



# DESIGN AND EVALUATION STUDIES OF *PHYLLANTHUS AMARUS* LOADED SUSTAINED RELEASE MICROPARTICLES BY COACERVATION AND PHASE SEPARATION PROCESS

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**ABSTRACT:** In the present study, three formulation of gelatin microparticles containing *Phyllanthus Amarus* were prepared by Coacervation phase separation method and characterized by optical microscopy and scanning electron microscopy. The microparticles were analyzed for drug entrapment, bulk density, angle of repose, particle size and *in-vitro* release pattern. The effect of process variables on microparticles size was studied and based on these preliminary studies, different batches of microparticles were prepared by altering the core: coat ratio of 1:1 and size range of 2-100  $\mu\text{m}$  gave the best sustained release effects. They were spherical in shape as evidenced by photomicrographs and scanning electron microscopy. The study concludes that the developed formulation F2 consisting 0.2 gm of *Phyllanthus Amarus* with 0.2gm gelatin was found to be promising microspheres for the treatment of some Struvite type of kidney stones.

**Keywords:** Microparticles, *Phyllanthus Amarus*, Sustained release, Coacervation phase separation method.

## INTRODUCTION :

The goals of sustained drug delivery are to conserve and maintain effective drug concentration, eliminate night time dosage, improve compliance and decrease side effects thus, optimizing drug therapy. Sustained release dosage forms provide a prolonged dosing of the drug from the product by supplying an initial amount of loading dose, perhaps one-half of the total dose release, followed by a gradual and uniform release of the remainder of the drug over the desired time period. *Phyllanthus amarus* L., known as stone breaker, is a plant

in the Phyllanthaceae family, belonging to the genus *Phyllanthus*. *Phyllanthus* has been used in Ayurvedic medicine for over 2,000 years and has a wide number of traditional uses including internal use for jaundice, gonorrhoea, frequent menstruation, and diabetes and topical use as a poultice for skin ulcers, sores, swelling, and itchiness. In this study, the factorial design was evaluated to study the importance of factors (concentration of ethanol and extraction process) on the concentration of flavonoids and phenolic compounds present in *Phyllanthus amarus* extracts. More than 50 compounds were identified in the *Phyllanthus amarus*, including alkaloids, flavanoids, lignans and triterpenes. This plant protects the kidney against stone formation via inhibiting growth of urate crystals. In herbal medicine, it has long played a role in treating several conditions, including urinary tract stones, dysentery, ulcers, swelling, diseases that mainly affect the genitals, particularly the urinary tract<sup>1</sup>.

These particles consist of core material, which is the drug, and a coating material. The coat material can be natural polymer gelatin and Span 20 as oil phase and Glutaraldehyde as cross-linking agent. Also literature survey revealed that not much work has been done on sustained release drug release of *Phyllanthus Amarus*, except for few workers.

## MATERIALS AND METHODS

**Materials:** *Phyllanthus amarus* was procured from Local area road side Anaikuttam. Span 20 were from Maruti chemicals, Ahmedabad, India. Gelatin from Rechem Laboratory chemicals Mumbai. Glutaraldehyde were collect from Ultimate Chem India Pvt. Ltd., Maharashtra and Purified water from Andavar plus drinking water, Chennai.

### Methods:

#### Collection of *Phyllanthus amarus*

The leaves of *Phyllanthus amarus* will be obtained locally and shall be identified and authenticated from Dr N.Senthikumar Head and Associate Professor of Botany ANJAC College, Sivakasi.

#### Extraction of plant materials

The *Phyllanthus amarus* extract was prepared by continuous soxhlet extraction using successive solvents based on their increasing polarity. 45gms of powdered drug was placed inside a thimble made of thick filter paper, and was loaded into the Soxhlet extractor. The solvent pot should not be overfilled and the volume of solvent in the still pot should be 3 to 4 times the volume of the soxhlet chamber and was heated to boil. The extraction was carried out continue sly until the colour of the solvent changes. The extracts was concentrated after recovering the solvents. The *Phyllanthus* extract was prepared with successive solvents with increase in polarity namely hexane, ethyl acetate, methanol, water. The prepared extracts were subjected to qualitative test for identify various phytochemical constituents as per standard procedure<sup>2</sup>.

