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RIBOFLAVIN PRODUCTION FROM COLOCASIA WASTE LEAVES

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ABSTRACT:
Riboflavin, commonly known as vitamin B2 and is used as dietary supplement. Riboflavin deficiency can lead to various disorder like sore throat, anemia etc. *Colocacia esculanta* is a flowering plant in the family Areacea can be used as source of Riboflavin. Industrial production of Riboflavin mostly relies on the microbial fermentation of flavinogenic microorganisms. Chemical synthesis of vitamin is being replaced by fermentation processes.
Key words: - Riboflavin, microbial fermentation, flavinogenic and Chemical synthesis.

INTRODUCTION:

Riboflavin, commonly known as vitamin B_2, it found in food and used as a dietary supplement, it is easily absorbed micronutrient with a key role in maintaining health in humans and animals(American society of health 1 august 2008). Riboflavin serves as a central component for cofactors like Flavin-adenine dinucleotide and Flavin mono-nucleotide (FAD & FMN) and is therefore required by all the flavoproteins (Lago & Kalpan, 1981). It is also used as a natural food coloring agent (Kalpan, 1981). Good sources of vitamin B_2 are milk, cheese, green leafy vegetables, yeast, almond and legumes. It helps in fortifying some foods like baby foods, breakfast, cereals and processed cheese (Graham et al, 2005).

Usually riboflavin deficiency occurs in endemic populations who exist on diets that lacks dairy products and meat (Oppenheimr S.J. et al, Boisvert W.A. et al, Wilson J.M et al, Powers HJ et al, Bates CJ et al).In addition to produce energy, riboflavin also works as an antioxidant by scavenging damaging particles in the body known as free radicals (Lim et.al, 2001).

Riboflavin deficiency leads to painful red tongue with sore throat. It may leads to oily skin, eyes can be itchy, watery, bloodshot and sensitive to light, anaemia and birth related deformities. High dose of Riboflavin appears to be useful alone or along with beta-blockers in the prevention of Migraine (Lim et al).*Colocacia esculanta* is a flowering plant in the family *Areacea* found in Southeast Asia and Indian subcontinents. Kingdom plantae, class angiosperms, subclass monocots, order alismatales, family araceae, subfamily ariodeae, species colocasieae, genus colocacia.

Bacillus subtilis is a facultative anaerobic, rod shaped, gram-positive bacteria (Christian Ehrenberg, 1935). Domain bacteria, phylum-firmicutes, class-bacilli, order-bacillales, family-bacillaceae, species-*B.subtilis*, genus Bacillus.Hence for preventing all such deformities or to overcome all the problems regarding deformities can be completely treated by plant source i.e Colocacia leaves and fermenting it with the help of an organism called Bacillus subtilis.

MATERIALS AND METHODS

Plant material:

Colocacia leaves obtained from outer part of the Nag River (Marshy place).

Source of organism:

B.subtilis obtained from sewage water. The pure bacterial culture can be obtained by performing serial dilution and bacterial species gram positive can be confirmed by performing gram staining.

Fermentation process:

10g of *Colocacia* leaves were dipped in absolute alcohol and immediately thoroughly washed with distilled water. Slurry of leaves was obtained by grinding 10g of leaves with 100ml distilled water in ice-cold mortar pestle. 10ml of slurry was taken as control and to the remaining 90ml of slurry following components were added-

Potassium phosphate	- 0.2g
Magnesium phosphate	- 0.1g
Zinc sulphate	- 0.05g
Ammonium sulphate	- 0.88g
Biotin	- 2 almonds (crushed)
PH	- 5-6 range (acidic)
Liquid broth of *B.subtilis*	- 10ml

Control flask was prepared similarly except bacterial organism in a proportionate ratio of 10ml. Both the flask was incubated at 35±2°C. Colorimetric analyses were done for control as well as treated at regular interval of 4 days in sterile condition.

Qualitative and Quantitative Estimation Of Vit B_2.

1) **Qualitative analysis: Thin layer chromatography (TLC)**

Control and treated samples along with standards were run simultaneously on TLC plate with below mentioned mobile phase.

Preparation of solvent system (50ml)

Chloroform: Ethanol: Acetone: Ammonia solution

RESULT & DISCUSSION:

Industrial production of Riboflavin mostly relies on the microbial fermentation of flavinogenic microorganisms. Chemical synthesis of vitamin is being replaced by fermentation processes because of economic and environmental considerations of the latter. Besides the economic advantages additional benefits of the microbial synthesis include the use of renewable sources, environmental-friendly approach and superior quality of final products (*Van Loon, 1996*).

In the present study *Colocacia* leaves were used. In the present *B.subtilis* was used to produce vitamin B_2 from *Colocacia* leaves, same result were obtained by *Seong Han Lim et al*, (2001) as they concluded that 3 kinds of riboflavin overproducers are known; *Bacillus subtilis*, and *Candida famata* utilize glucose as a carbon source. Riboflavin biosynthesis has been studied by *Catherence et al* (2004) in both gram positive and gram negative bacteria, in most detail in *B.subtilis* and *E.coli*. The possible mechanism of Riboflavin was studied by *Jefland et al.*, that in *B.subtilis*, strict transcriptional regulation of the *rib* operon takes place by means of m-RNA regulatory region transcribed from the 5'end of the *rib* operon. This regulatory m-RNA region is conserved in several bacteria and is predicted to fold into a specific secondary structure. It is reported that in *B.subtilis rib* A is the rate limiting enzyme in the riboflavin biosynthetic process in this bacterium and that increased expression of *rib*A leads upto 25% increase in Riboflavin yield.

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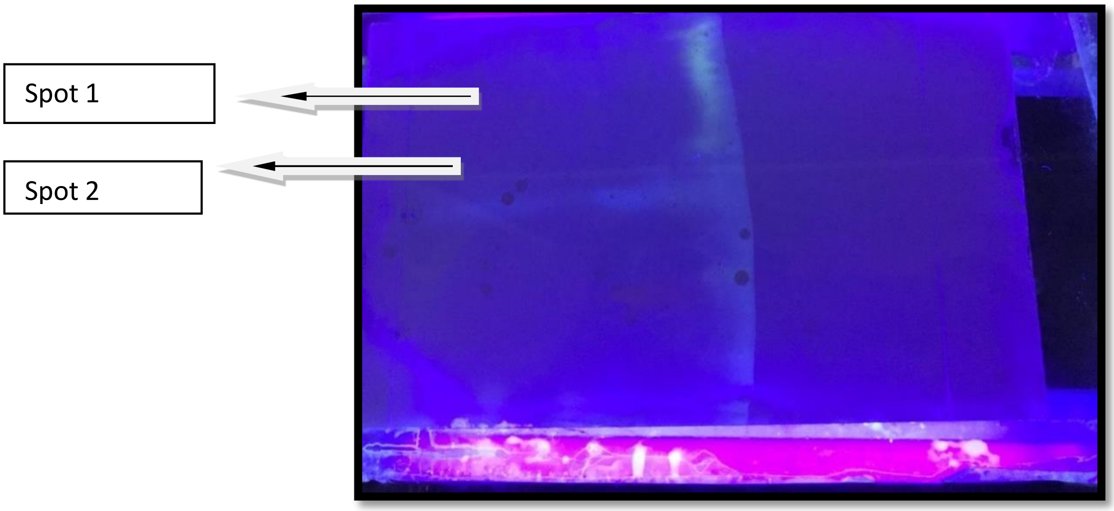


RF value table of fermentation broth

Fig: Thin Layer Chromatography plate Showing

Spot 1: Standard riboflavin

Spot 2: *Colocacia* broth

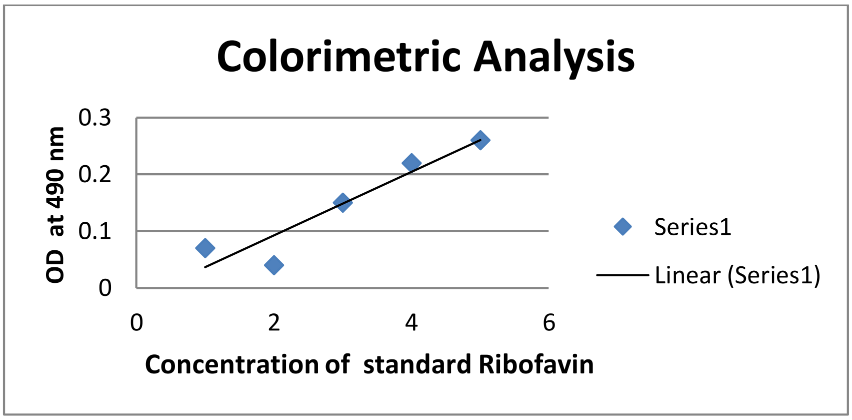
Sr.no.	**Sample**	**Rf value**
1.	Standard riboflavin	4.5 cm
2.	*Colocacia* broth	2.8 cm

Sr. no.	Chemicals	Concentrations in test tube (in ml)				
		1	2	3	4	5
1.	Standard Riboflavin	0.2	0.4	0.6	0.8	1.0
2.	1M NaOH	0.2	0.2	0.2	0.2	0.2
3.	0.1M KH_2PO_4	0.4	0.4	0.4	0.4	0.4
4.	Distilled water	0.8	0.6	0.4	0.2	0

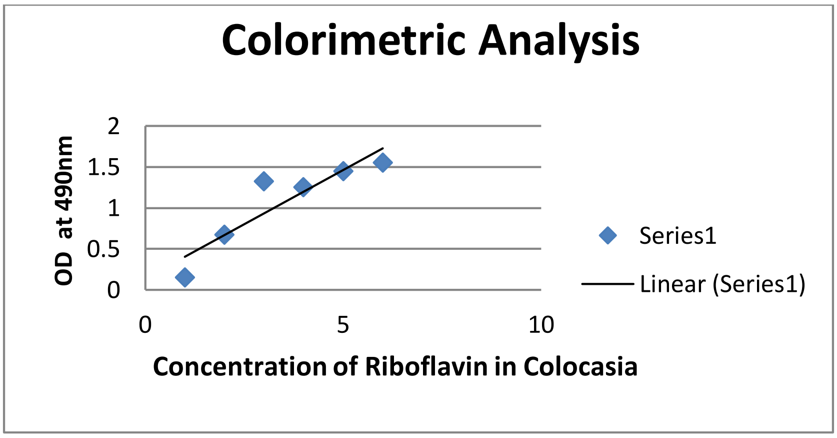
Colorimetric Analysis of Fermentation Broth for the Production of Riboflavin

1. Standard Riboflavin curve

Blank	**I**	**II**	**III**	**IV**	**V**
0.00	0.07	0.04	0.15	0.22	0.26



2. ***Colocacia*** **Broth curve**



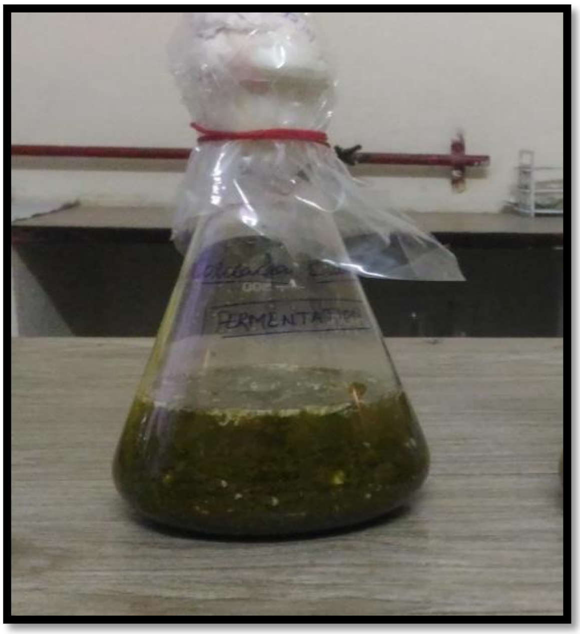


Fig:1 *Colocacia* slurry (fermentation broth)

Fig:2 Standard *Colocacia* slurry