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Anti-melanogenic Potential of Acalypha indica Ethyl Acetate Fraction on Zebra fish Embryos

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Abstract

Acalypha indica is a very well-known medicinal plant that is widely used for treatment of skin diseases and other ailments. Many studies have explored the medicinal properties of the different extracts from this plant, only very little information is available on its anti-melanogenic activity. Hence the present study is focused on the anti-melanogenic activity of *Acalypha indica*.The study was carried out *in-vivo* in zebrafish model for anti-melanogenic activity and also to find out the cytotoxicity of the extract on A375 melanoma cell line as *in vitro* anti-melanogenic model. Sequential extraction was carried out on whole plant using methanol as solvent by cold percolation method. Various fractions was prepared using Butanol, Hexane, Chloroform and Ethyl acetate, Aq. Ethanol with the increasing polarity of the solvent. To screen the active fraction; quantification of secondary metabolite, *in-vitro* antioxidant activity and the anti-melanogenic activity was performed. The active fraction was chosen and the *in-vivo* anti-melanogenic activity was studied in Zebra fish. Among the various fractions, the total polyphenols and flavonoids was high in Ethyl acetate fraction. The *in-vitro* antioxidant activity was screened using DPPH and ABTS assay. The activity was high in Ethyl acetate fraction with the IC_{50} value OD 42.75 and 14.55 µg/ml for the DPPH and ABTS activity respectively. Cytotoxicity study on A375 melanoma cell line was performed to find out the bioactivity of the sequentially fractionated extracts and the activity was found to be high in ethyl acetate fraction with the IC_{50} value of 56.6 µg/ml. Therefore the *in-vivo* activity was performed using the Ethyl acetate fraction in Zebra fish. Compared to positive Control (Group IV) the test group (Group II & III) exhibited substantial anti-melanogenic activity in phenotype based evaluation. Quantification of ocular melanin content also showed dose dependent depletion of melanin pigment. Melanin content of zebra fish embryos (whole organism) also showed the significant depletion of melanin. The heart rate was recorded for 30S and no significant variations among the experimental groups (Group II, III, IV, V) in the heart rate was observed. To study the active compound present in the extract, LC-MS analysis was carried out. LC-MS analysis of the ethyl acetate fraction showed the presence of 41 compounds with potent biological activity. Therefore further study has to be performed to elucidate the potential activity of *Acalypha indica* against hyperpigmentation.

Keywords: *Acalypha indica*, hyperpigmentation, Zebra fish, Melanoma cell line, Melanin content.

Introduction:

Melanin is the major pigment highly present in humans which is responsible for the

skin, hair and eye pigment. The synthesis of melanin in the body is called as melanogenesis, in this process the melanocyte cells present in the dermal layer secrete the melanin pigment (1). Melanin pigments are classified in to three types namely eumelanin, pheomelanin and neuromelanin. The biosynthesis of eumelanin and pheomelanin origin from the same step which is conversion of tyrosine in to dihydroxyphenylalanine (DOPA) in the presence of co-factor namely tyrosine hydroxylase and tetrahydrobiopterin. DOPA will then convert it to dopaquinone with the help of tyrosinase enzyme (2). The common precursor for the production of eumelanin and pheomelanin is dopaquinone, which undergoes various reactions to produce these two pigments (3). The former pigment eumelanin is responsible for black and brown colour whereas pheomelanin synthesis gives red and alkali colour (4). Skin and hair colour is due to the rate of dispersion of melanin pigment from the melanocyte cells. In light skin the melanosome (melanocytes containing organelle) are smaller clumped randomly while in darker skin, the melanosome are larger in size. The vital function of melanin is to shield the skin from ultra violet rays (4).

Skin hyperpigmentation is mainly due to the abnormal synthesis and transportation of melanin pigment which leads to the dark color of the skin. Hyperpigmentation is due to various reasons including post inflammation, spots due to ageing solar lentigo and melisma (5). In the melanin biosynthesis, tyrosinase plays a two catalytic activity namely hydrolysis of tyrosine to L-DOPA and conversion of L-DOPA to DOPA quinone through the oxidation process. Thus the down regulation of tyrosinase is the targeted therapeutic activity for the hyperpigmentation problems (6). Currently available depigmenting agents including hydroquinone, arbutin show mutagenic effects when used for prolonged period of time (5). Screening of natural products for tyrosinase inhibition activity is an alternative treatment for the hyperpigmentation problems.