### Phyto constituents study of *Phyllanthus Amarus* extract:

**Table 1: Phytochemical Screening Study of *Phyllanthus Amarus***

S.No	Phyto constituents	Tests	Results
1	Alkaloids	Dragendorff's test. Hager's test. Mayer's test. Wagner's test.	+  +  +

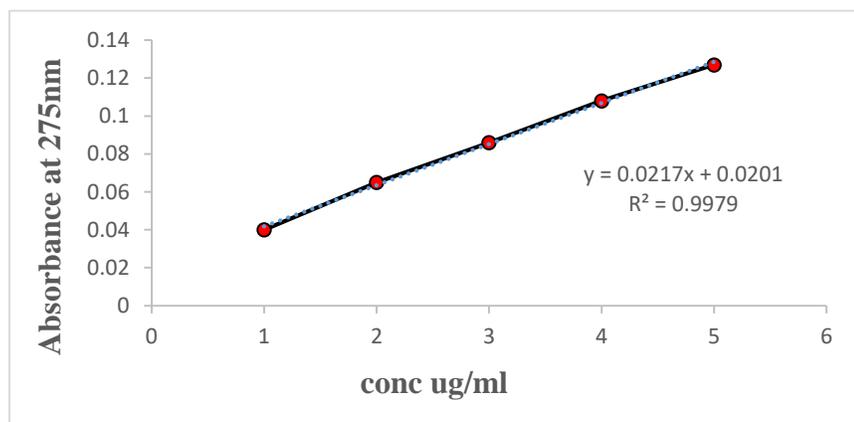
			+
2	Glycosides	Legal test Borntrager's  Modified Borntrager's	+ + +
3	Saponin	Foam test	+ +
4	Terpenoids	Liebermannburchard test Salkowski test	+ +
5	Flavonoids	Alkali test  Acid test	+  +

**STANDARD CURVE FOR *PHYLLANTHUS AMARUS***

**Preparation of (various concentrations) working stock solution**

Various concentrations of 1,2,3,4 and 5 µg/ml solutions was prepared by diluting 1,2,3,4 and 5ml of secondary solution to 10ml volumetric flask with phosphate buffer pH6.8. The absorbance was measured by using U.V spectrophotometric method at 275nm against blank was performed with phosphate buffer pH6.8.A standard curve was drawn by relating concentration (µg/ml) on X-axis and absorbance of *Phyllanthus amarus* at 275 nm on Y-axis. The standard curve was used to estimate the drug content from the *Phyllanthus amarus* micro particles.

**Figure:1 Standard Calibration Curve Data of *Phyllanthus amarus***



**PREFORMULATION STUDY**

**ORGANOLEPTIC PROPERTIES**

- a) **Colour:** A small quantity of extract was taken in butter paper and viewed.
- b) **Taste and odour:** Very less quantity of extract was used to assess the taste with help of tongue as well as smelled to get odour<sup>3</sup>

**SOLUBILITY ANALYSIS**

Solubility of extract in water, methanol, acid and ethanol was determined at room temperature. Aqueous and organic solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy<sup>4</sup>.

### GC-MS study

Chemical composition of the extract obtained from *Phyllanthus amarus* was analysed by Gas Chromatography-Mass Spectrometry.

### FT-IR STUDY

FT-IR studies of the pure *Phyllanthus amarus*, polymer and combination of crude drug and gelatin containing highest proportion were carried out to found any interaction between drug and excipients used in the formulation. FT-IR study was performed using IR spectroscopy (SHIMADZU).

