FORMULATION AND EVALUATION OF PHYLLANTHUS NIRURI HERBAL NANOPARTICLES FOR THE NATURAL TREATMENT OF SICKLE CELL ANAEMIA

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Abstract:

Sickle cell anemia is characterized by sickled-shaped erythrocytes, which is caused due to production of abnormal hemoglobin. It causes clogging of capillaries and blocks blood flow in the blood vessels, leading to severe complications like ischemia, hypoxia, and organ failure. Some marketed drugs are available for treating sickle cell anemia, like Hydroxy-urea, but due to their severe adverse effects, these medications are not given frequently. Therefore, herbal nanoparticles were designed as an alternative source to treat sickle cell anemia to solve these problems. The plant material was imported from the authorized market, and they were dried and powdered. The extraction process and isolation of the constituents were done simultaneously. The extracted active component was formulated into nanoparticles using five different formulations (F1-F5). Finally, the Phyllanthus niruri herbal nanoparticle was characterized, and the anti-sickling property was determined.

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1. Introduction:

Sickle cell anemia (S.C.A.) is defined as abnormally (Sickle) shaped red blood cells which are entirely demolished and eliminated from the systemic circulation, and it is characterized as Sickle hemoglobin (HbS) [1,2]. HbS is an irregular structural variation of normal adult hemoglobin (HbA) caused due to mutations in the H.B.B. gene that results in the substitution of valine over glutamic acid at the sixth position of the globulin subunit (S) of the hemoglobin molecule [2]. There is two most frequent type of S.C.A., first is SCA SS type, which is a homozygous type of S.C.A. that generate no HbA but primarily produce HbS with varying quantities of HbF and HbA2, and the second is SCA SC type which creates mostly HbS and HbC [3, 4]. The most common variant (> 70%) of S.C.A. found globally is a homozygous known as the "SCA SS" variant [5]. Hypoxia and cellular dehydration cause polymerization of HbS within the average red blood cells, thus leading to the deformation of normal erythrocytes into sickle-shaped erythrocytes. This sickling effect leads to occasional microvascular blockage, ischemia and reperfusion, and elevated production of vascular oxidases [6]. Several marketed drugs are available for treating sickle cell anemia, like Hydroxyurea (Droxia and Hydrea), L-glutamine oral powder (Endari), etc. Still, due to their severe adverse effects on the body, these drugs are not recommended frequently; therefore, Phyllanthus niruri herbal nanoparticles were prepared and examined as an alternative.

2. Material and methods:

1. Acetone (Yarrow chemicals, Mumbai)
2. Methanol (Desai chemicals company, Visakhapatnam)
3. α-Naphthol (Desai chemicals company, Visakhapatnam)
4. Seliwanoff's reagent (Yarrow chemicals, Mumbai)
5. Dragendorff's reagent (Yarrow chemicals, Mumbai)
6. Sodium nitroprusside solution (Yarrow chemicals, Mumbai)
7. Ferric chloride solution (Yarrow chemicals, Mumbai)
8. Ninhydrin reagent (Yarrow chemicals, Mumbai)
9. Phenolphthalein (Yarrow chemicals, Mumbai)
10. H.C.L. (Yarrow chemicals, Mumbai)
11. Catachein (Desai chemicals company, Visakhapatnam)
12. Quercetin (Desai chemicals company, Visakhapatnam)
13. Silica gel 60 (Yarrow chemicals, Mumbai)
14. Chloroform (Yarrow chemicals, Mumbai)
15. Methanol (Yarrow chemicals, Visakhapatnam)
16. Folin ceocalteu reagent (Yarrow chemicals, Mumbai)
17. 7.4pH phosphate buffer (Prepared in the GITAM laboratory)
18. S.L.S. buffer (Prepared in GITAM laboratory)
19. Chitosan (Ultrapure lab, Maharashtra)
20. Glacial acetic acid (Qualikems, Gujarat)
21. Tri-sodium poly phosphate (Qualikems, Gujarat)
22. Potassium dihydrogen orthophosphate (Qualikems, Gujarat)
23. Sodium hydroxide (Qualikems, Gujarat)

24. Distilled water (Prepared in GITAM laboratory)

3. Isolation of phyllanthus niruri:

The dried roots and stem part of *Phyllanthus niruri* were imported from Yucca Enterprises, Mumbai, and it was identified and verified by the Harshad M.Pandit, Ph.D. (Botany). The roots and stems were air-dried and mechanically milled using a hand mill to get fine powder, then stored in an air-tight container. The extraction process was carried out in Soxhlet's apparatus. Initially, a cotton ball was placed at the bottom of the device. A filtering disc was established, followed by 0.5kg of fine powdered material. Again, the filtered disc was set, and the power material refluxed for 48 hours using methanol as a solvent. Finally, the extract was condensed using a rotary evaporator at 65^{o}C to remove the remaining methanol from the crude extract, as shown in figure 2 [7].

4. Phytochemical screening of herbal extract [8-21]:

4.1. Screening test for carbohydrates:

4.1.1. Molisch's Test: Initially, test solution and α-naphthol were taken in a test tube, then a few drops of concentrated H_2SO_4 was added. The Reddish-violet color at the junction of two liquids indicates the presence of carbohydrates.

4.1.2. Seliwanoff's Test: Take 1ml of sample and add 5ml of seliwanoff's reagent, then boil the mixture in a hot bath for 5min. Rose color indicates the presence of carbohydrates.

