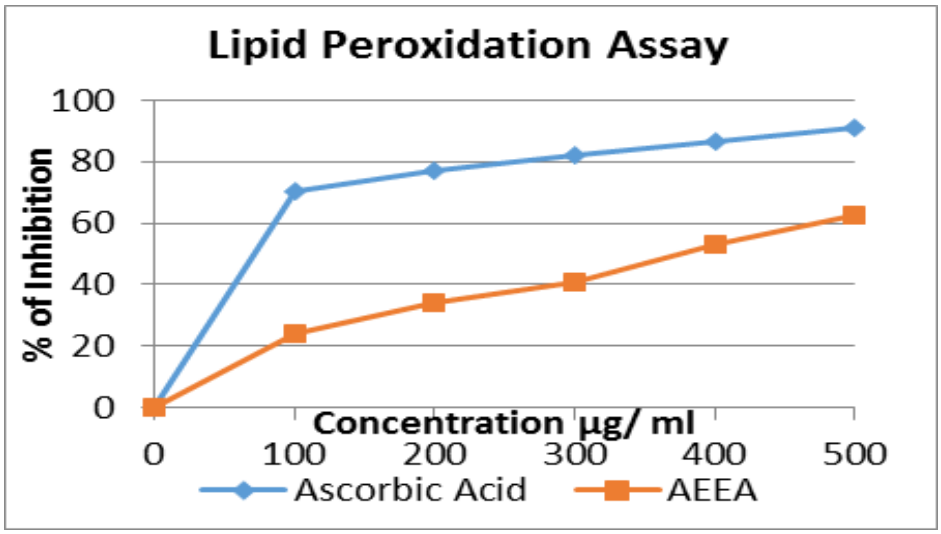


Fig. 7: Lipid Peroxidation Assay

CAM Model angiogenesis study the extract treated (100µl) group showed higher number of conspicuous blood vessels with greater thickness compared to control group. (Fig No: 8).

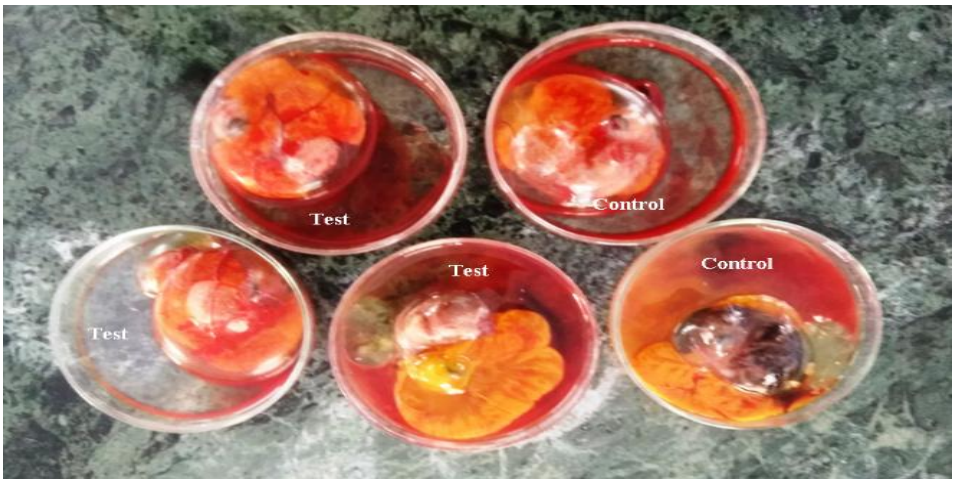


Fig. 8: CAM Model- Angiogenic Activity of AEEA.

Antimicrobial study was performed by Bioautography the Zone of Inhibition was correlated to Rf value HPTLC Analysis to corresponding Microorganisms. (Table No: 7 Fig No: 9).

Table No: 7: Contact Bioautography Rf Value of zone of Inhibition of AEEA.

Sample No	Micro Organism	zone of Inhibition Corresponding Rf Value
1	*Streptococcus pyogenes*	0.15, 0.47
2	*Staphylococcus aureus*	0.09, 0.66, 0.96
3	*Pseudomonas aeruginosa*	0.11, 0.46, 0.83
4	*Proteus vulgaris*	0.11, 0.63, 0.79
5	*Escherichia coli*	0.30, 0.59, 0.89
6	*Staphylococcus epidermidis*	0.55, 0.81