### PREPARATION OF MICROPARTICLES

**Table 2: Formulation of *Phyllanthus Amarus* Extract Loaded Micro particles by using Various Proportion of Gelatin**

S.NO	INGREDIENTS	F1 (1:0.5)	F2 (1:1)	F3 (1:1.5)
1	Core material (mg)	200mg	200mg	200mg
2	Coating material(mg)	100 mg	200mg	300mg
3	Span 20 (ml)	0.5ml	0.5ml	0.5ml
4	Glutaraldehyde	1.5 ml	1.5 ml	1.5 ml

### CHARACTERIZATION OF MICROSPHERES:

#### Production yield

After drying the obtained microspheres were weighed accurately. The % yield was determined by using the following formula,

$$\% \text{yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total mass of drug + Polymer}} * 100$$

#### Drug Entrapment Efficiency (DEE)

Entrapment efficiency was determined by dissolving 10 mg of microspheres in 2 ml DCM. When the particles were completely dissolved, the mixture was diluted up to 50 ml with phosphate buffer pH 6.8 and stirred for 1 h by using a magnetic stirrer for complete removal of DCM. The polymer was removed by filtration. The absorbance of the filtrate was measured at 275 nm, and the content was determined by using a calibration curve of *Phyllanthus amarus* extract and using the formula<sup>4</sup>.

$$\% \text{ Drug Entrapment} = \frac{\text{Calculated drug content}}{\text{Theoretical drug content}} * 100$$

### Flow Properties Studies<sup>5,6</sup>

Flow property depends on particle size, shape, porosity and density of microspheres.

### Particle Size Analysis

The particle size of the prepared microspheres was measured by the optical microscope with the aid of an eye-piece micrometer which was previously calibrated with the stage micrometer. The mean particle size and size distribution were determined<sup>7</sup>.

### SCANNING ELECTRON MICROSCOPY (SEM)

The samples were mounted on specimen studies using Double sided adhesive tape. The sputtering was done for nearly 3 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage. The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and the Working Distance WD is 10 mm<sup>8</sup>.

### In-vitro Dissolution Studies

#### Procedure

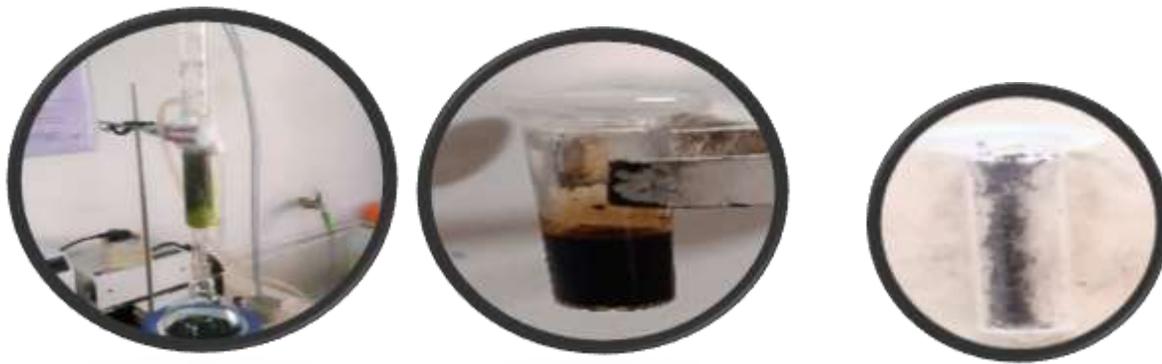
In-vitro release profile of the micro particles was evaluated using rotating basket dissolution apparatus. 900 ml of phosphate buffer (pH 6.8) maintained at 37±0.5°C is used as dissolution media, and the basket was rotated at a constant speed of 50 rpm. Accurately weighed amount of micro particles were placed in the baskets. Aliquots of samples were withdrawn at the interval of 1 hour for 8 hours in phosphate buffer pH 6.8. The samples withdrawn were filtered, diluted suitably and analyzed by spectrophotometrically for drug release<sup>9</sup>.

#### Anti-bacterial activity

The antimicrobial activity of the *Phyllanthus amarus* extract loaded micro particles was evaluated by agar well diffusion method. Bacteria were grown in Muller Hinton broth. After inoculation, plates were dried for 15 minute, and the wells were punched using sterile corn borers. Once wells were formed, they were filled with 100 µL gel and blank water. Plates were incubated for 24 h at 37 °C to allow gel to diffuse through the agar media to form zones of inhibition. The diameters of the zone of inhibition for different extracts against different bacteria were measured in millimeter for further analysis. An agar well (6 mm) showing zone of inhibition was considered as antimicrobial activity. All experiments were done in triplicate and the average values were used for drawing bar diagram<sup>10</sup>.