Acalypha indica comes under the family of Euphorbiaceae. This annual herb is commonly habituated in various countries such as Africa, South Africa, India and Srilanka (7). Traditionally this plant leaf is used externally in the treatment of ring worms and skin eruptions (8). Antibacterial activity of the plant fraction was studied for *Bacillus cereus* and *Aeromonas hydrophila.* Wound healing activity of this plant was studied in rats. Analgesic and anti-inflammatory activity of the methanolic leaf extract was studied against carrageenan-induced rat paw edema (9). With this scientific studies, the present study focused on the anti-melanogenic activity.

Materials and Methods:

Collection and authentication of plant

Extraction and fractionation of plant material: The coarse grounded plant material was extracted using methanol by cold percolation method. After 48 hours, the solvent was filtered and condensed using the rotary evaporator. The dried crude extract obtained was dissolved in water and fractionated using Butanol, Hexane, Chloroform, Ethyl acetate and Ethanol solvent with the increasing polarity of the solvent. The solvent was removed using rotary evaporator and the fraction was collected.

Phytochemical screening: The secondary metabolite in the respective fraction was qualitatively screened using the standard method.

Quantification of secondary metabolites

Estimation of polyphenol : 500 µg of the test samples Butanol fraction (B), (Hexane fraction (H), Chloroform fraction (CH), Ethyl acetate fraction (E) and Aq. Ethanol fraction (Aq.E) was dissolved with 0.5 ml of water and 0.2 ml of Folin-Ciocalteu's phenol reagent (1 : 1). After 5 min of incubation, 1 mL of saturated sodium carbonate solution (8% w/v in water) was added to the mixture and the volume was made up to 5 mL with distilled water. The reaction mixture was incubated in the dark for 30 min and the absorbance of blue color from

different samples were measured at 765 nm. The phenolic content was calculated as Gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of Gallic acid (10-320 µg/ml) (10).

Estimation of total flavonoids: 500µg of each test sample (H, CH, Aq. E, E, and B) was mixed with 0.5 mL of 2% $AlCl_3$ ethanol solution. After one hour of incubation at room temperature, the absorbance was measured at 420nm. Formation of yellow colour indicated the presence of flavonoid. A calibration curve was constructed, using quercetin (10 to 320 µg/ml) as standard. Total flavonoid contents were expressed equivalent to quercetin (mg/g) using the following equation based on the calibration curve: y = 0.0017x + 0.1365, where y was the absorbance (10).

In-vitro antioxidant activity of various fractions

DPPH scavenging activity: DPPH radical scavenging assay of test samples (H, CH, Aq.E, E and B) were done by modified method (11). In brief, 0.135 mM DPPH was prepared in methanol. Different concentrations (5, 10, 20, 40, 80, 160 and 320 µg/ml) of fractions were mixed with 2.5 ml of DPPH solution. The reaction mixture was vortexed thoroughly and kept at room temperature for 30 min. The Absorbance of the mixture was measured at 517 nm. Ascorbic acid was used as the reference standard. The ability of the sample to scavenge DPPH radical was calculated from the following formula:

% DPPH inhibition = [(OD of control - OD of test)/(OD of control)]×100

ABTS radical scavenging activity: ABTS radical scavenging assay of sample (B, H, CH, E and Aq. E) were performed according to the modified method (11). The ABTS (7 mM, 25 ml in deionized water) stock solution was prepared with potassium persulfate ($K_2S_2O_8$) (140 mM, 440 µl). Different concentrations of test samples and standard (Ascorbic acid) were mixed with the ABTS working solution (2.0 ml) and the reaction mixture was allowed to stand at room temperature for 20 min; then, the Absorbance was measured using an ultraviolet-visible spectrophotometer at 734 nm. The radical scavenging activity was given as ABTS radical scavenging effect. ABTS radical scavenging effect was calculated based on the formula

(%) = [(A0 - A1)/A0] ×100. Where, A0 is the control; A1 is the test

Maintenance of cell lines: Melanoma (A375) cell lines were procured from NCCS, stock cells was cultured in medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cell was dissociated with TPVG solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells were checked and centrifuged. Further 50,000 cells / well was seeded in a 96 well plate and incubated for 24 hrs at 37°C, 5% CO_2 incubator.

