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RESEARCH ARTICLE

COLUMN CHROMATOGRAPHY FRACTIONAL ANALYSIS OF *ERYTHRINA VARIEGATA* L. LEAF EXTRACT FOR ITS ANTIBACTERIAL EFFICACY.

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Abstract

Medicinal Plants are source of many noble drugs compound. Secondary metabolites are a source of biological active natural products with high potential to act as antibacterial and also as inhibitors and plant growth promoters. The progress in this area has great potential for industrial application especially in pharmaceuticals and nutraceuticals. This medicinal plants show the presence of Alkaloids, Flavanoids, Saponins, Tannins & Resins. The antimicrobial activity of the crude extract from the leaves of various medicinal plants are supposed to be due to the presence of various phytochemical constitutes present in them. Column chromatography is used to purify individual chemical compounds from mixtures of compounds. This also prevents cross-contamination and stationary phase degradation due to recycling. This method was used on chloroform extract for the leaf extract of *Erythrina variegata* using standard technique. The antibacterial sensitivity potential was assess and recorded on 14 fractions ranging from 1- 14 and compared with Negative control as "Chloroform" and Positive control as "Amikacin" treatment. The result confirmed high antibacterial potential in Chloroform extract of *Erythrina variegata* in comparison with other solvents tested such as n- Hexane, Dichloromethane, Ethyl acetate, Methanol and Aqueous. As the purification of fraction increased the antibacterial sensitivity of chloroform extract enhanced too. The pure solvent at fraction 14 was found to be nearby close to drug "Amikacin" antibacterial sensitivity, indicating the suitability of chloroform fraction derived from leaf extract of *Erythrina variegata* to be a potential source of development of newer medicine.

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Introduction:-

"*Erythrina variegata"* L. (syn. *E. indica* Lam.), common name- Indian Coral Tree/ Farad/ Parijata is a species of Erythrina native to the tropical and subtropical regions of the Indian subcontinents. In India, *E. variegata* is a thorny deciduous tree growing to 27 m tall. The leaves are pinnate with a 20 cm petiole and three leaflets up to 20 cm long and broad. The leaves extract as well as other plant parts extract of this plant have been found to exhibit antibacterial activity against *E. coli* and other bacterial cultures. This suggest that plants manifest relatively high levels of antibacterial action and may be sources of compounds that can be used to inhibit the growth of food borne pathogens (Kumar, 2006, Singh et.al., 2007 & Osho & Adetunji, 2010). Herbal drugs are prescribed widely even when their biologically active compounds are unknown. They are popular because of their effectiveness, minimal side effects in

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clinical experience and relatively low cost. The antibacterial compound found in plants may prevent bacterial infections by different mechanisms than the commercial antibiotics and therefore may have clinical value in treating resistant bacterial strains (Eloff, 1999).

The continuous search for new antibiotics and medicinal plants may offers a new source of antibacterial agents. This is indeed very important because *Escherichia coli, Pseudomonas aeruginosa, , Klebsella pneumoniae, Staphylococcus aureus,* and *Proteus vulgaris* are some of the important human pathogens that have developed resistance to anti-bacterials (Barbour et. al., 2004) and need to be treated with newer plant based medicine having potentialities of controlling the infection (Prescott, 2005). The present study is a sincere attempt to understand and assess the antibacterial potentialities of Erythrina leaf secondary metabolites against these infectious cultures of different bacteria. The research revealed that commonly found bacterial infecting Patnaites around the year are E. coli, Staphylococcus, Pseudomonas, Klebsella & Proteus. In this study of antibacterial assay, all the organisms responded to the plant extract and Zone of inhibition developed against bacteria in 14 different fractional separations by Column chromatography. The carefully processed Erythrina Leaves were subjected to Soxhlet apparatus by using different solvent and one most selected suitable solvent extract i.e Chloroform extract of Leaves were further fractional analyzed against different bacterial cultures and the results shows that it is a "Green medicine" for future.

Materials and methods:-

Collection of plant sample: The fresh leaves of *E. variegata* were collected from Agamkuan near RMRIMS, Patna.

Preparation of Leaf extracts: The leaves were washed thoroughly with tap water and in distilled water and then dried the leaves at room temperature. The dried leaves were ground to a fine powder in a mechanic grinder. About 100gm of powdered plant material was uniformly packed into a thimble (made up of muslin cloth) and extracted with 500ml of different solvents separately in Soxhlet Apparatus. Solvents used were n-Hexane, Chloroform, Dichloromethane, Ethyl Acetate, Methanol and Aqueous on the basis of their polarity. The process of extraction continues till the solvent in tube of an extractor become colorless. The extracts were filtered through muslin cloth or Whatman No.1 filter paper and collected in brown bottle separately. After that the different extract solvents were evaporated by Rota-vapour separately, where the temperature of water bath should be at 40^0C, and then Lyophilized by Lypholizer which gave rise to a solid mass of the extract. One such solvent extract i.e Chloroform extract of Erythrina Leaf is selected for Column chromatography antibacterial analysis. The solid mass was refrigerated for further use (Preeti Kumari et.al., 2017).