## RESULT AND DISCUSSION

All the results were presented in appropriate tables and figures. The below figures shows the herbal micro particles containing *Phyllanthus amarus* extract.



**Figure: 2 Herbal Micro particles Containing *Phyllanthus Amarus* Extract**

### **PREFORMULATION STUDIES:**

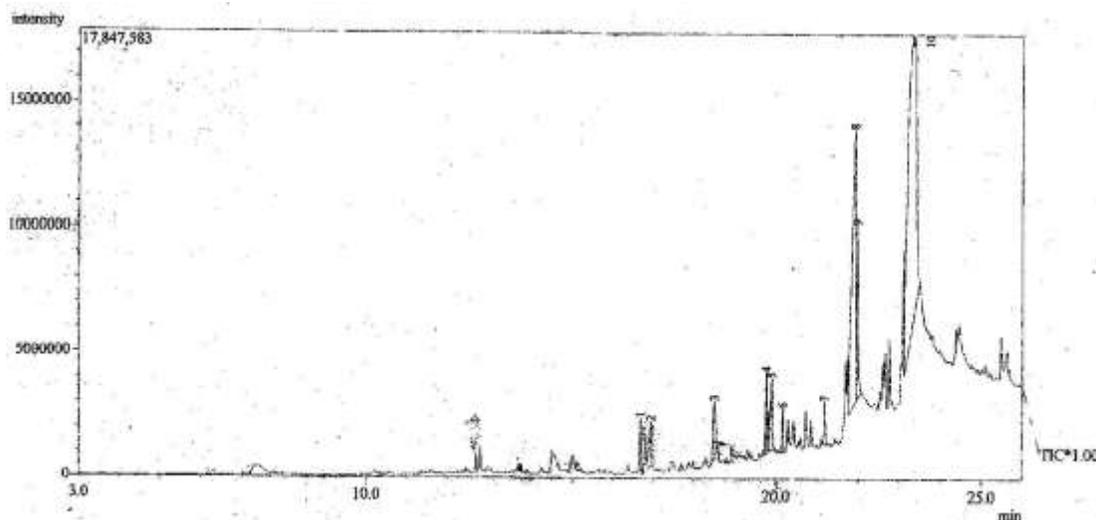
#### **Organoleptic Properties:**

The organoleptic properties of *Phyllanthus Amarus* extract was found to be Brownish green color, slightly odour and bitter taste was observed.

#### **Solubility studies :**

The solubility studies of *Phyllanthus Amarus* extract was found to be the extract insoluble in ether, chloroform and water soluble in ethanol slightly soluble in HCL.