4.1.3. **Osazone formation:** Test solution containing the solution of phenyl-hydrazine H.C.L., sodium acetate, and acetic acid was heated. Yellow color crystals indicate the presence of carbohydrates.

4.2. Screening test for Alkaloids:

4.2.1. Dragendorff's Test: Take a few milligrams of plant extract dissolved in 5ml of water. Then add 2M H.C.L. to create an acidic environment for the hydrolysis sample. Then add 1ml of dragendroff's reagent into the mixture. A Reddish brown color precipitate can confirm the presence of alkaloids.

4.2.2. Wagner's Test: The plant extract was acidified with H.C.L. (1.5%v/v) and add few drops of Wagner's reagent in the test tube. A Reddish brown color precipitate can confirm the presence of alkaloids.

4.2.3. Hager's Test: In 1ml of plant extract, add 1ml of Hager's reagent (picric acid). The presence of yellow color precipitate can confirm the presence of alkaloids.

4.3. Screening test for Glycosides:

4.3.1. Legal's Test: Plant extract was dissolved in 1ml pyridine, then a few drops of sodium nitroprusside solution was added. The presence of blood red color can confirm the presence of Glycoside.

4.3.2. Baljet's Test: Add 1ml of sodium picrate solution to the plant extract. The presence of blood red color can confirm the presence of Glycoside.

4.3.3. Forth formation test: to the plant, Extract 1ml of distilled water and Shake the test tube vigorously. The formation of Froth indicates the presence of Glycoside.

4.4. Screening test for Flavonoids:

4.4.1. Shinoda test: Alcoholic plant extract sample is taken in the test tube, and 5-10 drops of hydrochloric acid are added. Then add small pieces of magnesium to the test tube. If reddish pink color is observed, it indicates the presence of flavonoids,

4.5. **Screening test for Tannins:**

4.5.1. Ferric chloride ($Fecl_3$) Test: Add 1ml of ferric chloride solution ($Fecl_3$) to the plant extract. The greenish-black or dark blue color can confirm the presence of Glycoside.

4.5.2. Chlorogenic Test: Add 1ml of aqueous ammonia to the plant extract and then expose it to air for 1 minute. If green color is observed, it indicates the presence of tannins.

4.6. Screening test for proteins and amino acids:

4.6.1. Warming test: plant extract solution was heated in a boiling water bath for 2 minutes. If coagulation occurs, it indicates the presence of protein and amino acids.

4.6.2. Hydrolysis test: to the plant extract solution, add I ml of hydrochloride or sulphuric acid for hydrolysis reaction, then later add a few drops of Ninhydrin reagent, and the answer was boiled under a water bath. If a violet color is observed, it indicates the presence of proteins.

4.7. Screening test for lipids:

4.7.1. Saponification test: To the plant extract, add **a** few drops of 0.5N alcoholic K.O.H. and phenothiazine and heat in the water bath for 1-2 hours. Indication of foam formation confirms the presence of lipids.

4.8. Screening test for Ketones:

4.8.1. Test for pentose: Add hydrochloride acid to the sample solution containing a small amount of phloroglucinol. The red color indicates the presence of ketones.

4.9. Screening test for steroids:

4.9.1 Liebermann-Burchard test: Add I ml of chloroform solution to the plant extract solution, then the acetic anhydride and concentrated sulphuric acid are treated with chloroform plant extract. If a Green color ring is formed, it indicates the presence of steroids.

4.10. Screening test for Phenols:

4.10.1 Phenolic test: To the plant extract, add 1 ml of methanol, then take out a few milliliters of methanolic plant extract and add 1ml of water in a test tube, followed by adding 1-2 drops of ferric chloride solution ($Fecl_3$). The green color indicates the presence of phenol.

5.0 Determination of total phenolic content:

Take 0.5ml of plant extract, 5ml of distilled water, and 0.5ml of folic-ciocalteu (F.C.) reagent was prepared and kept aside for 3 minutes. Then add 1ml of 20% (w/v) sodium bicarbonate

(Na_2CO_3) into the mixture. Incubated the mixture for 1hr and observed it in a Calorimetric device at 725nm. The total phenol content is determined by using the equation given below,

$$Y = 1.7097 \ln(X) + 5.206$$

Where X=Absorbance and Y= Gallic acid equivalent weight (mg/ml)

6.0 Determination of total flavonoids:

Take 3ml of plant extract and mix with 3ml of aluminum chloride solution ($AlCl_3$), then constantly stir the mixture for 3 minutes. Finally, incubate the mixture for 1hr and observe under the calorimetric instrument at 415nm. The total Flavonoid content is determined by using the equation given below,

$$Y = 0.5001 \ln(X) + 3.442$$

Where, X=Absorbance and Y= Quercetin Equivalent weight (mg/g).

7.0 Determination of total Anthocyanin:

Take 0.5ml of plant extract, 3ml of vanillin-methanol (4%v/v), and 1.5ml of H.C.L. were mixed and stirred for 3min, then incubated the mixture for 1 hour and observed under a calorimetric instrument. The total Anthocyanin content is determined by using the equation given below,

$$Y = 0.0728 \ln(X) + 0.0171$$

Where X=Absorbance and Y= catechin Equivalent weight of (mg/g).

