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Evaluation of Anti Angiogenic Activity of Iron Nano Particles of Catharanthus Roseus

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ABSTRACT:

Catharanthus roseus is one of the plant well recognized in ayureveda. It is known for its antitumour, antimicrobial, anticancer activities it mainly consist of vincristine,vinblastine for treatment of various cancers.

Biosynthesis of iron oxide nanoparticles from C.roseus whole plant extract was carried out and their characterization as well as antiangiogenic activity were evaluated, color change,uv –visible spectron,Zp,DLS assements supported the biosynthesis and characterization of Feo_3 Mps .The invivo assement of anti angiogenic activity in Feo_3Mps treated zebra fish revalered their effectiveness in reducing a angiogensis it may useful for treatment to angiogensis.

Key Words:

Catharanthus roseus, ironoxide nanoparticle vincristine vinblastine Zp, DLS,UV-visible spectra.Anti angiogenic ,Anticancer activity.

INTRODUCTION:

A Natural product is a chemical organic compound, found in the nature, produced naturally by a living organism. These compounds possess pharmacological and biological activity, to combat various diseases. Human societies used natural products, since millennia. Natural products are the active components of many traditional medicines as well as modern medicines. They also have a great effect on culture of human's and they have been used throughout human history as condiments, pigments, and pharmaceuticals. They are lead compounds for drug discovery and the current importance of drugs from natural origin is undebatable.

AIM AND OBJECTIVES:

AIM:

The main aim of this study is invitro investigation of anti-angiogenic activity of ironoxide nanoform (colloidal) of methanol and ethanol extract of catharanthus roseus whole plant.

OBJECTIVES:

- To carry out the extraction process for the selected medicinal plant.
- To synthesis herbal mediated ironoxide nanoparticales using extracts of selected medicinal plant.
- To characterize the synthesized herbal mediated iron oxide nanoparticales using various

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characterization techniques.

- To evaluate the in-vivo anti-angiogenic activity of herbal mediated ironoxide plant extracts.
- Zibra fish model.
- Zibra fish fin generation model.

PLAN OF WORK:

- Literature survey, selection, collection, and authentication of the plant material.
- Extraction, preliminary phytochemical screening.
- Synthesis of ironoxide nanoparticals using whole plant catharanthus roseus
- Characterization of ironoxide nanoparticles with various techniques –
- UV
- DLS
- Zeta potential
- Evaluation of anti-angiogenic activity of ironoxide nanoparticals using whole plant of catharanthus roseus.

4. MATERIALS AND METHODS:

4.1. Materials:

Drugs, chemicals, solvents, plant, animals.

The entire chemicals used are of analytical grade and purchased from standard manufactures. Catharanthus roseus,

- Ethanol,
- Methanol,
- Acetone,
- Chloroform,
- Ferrous chloride,
- Ferric chloride,
- Zibra fish
- Lidocane

4.2. Plant Collection:

Collection and preparation of crude extracts of selected plants

The plant material of catharanthus roseus whole plant was collect during the month of December 2018 at JNTUH, kukatpally (village), malkajgiri-medchal (district), Telangana.

4.3. Drying and Powdering Of Plant Material:

- The whole plant were collected and shade dried completely for about 2-3 weeks.
- Then grind into powder.
- The powder was weighed using and electronic balance.

4.4. List of Instruments Used In Experiments:

- Macerator
- Rota evaporator
- Magnetic stirrer
- Magnetic beeds
- UV-visible spectroscopy
- Ultra sonicator
- INVESTIGATION AL DANIO RERIO
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4.5. Extractive Values:

The extractive value determines the amount of active chemical constituents extracted with solvents from a given amount of medicinal plant materials and herbal formulation.

PROCEDURE:

Accurately weighed 5g of the crude drug of catharanthus roseus was coarsely powdered and placed in a 250ml of conical flask and was macerated with 100ml of the solvent about 24hrs, shaking frequently for the first 6hrs and allowed to stand for 18hrs. The resultant mixture was filtered, from this taken 25ml of the filtrate. This filtrate was evaporated to dryness in a tarred porcelain dish and dried at 105℃. The dried residue was weighed and evaluated the percentage of W/W extractive value with reference to the air dried drug.