MTT assay: The monolayer cell culture of A375 melanoma cell line was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using respective media containing 10% FBS. In the 96 well microtiter plate, approximately 50,000cells/well was seeded and incubated. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations (20, 40, 60, 80 and 100 µg/ml) of all the fractions were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100µl of MTT (5 mg/10 ml of MTT in PBS) was added to each well. The plates were incubated for 4h at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed

to inhibit cell growth by 50% (IC_{50}) values is generated from the dose-response curves for each cell line (12). The same procedure was also performed on Vero cell line which served as control.

Estimation of anti-melanogenic activity of acalypha indica ethyl acetate fraction on Danio rerio (zebra fish) embryos

***Acclimatization and breeding of zebra fish*:** Zebra fish was procured from the local suppliers and housed in a home tank with the population density of each 1 fish per litre. The adult fishes were acclimatized for 12 days to the laboratory condition before breeding. On prior to the day of breeding, the male and female fishes were transferred to breeding tank in the ratio of 2:1. When the spawning was completed, eggs were gently procured from the breeding tank (13).

Grouping and dosing: The highly fertilized eggs were incubated with various concentration of ethyl acetate fraction in 96 well plate containing embryonic medium. The fertilized eggs were treated for 48 hours and the plate was stirred occasionally to ensure proper mixing and distribution of the fraction in the embryonic medium.

Group I: 100µg/ml of ethyl acetate fraction

Group II: 50 µg/ml of ethyl acetate fraction

Group III: 10 µg/ml of ethyl acetate fraction

Group IV: 0.2 mmol/ L 1-phenyl-2- thiourea (PTU), which served as positive control

Group V: only dilution water, which served as negative control.

Phenotypic evaluation of melanogenic inhibitory activity: Alteration in the development of embryo, changes in pigmentation and mortality of the embryo was noted. Melanin content, ocular melanin content of the treated embryos was estimated using IMAGEJ analysis software.

Melanin contents of zebrafish embryos: Embryos were digested in 1 N NaOH at 100°C and then vigorously vortexed to solubilize the melanin pigment. Optical density of the supernatant was measured at 405 nm (13).

Determination of melanogenic inhibitor's effects on heart rate: Compound toxicity of melanogenic inhibitors were determined by measuring the heartbeats of randomly selected 6 embryo per group for 30 S per 30 S were recorded at (27 ± 1°C) using an stereomicroscope. The Mean ±S.E / group were calculated. Tricaine (100 µg/ml in E3 media) was used to immobilize larvae (14).

LC-MS analysis: The number of compounds present in the ethyl acetate fraction was determined using LC-MS analysis. This analysis was performed using Thermo Scientific Vanquish instrument. The injection volume of the sample is 5µl and the solvent system used in pump A is 0.1% of formic acid in water, in pump B it is Methanol and pump C is Acetonitrile. The pump flow in the column is 6.00 ml/min^2. The mass fragments were compared with spectrum database.

Results and Discussion:

Qualitative phytochemical screening: Phytochemical analysis of all the fractions like n-hexane, ethyl acetate, chloroform, aq.ethanol and butanol showed the presence of various secondary metabolites. Among the various secondary metabolites, the ethyl acetate fraction contains flavonoids, phenols, steroids

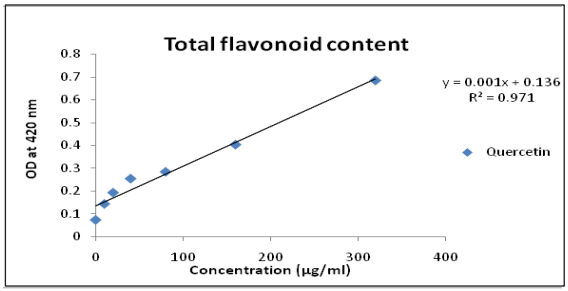
Table 1: Phytochemical screening of various fraction

Secondary Metabolites	Ethyl acetate	Butanol	Chloroform	Hexane	Aq. Ethanol
Alkaloids	-	-	+	+	-
Saponins	-	-	+	+	+
Tannins	-	-	-	-	-
Glycosides	-	-	+	+	+
Flavonoids	+	+	+	-	-
Phenols	+	+	+	+	+
Steroids	+	+	+	+	+
Terpenoids	+	+	+	+	+
Quinones	-	-	-	-	-
Proteins	-	+	+	-	+

and terpenoids. Chloroform fractions shows the presence of alkaloids, Saponins, Glycosides, flavonoids, phenols, steroids, terpenoids and proteins. Quinones and tannins were absent in all the fractions (Table 1).