8.0 assessment of the anti-sickling activity:

8.1. Blood samples:

The blood samples are collected from the sickle cell patients from the hospital. Those blood samples were brought to the laboratory and kept in the refrigerator. The tests were done one day before to ensure that these blood samples were taken from sickle cell patients.

9.0 isolation of pure compounds from the herbal extract:

Column chromatography is the most commonly used method for isolating active compounds from herbal extracts. It is mainly of two types,

Dry Technique: Firstly, the column is packed with dry silica powder. Then the mobile phase, along with suitable solvent, is passed through the dry powder silica so that it gets moist and settles down to make the column moist with solvent. [22]

Wet Technique: A slurry containing dry silica powder and the solvent is prepared, then it is poured over the column with the help of a funnel. The solvent must be added until the dry silica powder is dissolved [22].

10.0 Gallic acid:

Column chromatographic method for extraction and purification of Gallic acid from Phyllanthus niruri extract is designed in this study. The stationary phase is silica gel, and the mobile step is a solution of chloroform and methanol (40:60), respectively.

The differential scanning calorimeter (D.S.C.) technique is used to characterize each active compound extracted during the chromatographic process, and Infrared spectroscopy of these active compounds is done by using the Fourier transform (FTIR) [23].

11.0 preparation of the column:

The methodology used for the preparation of the column was the dry method. For the 30ml of the plant extract, around 600g of silica gel is taken. Initially, glass wool was placed at the bottom of Soxhlet, silica gel 60 was poured onto it, followed by the sample placed, and finally, the mixture of the continuous phase was poured over the sample. [24]

12.0 characterization of the isolated pure component:

12.1. Differential Scanning Calorimetry (D.S.C.): It is a thermo-analytical technique that measures the significant difference in heat raise of a test and the standard solution, respectively, as a function of temperature. During the entire experimental process, the Test and the standard are kept at the same temperature. The temperature program in this study is often designed so that the sample holder temperature increases linearly over time. The standard should have a well-defined heat capacity over the scanned temperature range. [25]

STA 7300 (HITACHI, Japan), operating with TA 700 software, is used to determine the extracts' D.S.C. spectra for active compounds. The temperature range set was around 30^0 C - 350^0C with a constant heating rate at 10^0 C min^{-1} for heating samples, under nitrogen flow rate with pin-holed aluminum pans. The following D.S.C. plots of the sample are shown in fig 13.

12.2. FTIR Spectroscopy: It is a technique primarily used in industry and academic institutions to study individual molecules' structural behavior in a given mixture. FTIR spectroscopy employs modulated mid-infrared radiation to probe a material. Infrared light is absorbed in specific frequencies which have a connection with the vibrational bond energies of the atoms in the molecule. Molecules can absorb energy when the bond energy of the vibration frequency and the frequencies of mid-infrared light are the same. Because molecule with various bonds vibrates at different frequencies, they thus absorb Infrared light at different wavelengths. The location (frequency) and strength of individual specific absorption bands contribute to the overall spectrum, thus forming the molecule's unique fingerprint. [26]

Perkin Elmer (I.R. Spectrophotometer model: Spectrum R.X.I.), having potassium bromide disc as standard, is used to determine FTIR spectra of plant extracts. The FTIR plot of plant extract is shown in fig 14.

13.0 calibration curve of phyllanthus niruri:

To prepare the stock solution (1mg/ml or 1000µg/ml), take 10 milligram of Gallic acid and dissolve it in 10 milliliters of distilled water. Then take 1ml of stock solution and transfer it into the 50ml volumetric flask along with 49ml of 7.4pH phosphate buffer, referred to as working solution (20µg/ml). Dilutions series of 0.25ml, 0.5ml, 0.75ml, 1ml, and 1.25ml respectively is prepared. Then add 0.1ml of folic catechu reagent to the test tube and keep aside for 6 minutes. Finally, add 1ml of 7% sodium carbonate solution and the absorbance is recorded every 90min in the triplicate form [27].

14.0 preparation of herbal extract nanoparticles:

A suitable method should be selected for the preparation of nanoparticles; therefore, in this study ionic-gelation method is used to prepare the herbal extract nanoparticles.

S. No	Ingredient (in percentages)	F-1	F-2	F-3	F-4	F-5
1.	Methanolic extract of Phyllanthus niruri	05	05	05	05	05
2.	Chitosan	0.25	0.5	1	1.5	2
3.	Acetic acid	1.6	1.6	1.6	1.6	1.6
4.	TPP	0.5	0.5	0.5	0.5	0.5

14.1. Ionic-gelation method:

The ionic gelation method is used to design the Phyllanthus niruri herbal nanoparticles. The solution is subjected to a magnetic stirrer to mix the active components in the solution; when the active ingredient of herbal extract (5%) has been completely dissolved, add Tri polyphosphate (T.P.P.). After this, the solution is agitated for another 2 hours before being centrifuged for 10 minutes at 2000 rpm. The supernatant is removed, and the pellets are re-suspended in phosphate-saline buffer, followed by centrifugation of pellets at 2000 rpm for 10 minutes to produce the herbal nanoparticles. Finally, this nanoparticle is rinsed with distilled water. Stirring is maintained for half an hour after the Opalescent hue is detected, and the pellet produced will be rinsed three times with distilled water. The nanoparticles are lyophilized and kept at 40^{o}C for further usage. The following nanoparticle formulation is given in table 1.