Extractive yield (%)W/W = W2-W1/weight of the drug taken X 100

Where,

W1 = weight of the empty china dish

W2 = weight of the empty china dish + residue

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4.6.Extraction Method :

1. Maceration : (methanol)

The 400g of catharanthus roseus drug was taken into macerator. And add 2000ml of methanol. After 2 weeks the extracted solution was filtered and further concentrated under reduced pressure by using rota evaporator.

2.Maceration : (ethanol)

The 400g of catharanthus roseus drug was taken into macerator. Then add 2000ml of ethanol. After 2 weeks the extracted solution was filtered and further concentrated under reduced pressure using rota evaporator.

Method 3:

Regeneration of Zebrafish fin assay:

- The adult fishes were obtained from the local suppliers.They were kept for feeding for two weeks,fishes were fed 2 times a day with coninues supply of air(oxygen).
- On the day of experiment the fishes were taken out from their tanks and placed in a 250ml of beaker containing 150ml of water.
- On experiment day fishes were taken out and given local anesthesia(lignocaine) given lignocaine-3.5ml in 250 ml of water.
- Then take the fishes and keep in the anesthetized water after 2min fishes were anesthetized and it was taken out immediately.
- If we slow to pick the fishes from anesthetized water then it will die.Here we have to be very carefull.
- Then keep the fishes in watch glass containing 30% of anesthetized water and 70% of fish water(normal water).
- Then their fin was cutted upto 50% br using razor blade or surgical scissor and imaged or seen under microscope.
- Here pre and post amputation images were collected before transferring the fishes into recovery beaker containing fish water.
- Test drug and standard drug were given to different groups and normal group will be maintained in similar conditions as like test and standard group.
- The drug is given in 150ml of water which is placed in 250ml of beaker .
- The fish water is changed every alternate day and dose was renewed.
- And it will be continues till 30 days till we get fully regenerated of zebrafish fin.
- The images were collected every 10^{th} day 20^{th} day 30^{th} day. The area of regeneration of zebrfish were calculated by using imagej software.
- By this software we get percentage regeneration was calculated and significance was obained satistically.

5. RESULTS AND DISCUSSIONS:

Extractive values:

Table 5.1.Determination of extractive values for useful for the evaluation of crude drugs

S.No	solvent	Color/consisitency	Extractive yield% w/w
1	Pet.ether	Blakish green/semi solid	2.48
2.	Chloroform	Dark blackish green / semi solid	3.8
3.	Ethyl acetate	Light blackish / semi solid	2.3
4.	Acetone	Blackish green / semi solid	4.3
5.	Ethanol	Deep blackish green liquid	12.5
6.	Methanol	Dark blackish green / liquid	11.6

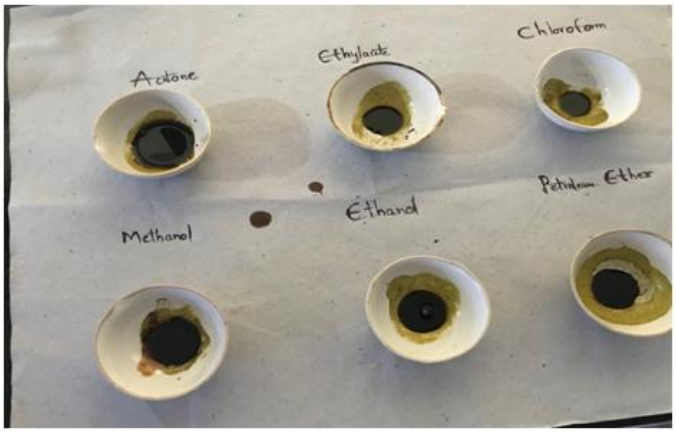

Figure 5.1: Crude extract with different solvent systems

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5.1.Preliminary phytochemical screening:

Table 5.2.Phytochemical screening was performed using standard procedures

S.no	Phytochemicals	Methanol	Ethanol
1	Carbohydrates	+	+
2.	Alkaloids	+	+
3.	Glycosides	-	+
4.	Tannins	+	+
5.	Flavonoids	+	+
6.	Saponins	+	+
7.	Steroids	-	-
8.	Terpenoids	-	-