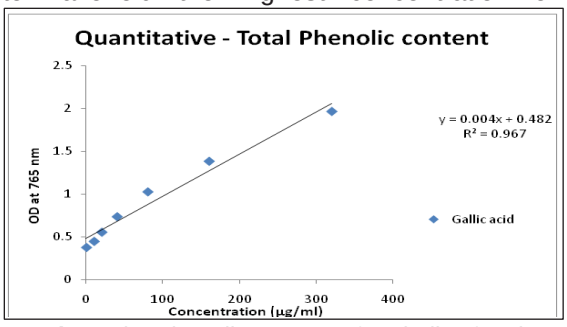
Quantification of secondary metabolites

Total flavonoid content: Total flavonoid content in the test samples were quantified using the calibration curve of standard Quercetin. Ethyl acetate fraction contains highest flavonoid content 165.00 mg/ml followed by Butanol (34.21 mg/ml), Chloroform fraction (27.74 mg/ml), Aq. Ethanol fraction (22.84 mg/ml) and Hexane fraction (15.78 mg/ml) of quercetin equivalents/g dry weight of the *fraction.*



Graph 1: Total flavonoid contents were expressed equivalent to quercetin (10-320 µg/ml) using the following equation based on the calibration curve: y = 0.0017x + 0.1365, where y was the absorbance.

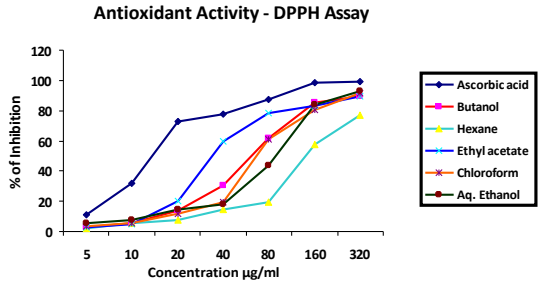
Total phenolic content: Total phenol content in the plant fractions was quantified using the calibration curve of Gallic acid. Similar to flavonoid the highest concentration of phenolic content was present in Ethyl acetate fraction (120.57 mg/ml). Aq. Ethanolic fraction contains very least phenolic content (2.89 mg/ml) followed by Hexane fraction (6.36 mg/ml). Butanol fraction contains 69.76 mg/ml and Chloroform fraction contains 41.25 mg/ml of Gallic acid equivalents/g dry weight of fraction.



Graph 2: The phenolic content of *A. indica* fractions was calculated as Gallic acid equivalents GAE/g of dry plant material on the basis of a standard curve of Gallic acid (10-320µg/ml)

In-vitro antioxidant activity

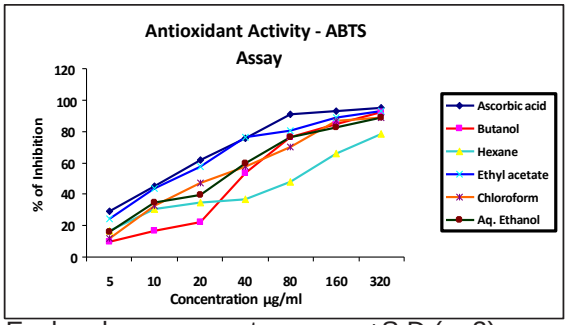
DPPH Radical Scavenging activity: The antioxidant activity of the various fractions were studied using the free radical DPPH (Graph 3).



Each value represents a mean±S.D (n=3).
Graph 3: DPPH scavenging activity of various fraction. Ascorbic acid was used as a standard.