Table 1: Formulation of herbal Nanoparticle Formulation employing methanolic extract of Phyllanthus niruri.

15.0 Preparation of isolated active ingredient (gallic acid) into nanoparticles:

The ionic-gelation method is used to prepare the isolated component nanoparticles. The procedure followed in the preparation is similar to the preparation of Phyllanthus niruri herbal nanoparticles. The formulation for preparing the gallic acid nanoparticle is shown in table 2.

Table 2: Formulation of the isolated active component (Gallic acid) Nanoparticle

S.No	Name of the ingredient (in percentages)	F1	F2	F3	F4	F5
1.	**Gallic acid**	5	5	5	5	5
	Chitosan	0.25	0.5	1	1.5	2
	Acetic acid	1.6	1.6	1.6	1.6	1.6
	TPP	0.5	0.5	0.5	0.5	0.5

16.0 Characterization of herbal Phyllanthus niruri nanoparticles:

16.1. Structural behavior studies of nanoparticles:

The structural behavior of herbal nanoparticles is done by using scanning electron microscopy. We placed 100l of the herbal nanoparticle formulation at the initial stage onto a glass slide (10mm). We dried them overnight inside a vacuum desiccator at room temperature up to the

S.E.M. examination. Using a gold sputter technology, the herbal nanoparticles are mounted on appropriate support to coat them with gold in a high vacuum evaporator. Finally, nanoparticle examination is done at various magnifications in 15kv [28].

16.2. Percentage Encapsulation Efficiency of Drug:

Drug encapsulation efficiency of herbal nanoparticles is determined by using the ultra-centrifugation technique. This technique removes additional nanoparticle constituents from the herbal nanoparticle at 12,000 rpm in half an hour. The pellets are dissolved in distilled water, and the supernatant is scanned with a UV-visible spectrophotometer. The drug encapsulation efficiency is calculated by using the following equation given below.

$$\%\ \text{drug entrapment efficiency} = \frac{\text{Amount of drug}}{\text{Amount of drug} + \text{polymer}} * 100$$

16.3. Production yield of nanoparticles:

The yield of nanoparticles is the total weight of the nanoparticles produced. It is determined by using the following equation given below,

$$\%\ \text{Yield Determination} = \frac{\text{Weight of drug}}{\text{Weight of drug} + \text{polymer}} * 100$$

16.4. Drug Dissolution Studies:

The in-vitro release studies of herbal nanoparticles are done using phosphate-saline buffer (P.H.- 7.4) at 37^{o}C. Herbal nanoparticles are dialyzed for 60 minutes against 50 ml phosphate-saline buffer with continuous shaking inside a dialysis bag. Aliquots are removed and simultaneously replaced by a new volume of aliquots in phosphate-saline buffer at regular intervals. The amount of active compound released is determined by measuring absorbance using a UV-visible spectrophotometer [29, 30].

16.5. Emmel's Test:

The concentration of extract (1mg/ml) is prepared using saline solution (0.9% NaCl) as a solvent. Using the above solution, we prepared a microscopic slide, where a drop of sickle cell anemia blood was placed in the slide, then added a few drops of 2% $Na_2S_2O_5$ to develop a hypoxic condition. After that, add a few drops of extract to avoid air bubble formation. Another slide referred to as negative control is prepared without using the extract under the same conditions. The slide edges are supercooled with the help of paraffin to maintain the hypoxic condition. The results were seen after one day and carried out in triplicate.

17.0 RESULTS & DISCUSSION:

17.1. Phytochemical Screening Results:

It showed that Phyllanthus niruri consists of Alkaloids, Tannins, and Phenols, while carbohydrates, Glycoside, and proteins were absent, as shown in Table 3.

17.2. Phenolic, Flavonoid, Anthocyanins content:

After the following tests are done, the Phyllanthus niruri contains the following phenolic, flavonoid, and anthocyanins, as shown in Table 4.

S. No	Name of test	Phyllanthus niruri
1.	Molisch's Test	-VE
2.	Seliwinoff's Test	-VE
4.	Osazone test	-VE
5.	Dragendroff's test	+VE
6.	Wagner test	+VE
7.	Hagers test	+VE
8.	Legal's Test	-VE
9.	Baljit's Test	-VE
10.	Froth formation test	-VE
12.	Shinoda test	+VE
13.	Ferric chloride test	+VE
14.	Chlorogenic Test	+VE
15.	Warming test	-VE
16.	Hydrolysis test	-VE
17.	Saponification test	-VE
18.	Test for pentose	-VE
19.	Liebermann-Burchard test	-VE
20.	Phenolic Test	+VE

Table: 3 Phytochemical Screening tests results of Phyllanthus niruri

S. No	Plant name	Total polyphenolic content mg/EGA	Flavonoid content mg/EQ	Anthocyanin content mg/E.C.
1.	Phyllanthus niruri	5.988	3.651	0.0044

Table: 4 Phenolic, Flavonoid, Anthocyanins content of Phyllanthus niruri

17.3. Emmel's Test:

The Emmel's Test was done in K.G.H. hospital, Visakhapatnam. The following Emmel's Test concluded that Phyllanthus niruri herbal nanoparticles had anti-sickling properties as represented in fig 1, 2.