### **GC-MS ANALYSIS:**

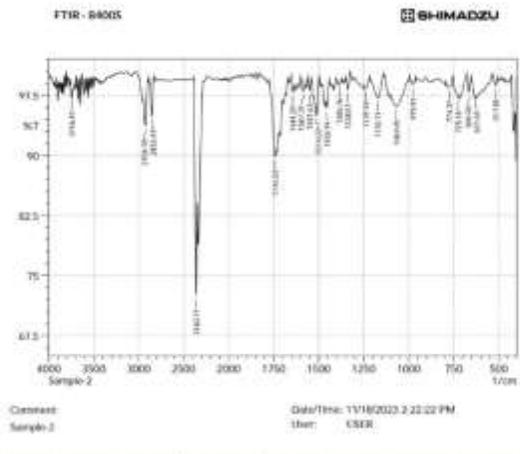


**Figure 3: Gas Chromatography Mass Spectroscopy of *Phyllanthus amarus***

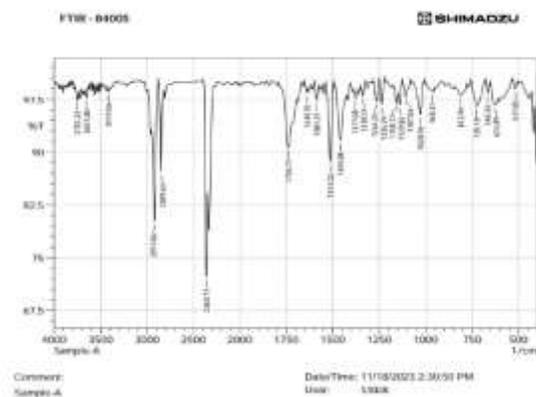
*Phyllanthus amarus* plant contains several chemical compounds. In GC-MS study, six chemical compounds were separated and identified as antimicrobial activity. They are –Heptadecene, Hexadecanoic acid, 1-methylethyl ester, 9,12,15-Octadecatrienoic acid, Ascorbic acid 2,6-dihexadecanoate, Methanesulfonic acid, 2-(2-hydroxy-hexahydropentalen-3a-yl)-ethyl ester and Dodecanoic acid.

### **FT - IR SPECTRAL STUDIES:**

FT - IR studies of pure *Phyllanthus amarus* extract and combination of drug and excipients was carried out to found any interaction between drug and excipients used in the formulation..



IR Spectrum of *Phyllanthus amarus* Extract



IR Spectrum of formulation F2

**ESTIMATION OF PERCENTAGE DRUG YIELD:**

**Table 3: The Percentage Drug Yield of *Phyllanthus Amarus* Micro Particles**

S.No	Formulations	Drug : Polymer	Percentage drug yield(%)
1	F1	1:0.5	81.48
2	F2	1:1	93.75
3	F3	1:1.5	70.27

**Discussion:**

The amount and percentage drug yield formulations were estimated by UV spectrophotometric method. The percentage drug yield of all formulations were found in the range of 70.27 % to 93.75%. The F2 showed maximum percentage drug yield 93.75% than other formulations.

**DRUG ENTRAPMENT EFFICIENCY (DEE):**

**Table 4: Drug Entrapment Efficiency of *Phyllanthus amarus* micro particles**

Formulations	Absorbance at 275 nm	Theoretical yield (mg)	Practical yield (mg)	%Drug Entrapment Efficiency
F1	0.054	20	16.41	82.05
F2	0.071	20	18.24	91.2
F3	0.049	20	15.65	78.25

**Discussion:**

The Drug Entrapment Efficiency of *Phyllanthus amarus* micro particles formulations were found in the range of 78.25 % to 91.20%. The F2 showed maximum percentage drug yield 91.20% than other formulations.

**FLOW PROPERTIES PHYLLANTHUS AMARUS MICRO PARTICLES**

Flow property depends on particle size, shape, porosity and density of micro particles shown in table 14

**Table 5: Flow Properties *Phyllanthus amarus* micro particles**

Formulations	Bulk Density	Tapped density	Car's index	Hausner's Ratio	Flow Property	Angle of repose
F1	0.170	0.196	13.26	1.152	Good	34°99'
F2	0.174	0.203	14.20	1.170	Good	33°42'
F3	0.169	0.200	15.5	1.186	Good	30°11'

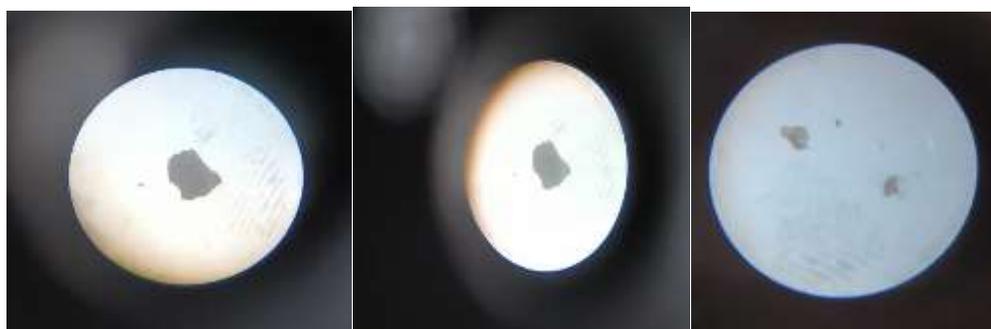
**Discussion:**

All the formulation shown in good flow properties.