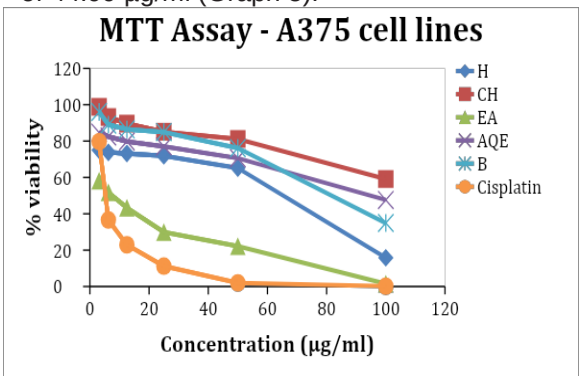
Chloroform fraction showed best IC_{50} value 42.55 µg/ml followed by Butanol fraction 57.36 µg/ml. Hexane fraction showed less activity among all the other fraction with the IC_{50} value of 154.98µg/ml. Ethyl acetate and Aq. Ethanol fraction showed moderate activity with the IC_{50} value of 63.31, and 68.10 µg/ml. The standard ascorbic acid showed the IC_{50} value of 16.77 µg/ml.

ABTS Scavenging activity: The antioxidant activity of the fraction was studied based on the decolorization of ABTS free radical. Among all the fraction, Chloroform fraction showed best IC_{50} value (14.75 µg/ml). Ethyl acetate and Aq. ethanol fraction showed the IC_{50} value of 28.37 and 27.53 µg/ml respectively. Hexane fraction showed least scavenging activity with the IC_{50} value of 59.43 µg/ml and Butanol fraction showed the scavenging activity with the IC_{50} value of 38.65µg/ml. The ABTS scavenging activity was high when compared with DPPH activity. (Graph 4)



Each value represents a mean±S.D (n=3).
Graph 4: ABTS radical scavenging activity of various fraction. Ascorbic acid was used as a standard.

Cytotoxicity activity in A375 melanoma cells: The cytotoxicity effect against A375 Melanoma cell lines were studied for all the fractions at different concentrations (20, 40, 60, 80 and 100 µg/ml) using MTT assay. Among the fractions studied, Ethyl acetate fraction inhibited the cell proliferation with the IC_{50} value of 56.6 µg/ml followed by Hexane fraction with the IC_{50} value of 44.99 µg/ml (Graph 5).



Graph 5: Cytotoxic effects of various fraction against A375 melanoma cells. Cell viability was calculated and ethyl acetate fraction showed potent cytotoxic effect on A375 melanoma cells.

Toxicity study in Vero cell line: The cytotoxicity effect against Vero cell lines were studied for all the fractions at different concentrations (20, 40, 60, 80 and 100 µg/ml) using MTT assay. The fractions tested did not show any cytotoxicity on Vero cell line. The cells showed distinct morphology. No cell distortion or rounding of cells were observed in all the fractions tested.

Anti-melanogenic activity of A. indica ethyl acetate fraction in zebra fish embryos: To further evaluate the anti-melanogenic property of *A. indica* ethyl acetate fraction, *in-vivo* study was performed on Zebra fish embryo. In Group I; 100% mortality was observed and in group II and III the mortality was observed in 33 and 55 hour of post fertilization of zebrafish embryo. Similarly delay in hatching was observed in group II, III and IV. There was dose dependent melanin pigment depletion observed in treated embryos. Compared to Positive Control (Group IV) the test group (Group II & III) exhibited substantial anti- melanogenic activity in phenotype based evaluation. Quantification of ocular melanin content also showed dose dependent depletion of melanin pigment. Melanin content of whole zebra fish embryos also showed significant depletion of melanin. The heart rate was recorded for 30S and no significant variations in heart rate were observed among the experimental groups (Group II, III, IV, V).

Table 2: Heart rate of Experiment al Embryo

	Group I	Group II	Group III	Group IV	Group V
Fish 1	mortality	74	74	74	76
Fish 2	mortality	72	75	73	74
Fish 3	mortality	mortality	73	75	75
Fish 4	mortality	mortality	74	73	75
Fish 5	mortality	mortality	76	74	76
Fish 6	mortality	mortality	74	72	74
Mean		73.000	74.333	73.500	75.000
Std. Dev		1.000	0.943	0.957	0.816

LC-MS *analysis of ethyl acetate fraction*: LC-MS analysis of ethyl acetate fraction showed the presence of 41 compounds. The important compound with potent biological activity was shown in table 3.