17.3.1. Control Group of Sickle cell anemia patients:

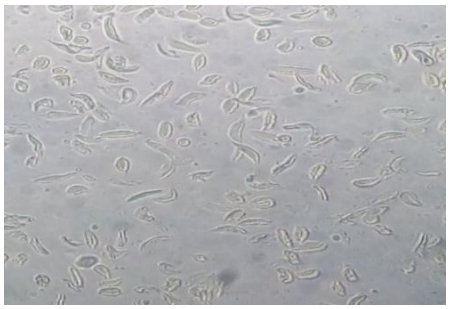
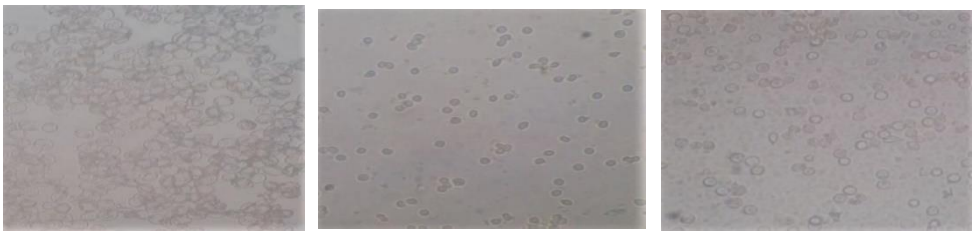


Figure 1: Slides representing the control group of R.B.C. of Sickle cell anemia patient

Phyllanthus niruri plant showing anti-sickling property:



n=1	n=2	n=3

Figure2: Phyllanthus niruri showing anti-sickling property (Emmel's Test)

17.4. Differential Scanning Calorimetry plot of Phyllanthus niruri:

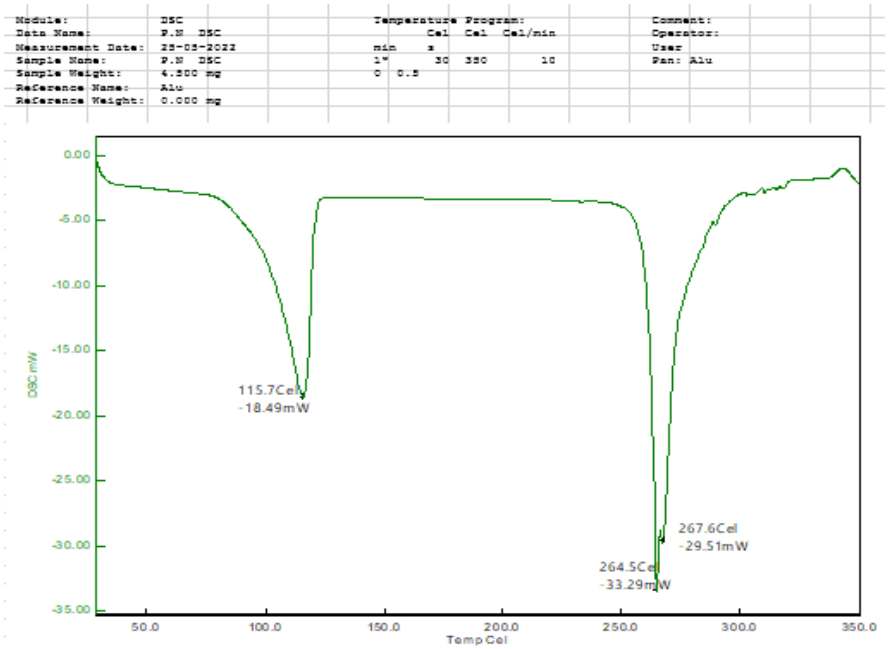


Figure 3: D.S.C. plot of pure component obtained from Phyllanthus niruri extract

17.5. Fourier transmission infrared spectroscopy analysis of Phyllanthus niruri:

The peaks of pure components obtained in the FTIR analysis represent the functional group present in the Gallic acid. Therefore, the isolated pure part obtained from Phyllanthus niruri is assumed to be Gallic acid. The FTIR peaks of different functional groups for their wavelength obtained from Phyllanthus niruri are given in table 5.