**PARTICLE SIZE ANALYSIS:**

Particle size determination was done by optical microscopy. The size range of about 100 particles was determined by microscopy. The eyepiece micrometer was first calibrated to calculate the factor.

The particle size range of three formulation are shown in below figures.



**Figure 4: Microscopic Images of Micro particles**

**Table 6: Particle Size Analysis of Different batches of Micro particles**

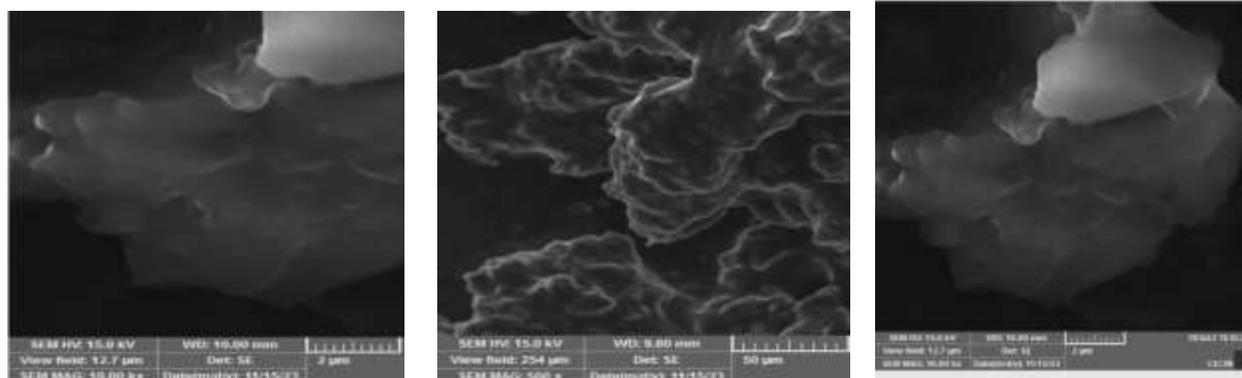
S.No	Formulations	Drug : Polymer	Particle size (µm)

1	F1	1:0.5	51.48
2	F2	1:1	43.75
3	F3	1:1.5	60.27

**Discussion:**

The particle size range of all three formulation were found t be 43.75-60.27( $\mu\text{m}$ ) with in official range particle size

**SCANNING ELECTRON MICROSCOPY (SEM)**



**Figure 5: SEM Photographs of *Phyllanthus Amarus* Micro particles**

**Discussion**

Scanning electron microscopy was carried out the optimized formulation F2 (ratio1:1), it has a smooth surface without lumps. The size range from 2 to 50  $\mu\text{m}$ .

**IN VITRO DISSOLUTION STUDIES OF MICROPARTICLES:**

**Table 7: In Vitro Dissolution Study of *Phyllanthus Amarus* Extract Loaded Microparticles**

S.NO	Time (H)	F 1(1:0.5)%	F 2(1:1)%	F 3(1:1.5)%
1	1	10.13	9.44	9.03
2	2	15.92	13.72	19.38
3	3	26.83	26.14	30.84
4	4	38.71	40.08	48.92
5	5	51.68	59.82	55.68
6	6	63.55	68.93	67.83
7	7	68.02	83.15	79.56
8	8	75.71	92.53	85.91