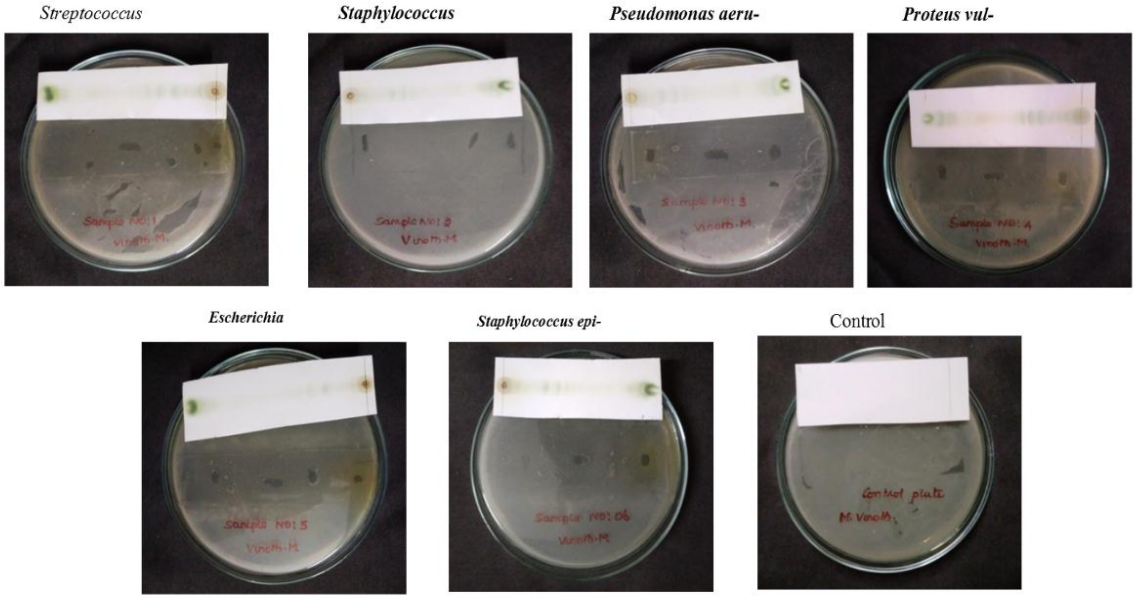


Fig. 9: Contact Bioautography of AEEA.

DISCUSSION

AEEA contains Alkaloids, Carbohydrates, Proteins & amino acids, Phenols, Tannins and Flavonoids. HPTLC screening studies showed the presence of 9 compounds at 520nm, 8 compounds at 254 nm and 6 compounds at 366 nm at wavelength. The Rf value obtained from the Dragendorff Reagent visualized TLC matched to HPTLC screening Rf values of 0.36, 0.47 & 0.67at 520 nm. Rf value obtained from Formaldehyde in Sulphuric acid Reagent visualized TLC matched to HPLTC screening Rf values of 0.19, 0.46, 0.55 and 0.65 at 520 nm, 0.18, 0.32, 0.35 and 0.55 at 366nm, 0.30, 0.36, and 0.55 at 254 nm florescence quenching seen to coincide with spots of Rf value 0.31(3rd spot), 0.35(4th spot) & 0.55(7th spot) in formaldehyde in sulphuric acid reagent identification, so they may be flavonoids. HPTLC study also confirm the presence of Alkaloids & Flavonoids. Estimation of Total Flavonoids Content of AEEA was 57.2 ± 0.26 mg Quercetin Equivalents / g Plant Extract. Total Phenolic content of AEEA 217.5 ± 0.5 mg Gallic acid equivalents /g plant extract. More than 5000 plant polyphenols have been identified and several of them are known to possess a wide spectrum of biological activities such as antioxidant anti-inflammatory, anti-microbial, anti-carcinogenic, anti-HIV, cardio protective and Neuron protective influence etc.[17] In view of their wide range of pharmacological activities they have a great therapeutic potential. In-vitro antioxidant activity of AEEA. In Nitric oxide Scavenging Assay. IC50 value of AEEA is 294.18± 11.25 µg/ ml. Reducing Power assay was also performed and the concentration Vs OD value the reducing power of our drug (AEEA) is 75% compared to standard drug. In Lipid Peroxidation assay IC50 Value of AEEA was 226.18± 4.04 µg/ ml. Results of antioxidants study shown AEEA have antioxidant activity. Oxygen is necessary for human life, it can also damage cells when certain chemical processes create oxygen free radicals.[18] Maintenance of redox homeostasis plays an important role in disease prevention. Oxidative stress is generated by unbalance between reactive oxygen species (ROS) and antioxidants. Excess of ROS leads to various biological reactions such as lipids, proteins and nucleic acids degradations thus may formation of oncogenes, mutagens formation, and induction of inflammation. Oxidative stress is suggested to play a major role in pathogenesis of cardiovascular diseases, Neuron degeneration, cancers, immune disorders, diabetes, aging, and others.[19] Angiogenesis is fundamental a highly regulated process biological processes for development, reproduction tissues and wound repair.[20] In this study shape, size, appearance, and number of blood vessels is compared with control group eggs the test group eggs shows the increased blood vessels thickness, and clearly visible and also shows the more breaches of blood vessels adhesion on the surface of shell. From the observation AEEA have

no irritant effect to fertilized chick (in vivo), and also AEEA having the significant angiogenesis property. The observation pictures are depicted. The main advantage of Bioautography is the fast and cost-effective screening of active constituent responsible for antimicrobial activity, and even it can be carried out in with a simple laboratory equipment's. In this study zone of inhibition & Rf values are correlated into HPTLC screening Rf values 0.30, 0.46, 0.55, 0.66, 0.80, and 0.92. Zone of inhibition of may be due to corresponding compound percent in Rf vales. The constituent's percent in extract possess antimicrobial activity against following most common wound pathogen such as *Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, Staphylococcus epidermidis.*

CONCLUSION

The results of present study shown alcoholic extract *Elytraria acaulis* (L.Fil.) Lindau having anti-oxidant, angiogenic and anti-microbial properties. Anti-oxidant property, angiogenic and anti-microbial activities promoting wound healing, So AEEA may have wound healing property, Further studies needed to confirm its wound healing property and also may have Ischemic cardio protective and Neuroprotective effect because AEEA having anti-oxidant property, angiogenic and anti-microbial activity other Pharmacological activity.

REFERENCES

1. Dibakar Mishra. Cattle wounds and ethnoveterinary medicine: A study in Polasara block, Ganjam district, Orissa, India. Indian Journal of Traditional Knowledge, 2013; 12(1): 62-65.
2. Deepak M. Kasote, Surendra S. Katyare, Mahabaleshwar V. Hegde, and Hanhong Bae. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. Int J Biol Sci, 2015; 11(8): 982–991.
3. N. Balasundram. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses, 2006; 99: 191–203.
4. Yongfeng Fan & Guo-Yuan Yang. 2007. Therapeutic Angiogenesis for Brain Ischemia: A Brief Review. J Neuroimmune Pharmacol, 2007; 2: 284–289.
5. Nasir B, Fatima H, Ahmed M and Haq IU. Recent Trends and Methods in Antimicrobial Drug Discovery from Plant Sources. Austin J Microbiol, 2015; 1(1): 1002.

6. MM Suleimana,a LJ McGaw, V Naidoo, and JN Eloff. Detection of Antimicrobial Compounds by Bioautography of Different Extracts of Leaves of Selected South African Tree Species. Afr J Tradit Complement Altern Med, 2010; 7(1): 64–78.
7. Yamunadevi Mariswamy, Wesely Edward Gnaraj, Johnson M. Chromatographic fingerprint analysis of steroids in Aerva lanata Linn by HPTLC technique. Asian Journal of Tropical Biomedicine, 2011; 6: 428-433.
8. Saumya S M & Mahaboob Basha P. In Vitro Evaluation Of Free Radical Scavenging Activities of Panax Ginseng And Lagerstroemia Speciosa: A Comparative Analysis. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3(1): 165-169.
9. Saumya S M & Mahaboob Basha P. In Vitro Evaluation Of Free Radical Scavenging Activities of Panax Ginseng And Lagerstroemia Speciosa: A Comparative Analysis. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3(1): 165-169.
10. P. Jayanthi and P. Lalitha. Reducing Power Of The Solvent Extracts Of Eichhornia Crassipes (Mart.) Solms. International Journal of Pharmacy and Pharmaceutical Sciences, 2011; 3(3): 126-128.
11. Rohan Sharadanand Phatak, Asha Krishnaji Pratinidhi, Anup Subhash Hendre. Evaluation of antioxidant and free radical scavenging activities of spices mixture extract as additive with reference to synthetic antioxidant. Der Pharmacia Lettre, 2015; 7(2): 27-34.
12. C.C Barua, A. Talukdar, S A Begum, D.K Sarma, D.C Pathak, A.G Barua and R.S Bora. Wound healing activity of Ethanolic extract of Alternanthera brasiliana Kuntz using in vivo and in vitro model. Indian Journal of Experimental Biology, 2009; 47: 1001-1005.
13. Lalitha Vaidyanathan, Devi Thanikachalam, Lokeswari T S Sivaswamy. Evaluation of Wound Healing Potency of Cassia auriculata Flower Extracts Using Chick Embryo Wound Model. International Journal of Pharmaceutical Sciences Review and Research, 2014; 27(2): 222-227.
14. Mounyr Balouiri, Moulay Sadiki, Saad Koraichi Ibnsouda. Methods for in vitro evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 2016; 6: 71-79.
15. Subramanion L. Jothy, Zuraini Zakaria, Yeng Chen, Yee Ling Lau, Lachimanan Yoga Latha, Lai Ngit Shin and Sreenivasan Sasidharan. Bioassay-Directed Isolation of Active Compounds with Anti-yeast Activity from a Cassia fistula Seed Extract. Molecules, 2011; 16: 7583-7592.

16. Nagaraja, B.K. R. Sripathy, C.N. Ramchand and M. Sundararaman. Bioautography Guided Identification of Anticandidal Compounds from A. terreus st. American Journal of Infectious Diseases, 2011; 7(4): 91-97.
17. Mohammad Asif & Elham Khodadadi. Medicinal uses and chemistry of flavonoid contents of some common edible tropical plants. Journal of Paramedical Sciences (JPS), 2013; 4(3): 119-138.
18. V. Lobo, A. Patil, A. Phatak, and N Chandra. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev, 2010; 4(8): 118–126.
19. Taibur Rahman, Ismail Hosen, M. M. Towhidul Islam, Hossain Uddin Shekhar. Oxidative stress and human health. Advances in Bioscience and Biotechnology, 2012; 3: 997-1019.
20. Manisha Bisht, D.C. Dhasmana, S.S. Angiogenesis: Future of pharmacological modulation. Bist. Indian J Pharmacol, 2010; 42(1): 2–8.