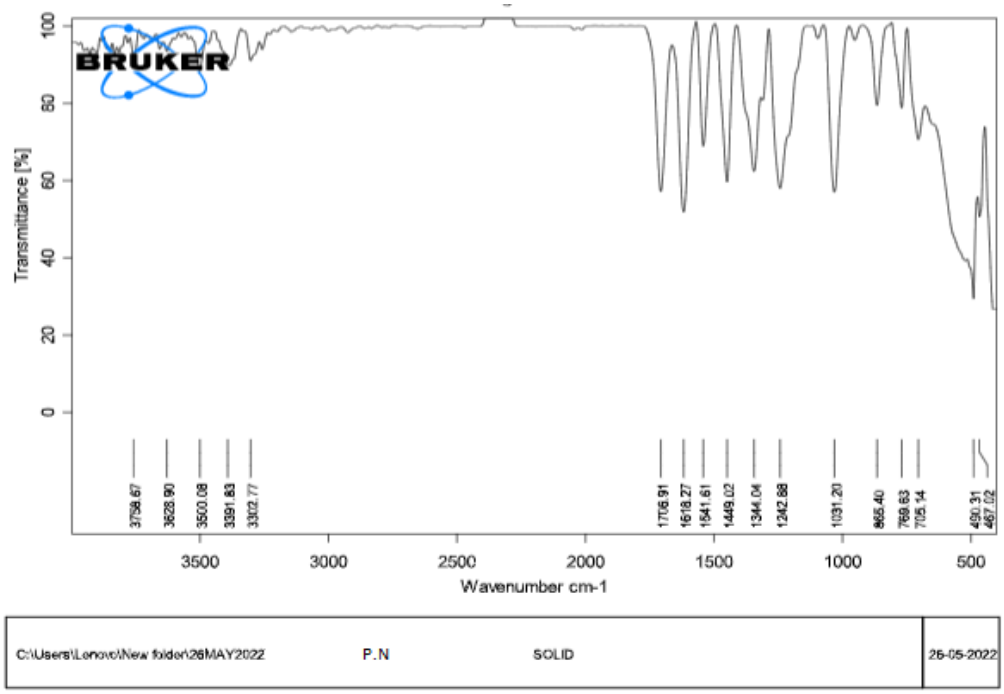


Figure 4: FTIR plot of pure component obtained from Phyllanthus niruri.

Table 5: FTIR analysis of Phyllanthus niruri

S.No	Group	Wavenumber
1.	C=O	1706.91cm^{-1} ,1618.27cm^{-1}
2.	C=C	1541.61cm^{-1} ,1449.02cm^{-1}
3.	C-OH	1344.04cm^{-1}
4.	C-O	1242.68cm^{-1}
5.	C=H	467.02cm^{-1} ,436.76cm^{-1}

17.6. Calibration curve for the pure compounds isolated from the herbal extract:

The calibration curve of Gallic acid (pure compound) is constructed, and it represents a linear plot between absorbance (Y-axis) and concentration (X-axis); thus, the curve follows the Beer-Lambert law as shown in fig 5.

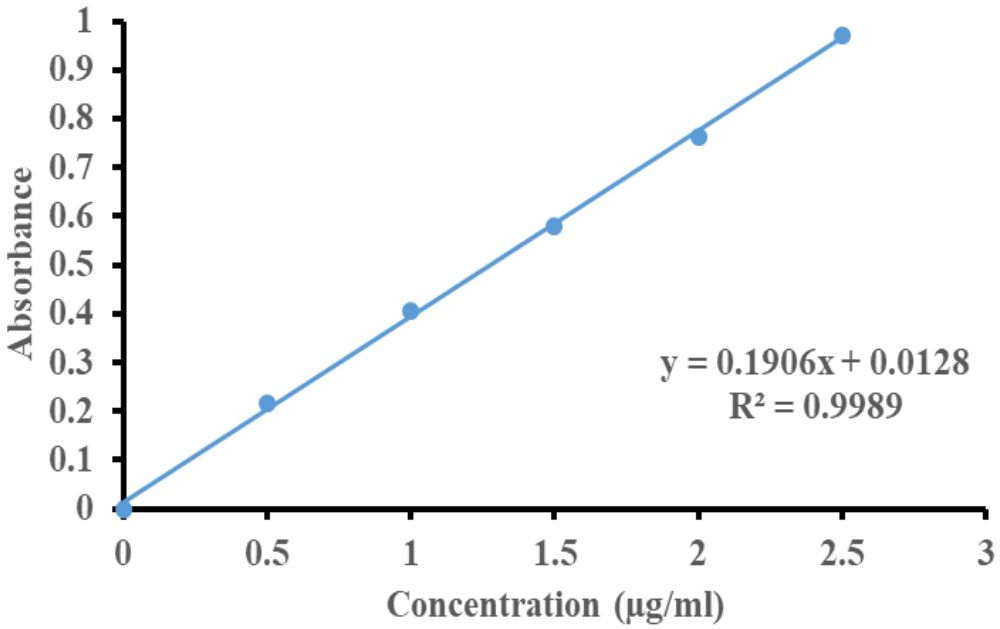


Figure 5: Calibration curve of Gallic acid.

17.7. Scanning Electron Microscopy (S.E.M.) studies of Phyllanthus niruri:

S.E.M. images of Gallic acid nanoparticle formulation five are shown in figure 6, where the size of Gallic acid nanoparticle in formulation 5 is in the appropriate range (49.9-86.42nm).

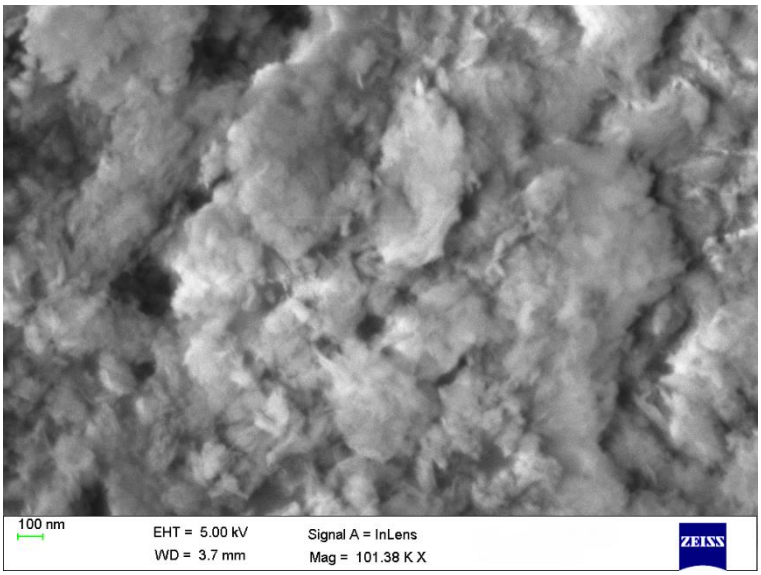


Figure 6: S.E.M. of Gallic acid nanoparticles of (F5)

17.8. Drug encapsulation efficiency of herbal nanoparticles:

The percentage encapsulation efficiency of herbal nanoparticles is calculated, and the following results are shown in Table 6.

Table 6: Percent Drug encapsulation efficiency, n=3, mean= ± S.D

S. No	Name	% Drug Encapsulation efficiency
1.	Gallic acid	49.3±1.56

17.9. Production yield of Phyllanthus niruri extract herbal Nanoparticles:

The percentage yield of herbal nanoparticles of the different formulations is calculated, and their results are summarized in table 7.

Table 7: Production yield of herbal nanoparticles, n=3, mean= ± S.D

S.NO	Extract	F1	F2	F3	F4	F5
1.	Phyllanthus niruri	7.28±1.56	8.87±1.32	19.71±1.35	45.97±1.26	80.45±0.64

17.10. Production yield of isolated Gallic acid Nanoparticles:

The percentage yield of Gallic acid nanoparticles derived from different formulations is calculated, and it was found that formulation 5 produced more yield (92.52±0.53) compared to other formulations. The results are summarized in table 8**.**

Table 8: Production yield of Gallic acid nanoparticles, n=3, mean±S.D

S.No	Isolated component	F1	F2	F3	F4	F5
1.	Gallic acid	8.74±1.43	9.80±1.23	20.13±1.53	48.88±1.35	92.52±0.53

***17.11. In-vitro* release study of Phyllanthus niruri:**

17.11.1. Cumulative percent release of Gallic acid: The cumulative percent release studies of different formulations were performed. A plot between percent drug release (Y-axis) and Time (X-axis) is shown in figure 7.

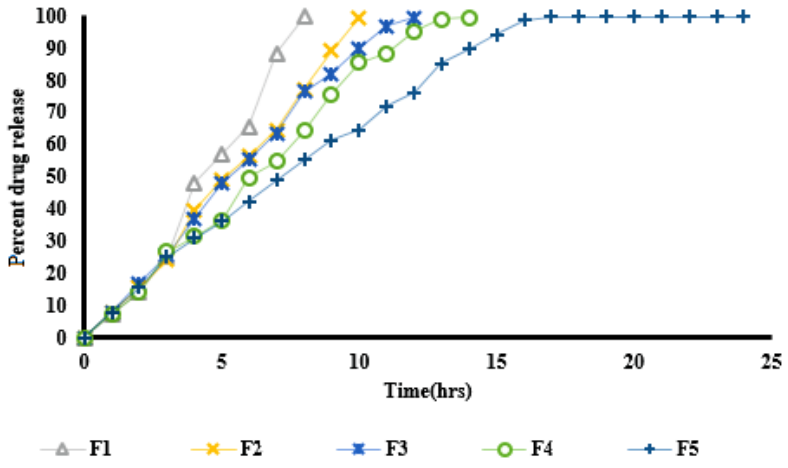


Figure 7: % Drug Release vs. Time plot of Gallic acid (F1-F5) nanoparticles.

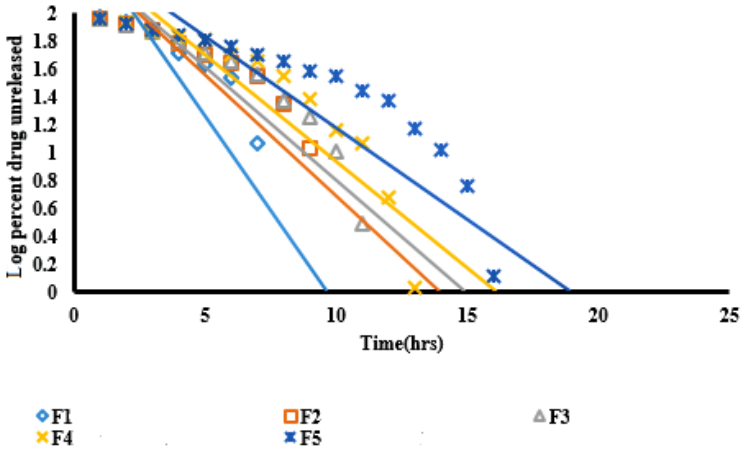


Figure 8: Log percent unreleased vs. Time percent release plots of Gallic acid (F1-F5) nanoparticles

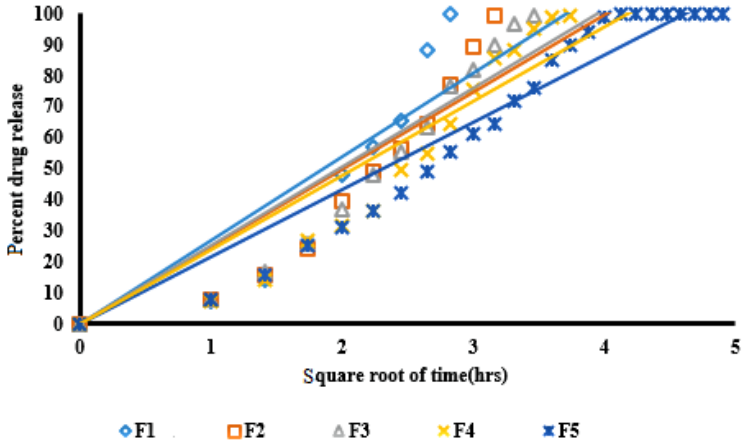


Figure 9: Percent drug release vs. Square root of time plots of Gallic acid (F1-F5) nanoparticles

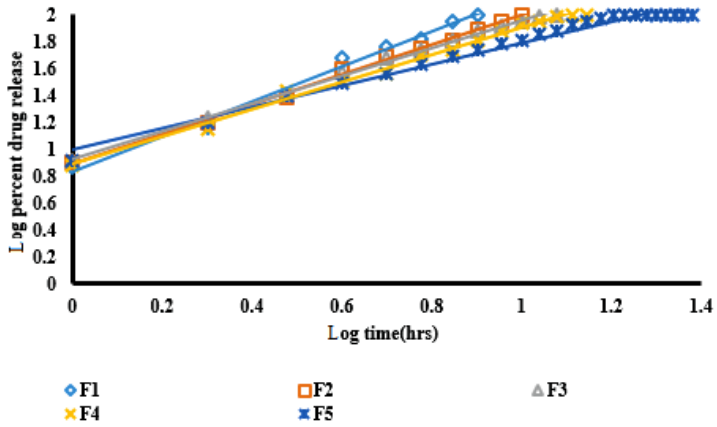


Figure 10: Log percent drug release vs. Log time plots of Gallic acid (F1-F5) nanoparticles

17.12. Analysis of Release Kinetics Data of Gallic acid nanoparticles:

The following release kinetics studies are done per the zero order, first order, and the Higuchi and Peppas model. The correlation coefficient (R^2), regression analysis, and 'n' value of the Peppas equation are calculated, and the results are summarized in tables 14, 15, and 16, respectively.

Table 9: Determination of Correlation Coefficient (R^2) Values of Drug Release

S. No	Isolated constituent	Test Formulation	Zero-order	First order	Higuchi	Peppas equation
1.	Gallic acid	F-1	0.9796	0.9904	0.8797	0.9915
		F-2	0.9895	0.9813	0.9626	0.9713
		F-3	0.9834	0.9872	0.9805	0.9864
		F-4	0.9872	0.9477	0.9412	0.9956
		F-5	0.9979	0.9792	0.9773	0.9407

Table 10: Regression(r) values for the Gallic acid nanoparticles

S. No	Isolated component	Test Formulation	Zero-order	First order	Higuchi	Peppas equation
1.	Gallic acid	F-1	0.939	0.941	0.965	0.945
		F-2	0.981	0.963	0.986	0.936
		F-3	0.987	0.972	0.971	0.981
		F-4	0.945	0.984	0.936	0.954
		F-5	0.963	0.991	0.962	0.963

Table 11: 'n' value in peppa's equation

S. No	Isolated component	Formulation	'n' in Peppas equation
1.	Gallic acid	F1	0.9861
		F2	0.9662
		F3	0.9025
		F4	0.9389
		F5	0.9469

The above results found that the formulation 5 (F5), of Gallic acid nanoparticles showed desired controlled release (99.6±0.38) for 24 hours compared to other formulations. All formulations follow zero order and Peppas n value indicate non-fickian diffusion.

17.13. Emmel's Test for the Gallic acid nanoparticles:

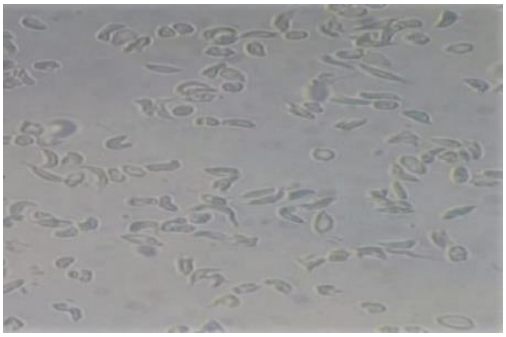


Figure 11: slide representation of the blood of sickle cell patients (control group)

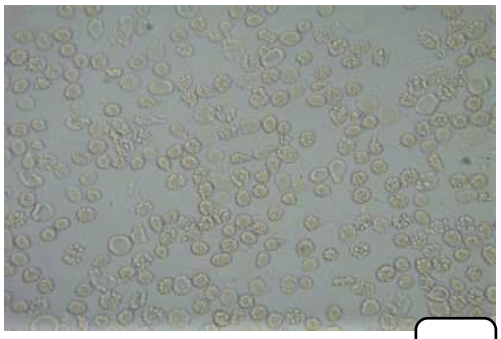


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Figure 12: Anti-sickling activity shown by Gallic acid nanoparticles.

The Emmel's Test is done on the Gallic acid nanoparticles, and these Gallic acid nanoparticles were able to normalize the shape of R.B.C. from sickle form.

Conclusion:

The design of Phyllanthus niruri containing herbal nanoparticles was prepared successfully, and all the tests were performed and showed satisfactory results. Thus we conclude that the plant Phyllanthus niruri containing Gallic acid as its active constituents has anti-sickling properties. They can be formulated into nanoparticles and produce an effective and economical treatment of sickle cell anemia with fewer side effects.

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Conflict of Interest: Authors declare there was no conflict of interest.

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