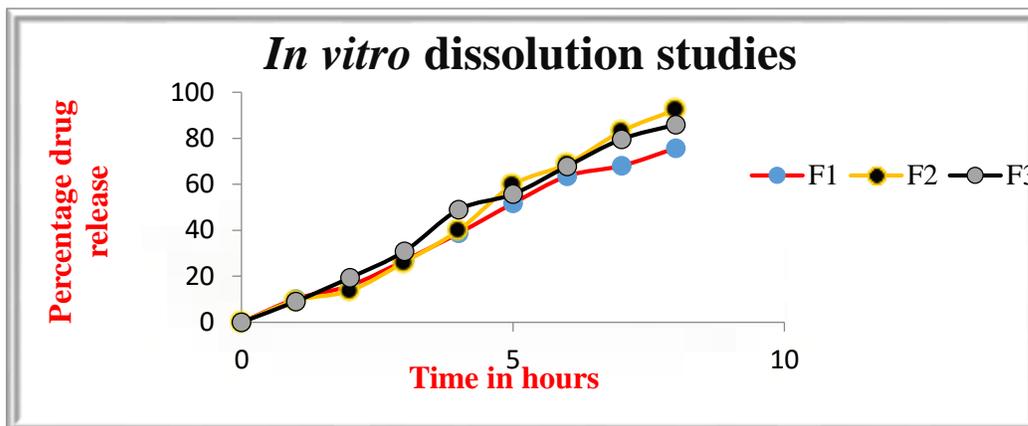


Figure 6: In vitro dissolution profiles of different batches

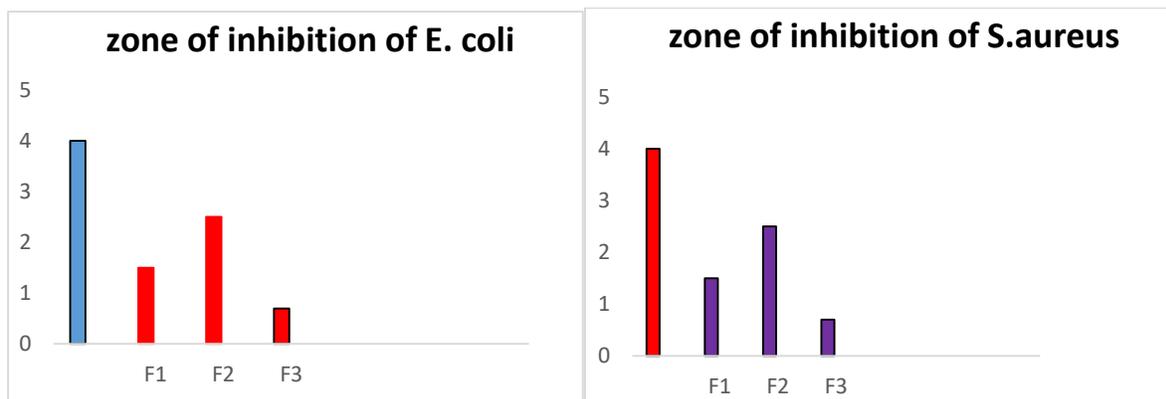
**Discussion:** In vitro drug release of *Phyllanthus amarus* micro particles formulations were carried out as per the procedure. The percentage release of drug from different Formulation F2 (1:1) produce maximum drug release 92.53% at the end of 8 hours.

**ANTI-BACTERIAL ACTIVITY OF *PHYLLANTHUS AMARUS* MICRO PARTICLES FORMULATIONS:**

Gram positive bacteria *Staphylococcus aureus* and Gram negative bacteria *E.coli* was used by Agar well diffusion method. Anti-microbial activity of *Phyllanthus amarus* micro particles results were presented in below Tables and Figures.

S.No	Bacteria used	Antibiotics	(STD) zone	F1 zone	F2zone	F3zone
1	Escherichia coli	Amikacin	4	1.5	2.5	0.7
2	Staphylococcus Aureus	Erythromycin	4	1.3	2.3	1.0





**Figure 7: Anti- Bacterial Activity of *Phyllanthus amarus* Micro particles Formulations**

## Discussion

The study of *Phyllanthus amarus* extract loaded microspheres was done by results showed good anti - bacterial activity.

## CONCLUSION

Formulation and *in vitro* evaluation of microspheres containing *Phyllanthus amarus* extract and study of its Anti-bacterial activities are successfully carried out. The technique chosen microencapsulation with gelatin by coacervation phase separation with temperature change and cross linking with glutaraldehyde was able to sustain the release effectively. Because of good results of micro particles having core: coat ratio of 1:1 and size range of 2-100  $\mu\text{m}$  gave the best sustained release effects. The study concludes that the developed formulation F2 consisting 0.2 gm of *Phyllanthus Amarus* with 0.2gm gelatin was found to be promising microspheres for the treatment of some Struvite type of kidney stones.

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