Table 3 :LC-MS analysis of A.indicaethyl acetate fraction

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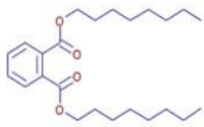
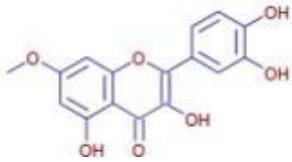
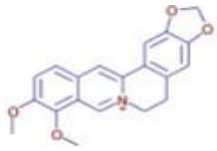
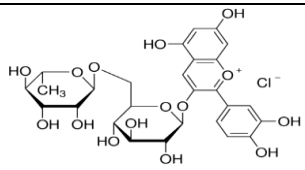
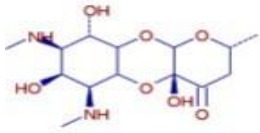
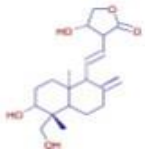
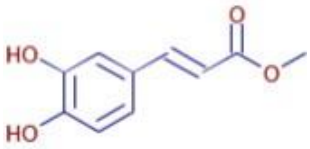
LC-MS Data of Acalypha indica ethyl acetate fraction

S.No	Compound	Structure	Biological Activity
1	Dioctyl phthalate		Anti-microbial activity and pro-inflammatory activity (20).
2	(-)-Erythromycin		Potent antibiotic against gram positive bacteria (21).
3	Rhamnetin		Flavonoid compound which possess anti-bacterial, anti-cancer and anti-inflammatory activity (22).
4	Berberine		isoquinoline alkaloids. Possess anti-bacterial activity, anti-diabetic, anti-cancer, neuroprotective, anti-inflammatory and anti-atherosclerotic actions (23)
5	Keracyanin		Anthocyanin. Shows potent anti-oxidant activity (24) .
6	Spectinomycin		Anti-bacterial activity against gram negative bacteria (25)
7	Andrographolide		Anti-inflammatory, antiallergic, anti-platelet aggregation, hepatoprotective, and anti-HIV activities (26).
8	Methyl caffeate		Anti-bacterial and Anti-mycobacterial activity (27).

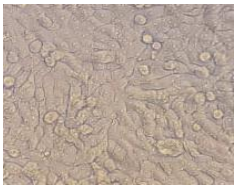
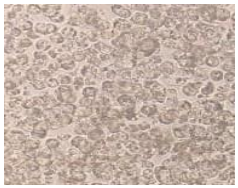
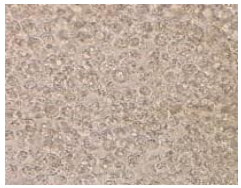
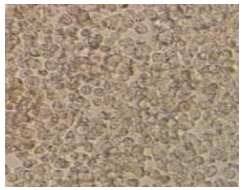
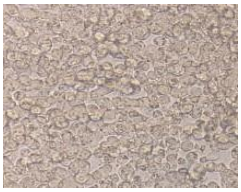
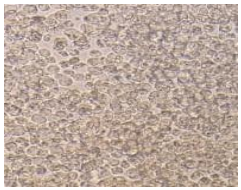
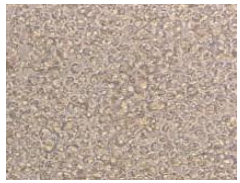
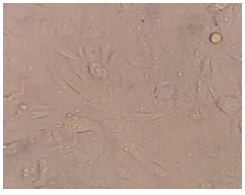
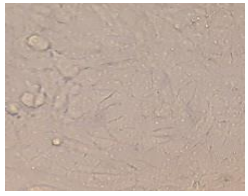
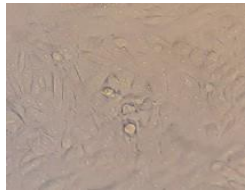
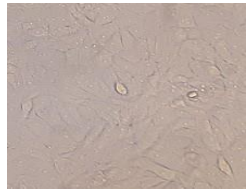
Control	Hexane fraction	Chloroform fraction	Ethyl acetate fraction
			
	(100 µg/ml)	(100 µg/ml)	(100 µg/ml)
Aqueous Ethanol fraction	Butanol fraction	Cisplatin	
			
(100 µg/ml)	(100 µg/ml)	(100 µg/ml)	

Fig 1: Phase contrast microscopic images of A375 melanoma cells. In control cells distinct morphology was observed. In treated cells, distorted cell morphology was observed.

Control	Hexane fraction	Chloroform fraction	Ethyl acetate fraction
			
	(100 µg/ml)	(100 µg/ml)	(100 µg/ml)

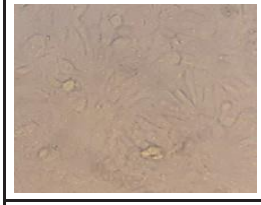
Aqueous Ethanol fraction	Butanol fraction
	
(100 µg/ml)	(100 µg/ml)

Fig 2:Phase contrast microscopic images of Vero cell line treated with different fraction at 100 µg/ml. Normal cell morphology was observed in control and treated cells.

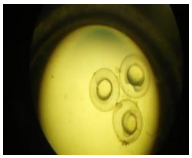
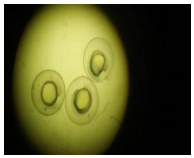
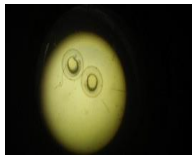
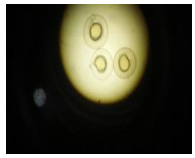
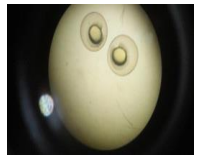
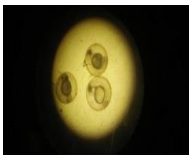
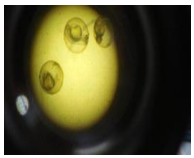
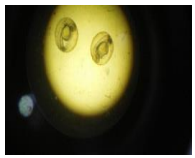
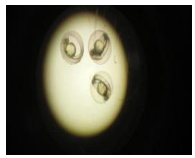
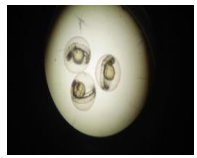
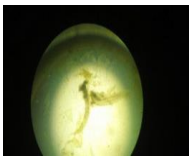
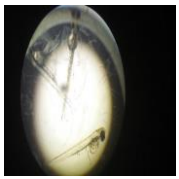
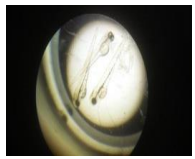
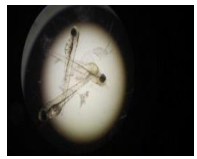
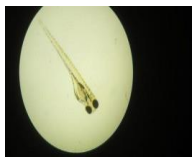
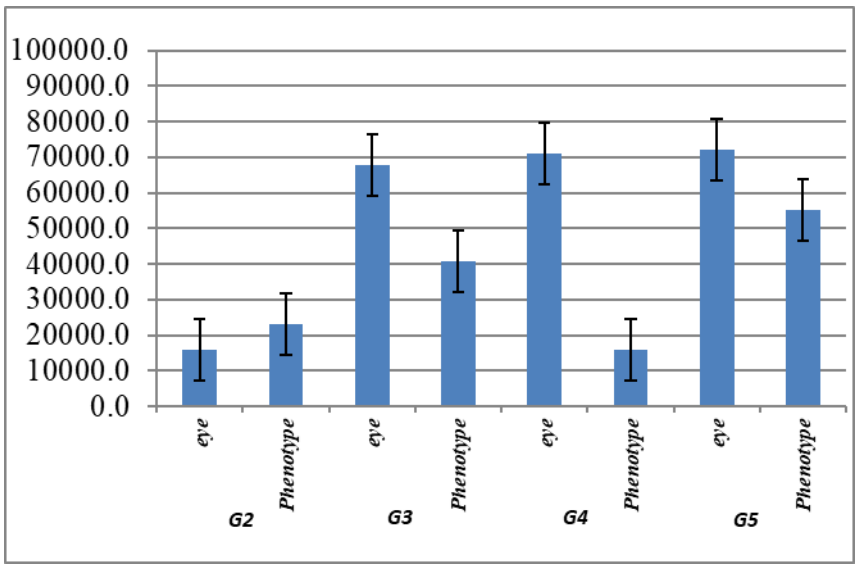
	Group I	Group II	Group III	Group IV	Group V
7 HPF					
31 HPF					
55 HPF					
127 HPF					

Fig 3: Anti-melanogenic activity of the *A.indica* ethyl acetate fraction on zebra fish embryos



Graph 6: Phenotype & ocular based melanin evaluation

Cosmetic industries are focused on the development of various products towards hyperpigmentation. Natural products development are found to be the area of interest for exploring various properties of plants for human use. One of such property under study is anti-melanogenic or anti hyperpigmentation property. Hyperpigmentation is mainly due to the upregulation of melanin production due to prolonged exposure of sun, melasma and other skin disorders. Melanin biosynthesis can be inhibiting by preventing exposure of UV, impeding the metabolism of Melanocyte and downregulating the tyrosinase enzyme activity (15). Inhibition of tyrosinase enzyme is the key research in the cosmetic industry and dermatology field to treat the skin pigmentation disorders.

The present study focused on the anti-hyperpigmentation property of Acalypha indica. In Tamilnadu, the plant is commonly called as Kuppaimeni and the leaf is traditionally used to treat the sore gums and skin disease like scabies (16). The study showed the presence of various secondary metabolites in present in the plant. Fractionation process was carried out using various solvents and all the fraction showed the presence of polyphenols which was concordant with the previous fraction (Chekuri et al., 2016). Free radicals provoke the hyper pigmentation process thus studies are carried out to explore the role of antioxidants in suppressing hyperpigmentation activity (17). *In-vitro* antioxidant studies such as DPPH and ABTS assay done using the various fractions showed good scavenging activity. When compared with DPPH, the radical scavenging activity was more effectively exhibited by ABTS assay on all the fractions tested and the IC_{50} value for ethyl acetate was 28.37 µg/ml. Cytotoxicity of all the fraction was studied in A375 melanoma cell line in order to study the impact of the fractions on melanin producing cells. The cell viability was dose dependently reduced in ethyl acetate fraction treated cells. The result was concordant with previous study where the ethyl acetate fraction showed stronger effects when compared to the other fraction of *V. Odorata.* The activity is based on the presence of secondary metabolites in ethyl acetate fraction which usually contains the medium polarity compounds like polyphenols and flavonoids (18).

In order to confirm the antimelanogenic activity, further studies were carried out in the zebrafish embryos. Zebra fish is an ideal model to study the anti-melanogenesis because the eggs are transparent which is helpful for visualization and easy to handle (19). The result provides the evidence that the exposure of embryos to Ethyl acetate fraction of *Acalypha indica* induces mortality up to the 50 µg/ml concentration within 48 hours of exposure. In the entire test concentration exposed groups delayed hatching was observed when compared with the control group. The results of Phenotype based evaluation, Estimation of ocular melanin content, and Melanin contents of entire zebrafish embryo showed dose dependent anti-melanogenic effect on *Acalypha indica* ethyl acetate fraction exposed embryos. The *Acalypha indica* ethyl acetate fraction exhibited significant anti- melanogenic effect compared to the positive control, phenylthiourea (PTU). Determination of toxicity of melanogenic inhibitors effects on heart rate showed that *Acalypha indica* does not significantly affect the heart rate.

LC-MS analysis showed the presence of different secondary metabolite including flavonoids, phenolics and anthocyanin. The presence of these compounds helps in the potent antimelanogenic activity of *Acalypha indica* ethyl acetate fraction.

Conclusion:

This study evaluated the antimelanogenic activity of *Acalypha indica* in zebrafish and cytotoxicity on A375 melanoma

cell line. The total phenolic and flavonoid content of the fraction was quantified and *in-vitro* anti-oxidant activity was studied. The ethyl acetate fraction exhibited dose dependent anti-melanogenic activity in zebra fish model which was estimated by ocular melanin content, phenotype based evaluation and quantitative estimation of melanin content of zebra fish embryo. Future prospects of the study would be; Isolation of potent antimelanogenic compound from ethyl acetate fraction and studying the mechanism of antimelanogenesis.

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