Selected test microorganisms: Extracts were tested against pathogenic microbes, including the bacteria *Escherichia coli, Pseudomonas aeruginosa, Klebsella pneumoniae, Staphylococcus aureus,* and *Proteus vulgaris*.

Antibacterial Assay: Antibacterial activity of plant extracts was carried using agar disc diffusion method with some minor modifications. 200µl bacterial cultures were spread in nutrient agar culture plate by glass rod spreader. Disc of 6mm containing extract were diffused in the plate and after incubation, zone of inhibition were read.

Column Chromatography fractional analysis: Column chromatography is an isolation technique in which the Phyto-constituents are being eluted by adsorption (Fair et.al., 2008).

The principle involved in this separation of constituents is adsorption at the interface between solid and liquid. The component must have various degree of affinity towards adsorbent and also reversible interaction to achieve successful separation. No two compounds are alike in the above aspect. Low affinity compounds will elute first. The columns of different sizes were used for the present studies. Since the chloroform extract was found to possess significant pharmacological activity when compared to other extracts, and then an attempt was made to fractionate the chloroform extract by column chromatography. The elution was done by using solvents of different polarity like n-hexane, ethyl acetate, dichloromethane, methanol and Aqueous. The Column Chromatography was run on Chloroform extract with dry packing material in which Aluminium oxide was used as packing material.

Procedure Followed (Column Chromatography) (Amrita, 2016):-

Prepared the Column: Placed the column in a ring stand in a vertical position, A plug of glass wool was pushed down to the bottom of the column, Prepared slurry of silica gel / aluminium oxide with a suitable solvent & pour

gently into the column and Opened the stop cock & allowed some solvent to drain out. The layer of solvent covered the adsorbent; otherwise cracks developed in the column.

Adding the Sample to the Column: Dissolved the sample mixture in a minimum amount of solvent (chloroform). Removed the solvent by placing the mixture in a rotary evaporator The Rotavapour works at a low temperature and low pressure. Then, placed the dry powder on a piece of weighing paper and transfer it to the top of the column through the funnel.

Developing the Chromatogram: Attached a dropping funnel filled with solvent on to the column. Add solvent continuously from the funnel to the top of the column. Opened the stopcock carefully and the components of the mixture run down the column forming separate bands.

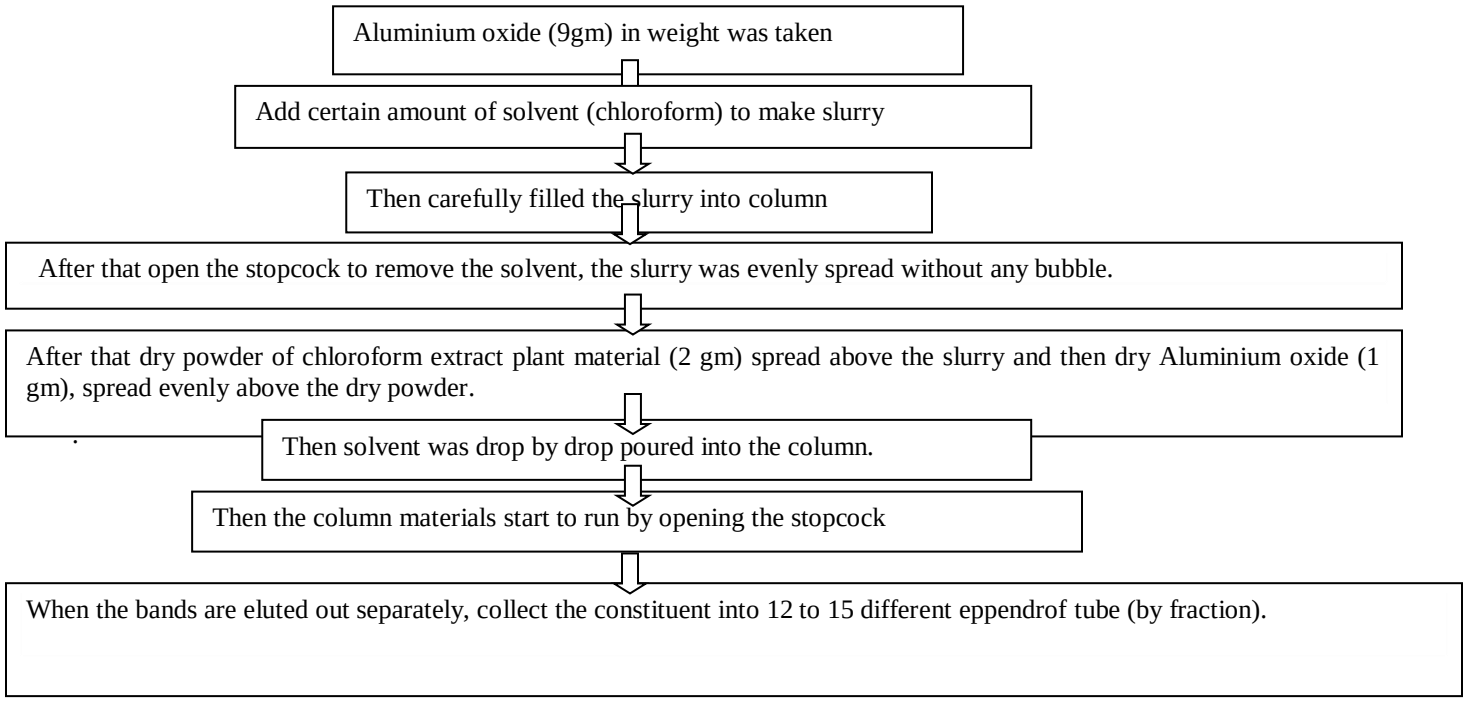
Recovering the Constituents: Continued running the solvent till the bands which eluted out separately, Collected the constituents (Evaporate the solvent by placing the mixture in a rotary evaporator).