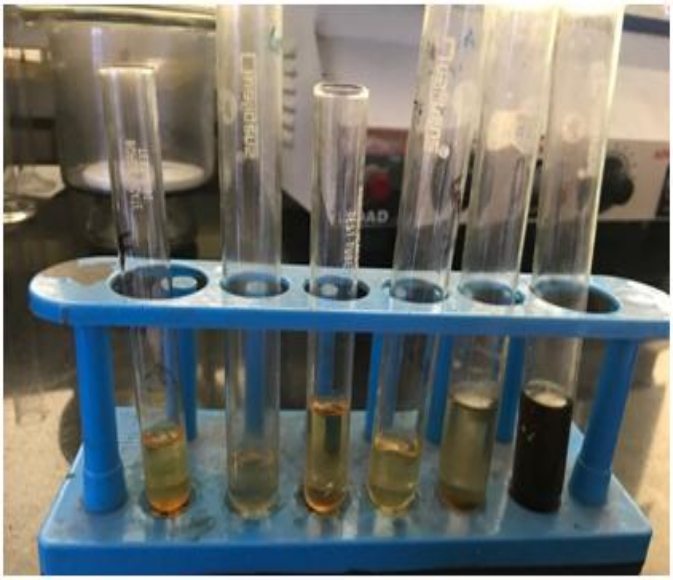

Figure 5.1: Preliminary phytochemical tests

5.2. ESTIMATION OF TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT:

Total phenolic content estimation:

Phenolics are major group of compounds that are used as free radical scavengers or anti-oxidants .The amount of total phenolic content was determined by Folin-Ciocalteau method .Gallic acid was used as standard compound.The calibration curve for gallic acid was represented diagrammatically(figure:1) using equation y=0.005x+0.008,R^2 =0.998,where "y" is absorbance at 760nm and "x" is the Gallic acid equivalent (GAE).The total phenoloc content of the ethanolic whole plant extract of C.roseus was found to be 0.50366 mg GAE/0.05g of extract (Table 5.3)

Table 5.3:The total phenolic content of ethanolic leaf extract of C.roseus:

S.no	Plant name	Part	Extract	Unknown concentration (µg/ml)	Phenolic content mg GAE/0.05g of extract
1.	C.roseues	Whole plant	Ethanolic	25.168	0.50366

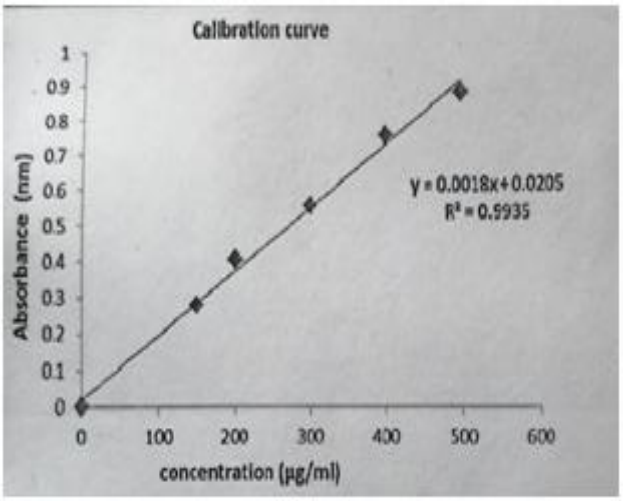

Figure 5.2 : Calibration curve of phenolic content

Total flavonoid content estimation:

The concentrations of flavonoid in ethanolic whole plant extract of C.roseuses was determined using spectro – photometric method with aluminium chloride .Quarcetin was used as standard compound.The calibration curve for Quercetin was represented diagrammatically (figure:2) using equation y=0.0018x+0.0205, R^2 =0.9935,where y is absorbance at 510nm nd x is the Quarcetin equivalent (QE). The toital flavonoid content of the ethanolic whole plant extract of C.roseuswas found to be 3.0704mg QE/0.025 g of extract.(Table:5.4)

Table 5.4. The total flavonoid content of ethanolic extract whole plant extract of C.roseus:

S.no	Plant name	Part	Extract	Unknown concentration(µg/ml)	Flavonoid content mg QE/0.025 g of extract
1.	C.roseus	leaf	Ethanolic	30.7040	3.0704