Antibacterial sensitivity test (AST):-

It is usually carried out to determine which antibiotic will be most successful in treating a bacterial infection. Small wafers containing antibiotics are placed onto a plate upon which bacteria grows. If the bacteria are sensitive to the antibiotic, a clear ring, or zone of inhibition, is seen around the wafer indicating poor growth (L. Barth Reller et.al, 2009). 14 fractions have been taken after column chromatography in different eppendof tube, All tubes were then lyophilized in lyophilize to get powder form of plant extract, The dry column plant extract was then dissolved in chloroform solvent in 1mg/ml concentration, Then sensitivity tests were done on 5 different bacterial culture i.e., *Escherichia coli, Pseudomonas aeruginosa, Klebsella pneumoniae, Staphylococcus aureus,* and *Proteus vulgaris* by disc diffusion method, 14 disc was placed in two different Petri plate by numbering 1 to 8 and 9 to 14 with positive (Drug= Amikacin) and negative (Chloroform), Data were recorded in tabular form of plant sensitivity of bacterial by measuring zone of inhibition separately.

Figure: Flow chart of experimental procedure (Column Chromatography Syringe).

The chloroform extract was subjected to aluminium oxide column chromatography for the isolation of the phyto-constituents. An appropriate column sized 2.5cm diameter and 10cm length was used.



Results and discussion:-

The results show that antibacterial activity against tested bacteria is an indication that the plant is a potential source for production of drugs with a broad spectrum of activity and can be used as antibacterial agents in novel drugs formulation for the treatment of gastroenteritis, typhoid fever, urinary tract infection and wound infections. In addition, there is need for the characterization and isolation of the active ingredients. Keeping this in view the chromatography fraction analysis was carried out using leaf extract of *Erythrina variegata*. The dried powder material was extracted successively in a Soxhlet apparatus. The extracts were filtered while hot and concentrated under reduced pressure. The practical and % yields of the extracts were calculated. Different types of polar solvents like n-Hexane [non- polar solvent], Chloroform [slightly Polar] and dichloromethane [moderate], methanol [Polar], Aqueous [low polar solvent] were used for the extractions (Sofia Zazouli et. al., 2016). These extracts were used for the detection of phytochemical analysis. Herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of fruits and vegetables has been attributed by phytochemicals, which are the non- nutrient plant compounds. This Column chromatography fraction is done in selected chloroform solvent extract in concentration of 1mg/ml. The result of fractional antibacterial column chromatography analysis has been presented in Table hereunder.

Table 1: Fractional Antibacterial Column Chromatography Analysis:

B.C	1	2	3	4	5	6	7	8	9	10	11	12	13	14	C	D
E.coli	8mm	8mm	8mm	9mm	9mm	9mm	9mm	9mm	10mm	10mm	11mm	11mm	12mm	12mm	7	30
Pseudo Monas	9mm	9mm	9mm	9mm	9mm	10 mm	10mm	9mm	11mm	11mm	11mm	11mm	12mm	12mm	7	30
Klebsella	9mm	9mm	9mm	9mm	9mm	9mm	9mm	9mm	10mm	10mm	10mm	10mm	11mm	11mm	7	30
Staphylo Coccus	8mm	8mm	8mm	8mm	9mm	9mm	9mm	9mm	10mm	10mm	11mm	11mm	12mm	12mm	7	30
Proteus	9mm	9mm	9mm	9mm	10mm	10mm	10mm	10mm	11mm	11mm	12mm	12mm	12mm	12mm	7	30

Note: (1-14) Different fractions; D= +ve Control (Amikacin) Drug and C= -ve Control (chloroform)

The results above revealed that the 9 to 14 Column chromatography fractions shows better zone of inhibition as compared to 1 to 8 in different bacterial culture. This means that as the *Erythrina variegata* extract compound purified its antibacterial sensitivity activity increase. Amikacin drug shows same zone of inhibition in all bacterial culture. The Antibacterial Sensitivity of Column chromatography fractional analysis is given in Plate 1 and 2 as here under.

Photo 1-10 Column Chromatography Fractional Sensitivity Analysis and **Photo-11** Different Bacterial Culture are here under.

Plate 1:-Photo 1- 6 Chromatograph Fraction Sensitivity analysis of Leaf Extract:

Photo 1 (1 - 8)…. *Escherichia coli*

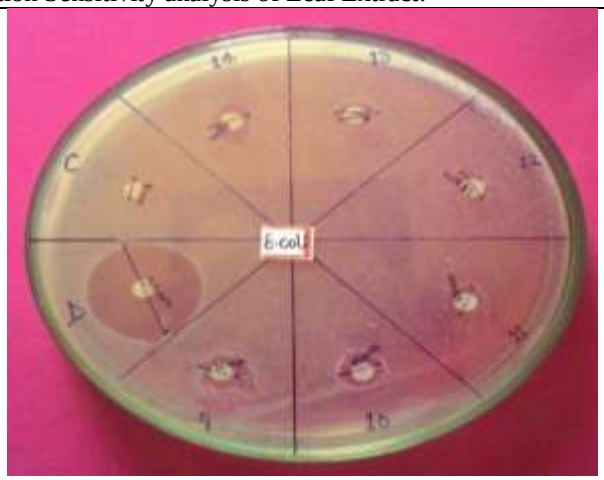


Photo 2 E. coli (9 – 14);

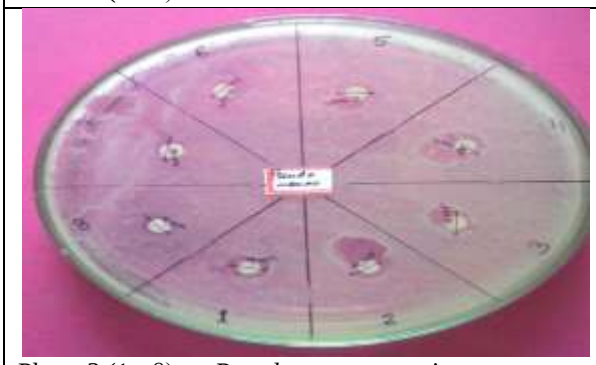


Photo 3 (1 - 8)…. *Pseudomonas aeruginosa*

Photo 4 Pseudomonas (9 – 14);

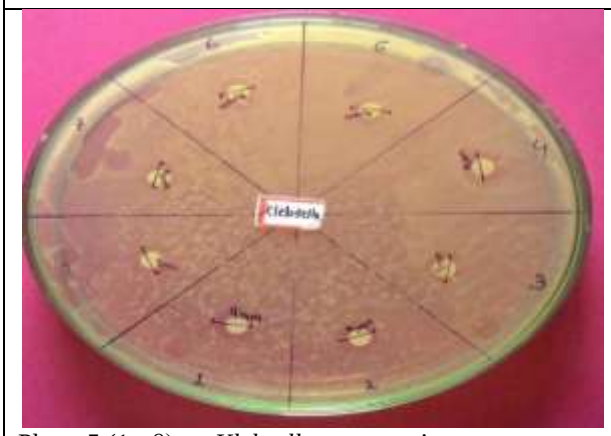


Photo 5 (1 - 8)…. *Klebsella pneumoniae*

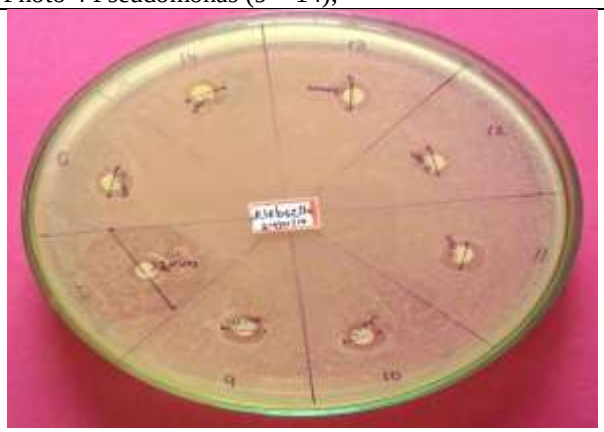


Photo 6 Klebsella (9 – 14);

Note: Second plate 9 to 14 with +ve Control (Amikacin) Drug and -ve Control (chloroform)

Plate2:- Photo 7-10 Chromatograph Fraction Sensitivity analysis of Leaf Extract:
Photo 11: Different Bacterial culture

Photo 7 (1 - 8)…. *Staphylococcus aureus*

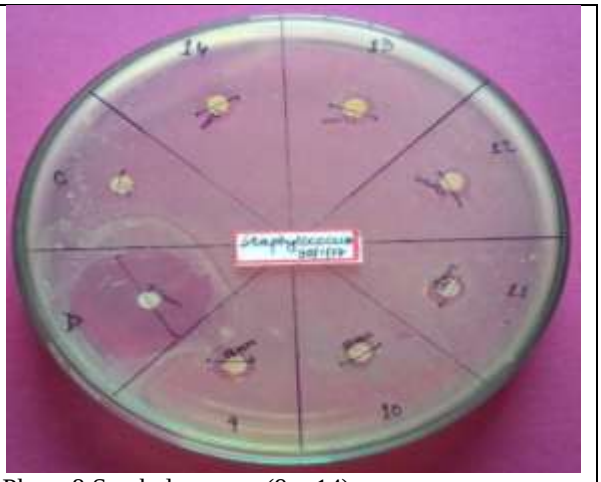


Photo 8 Staphylococcus (9 – 14);

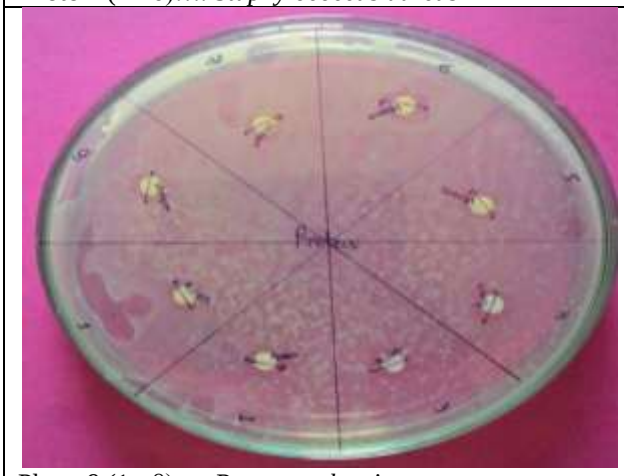


Photo 9 (1 - 8)…. *Proteus vulgaris*

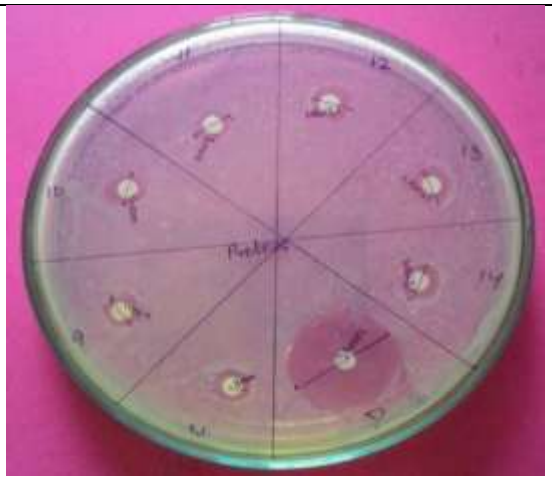


Photo 10 Proteus (9 – 14);

Photo 11 : Bacterial Cultural Plate (E.coli, Proteus, Staphylococcus and Klebsella)

Note: Second plate 9 to 14 with +ve Control (Amikacin) Drug and -ve Control (chloroform)

Conclusion:-

The antibacterial activity of the Chloroform solvent extract from the leaves of E. variegata may be due to the presence of various phytochemical constituents indulged in them. Therefore, *Erythrina variegata* leaf extract shows

that it is further used as a source of pharmaceutical materials required for the preparation of new antibacterial drugs. Plant extracts have great potential as antibacterial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment.

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