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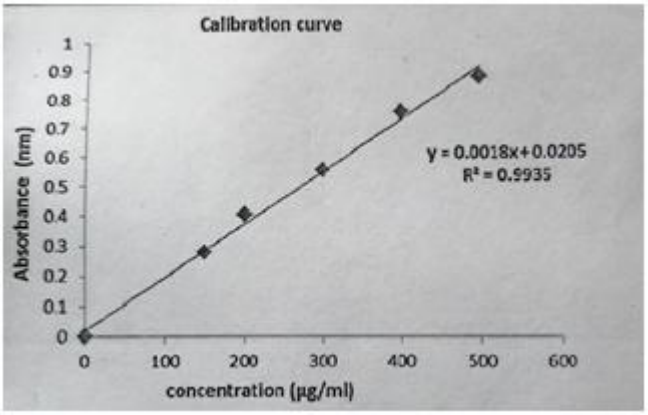

Figure 5.3 :Calibration curve of flavonoid content

SUMMARY AND CONCLUSION:

The medicinal plants are used for treatment of many diseases. these plants are various active chemical constituents which are isolated and subjected to different phytochemical screening in order to determine the specific chemical constituents responsible for the activity. The obtained compound is subjected to the experiment to known the therapeutic dose for the compound.

Catharanthusroseus whole plant was collected. They were shade dried completely for about two weeks and dried powder was subjected to find out the extractive values of powder with different solvents.

Extractive values were determined using the solvents like pet ether, toluene, benzene, ethyl acetate, ethanol and methanol. Methanol and ethanolic extract was found ton higher extractive value than other extractive value. It indicates that the compound resent in the catharanthusroseus more polar nature.

Ethanolic extactof catharanthusroseus whole plant was subjected to preliminary analysis. The qualitative analysis by phytochemical screening shown the chemical constituents like alkolides, carbohydrates, glycosides, flavonoids and tannins. The qualitative analysis was explained by total phenolic content and total flavonoid content estimation.

Iron oxide nano particles were successfully synthesized by catharanthusroseus whole plant (ethanolic extract). these iron oxide nano particles were characterized confirmed by DLS and ZP.

Anti angiogenic activity was determined by using zebra fish (danio rerio). Ethanolicextact of catharanthusroseus iron oxide nano particles shown the potent activity at the concentration of 200 micro gram/100 Ml.

Thus from above study it has proved that catharanthusroseus can be used for treatment of angiogenesis.

REFERNCES

[1] Zygmunt M, Herr F, Munstedt K, Lang U, Liang OD. Angiogenesis and vasculogenesis in pregnancy. Eur J ObstetGynecolReprod Biol. 2003;110:S10–S18.

[2] Carmeliet P. Angiogenesis in health and disease. Nat Med . 2003;9:653–660.

[3] Charnock-Jones DS, Kaufmann P, Mayhew TM. Aspects of human fetoplacentalvasculogenesis and angiogenesis. I. Molecular regulation. Placenta . 2004;25:103–113.

[4] Fraser HM, Duncan WC. Vascular morphogenesis in the primate ovary. Angiogenesis. 2005;8:101–1016. United Nations. Questions About Nanotechnology. 2012; Return to citation in Text:

[5] Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology. Federal Drug Administration: USA, 2011Return to citation in text:

[6] ISO/TS 80004-1:2010, Nanotechnology – Vocabulary – Part 1: Core Terms. International Organization for Standardization: Geneva, Switzerland, 2010; (accessed July 17, 2017). Return to citation in text:

ISSN No: 2348-4845
International Journal & Magazine of Engineering, Technology, Management and Research
A Peer Reviewed Open Access International Journal

[7] Babay S, Mhiri T, Toumi M (2015) Synthesis, structural and spectroscopic characterizations of maghemite g-Fe2O3 prepared by one-step coprecipitation route. J MolStruct 1085:286-293. doi: 10.1016/j.molstruc.2014.12.067

[8] Boldyrev VV (2006) Thermal decomposition of ammonium perchlorate. ThermochimActa 443(1):1-36. doi: 10.106/j.tca.2005.11.038

[9] Bumajdad A, Ali S, Mathew A (2011) Characterization of iron hydroxide/oxide nanoparticles prepared in microemulsion stabilized with cation/non-ionic surfactant mixtures. J Colloid Interface Sci 355(2011):282-292. doi: 10.1016/j.jcis.2010.12.022 Callister Jr WD (2012) Ciência e engenharia de materiais: umaintrodução. Rio de Janeiro: