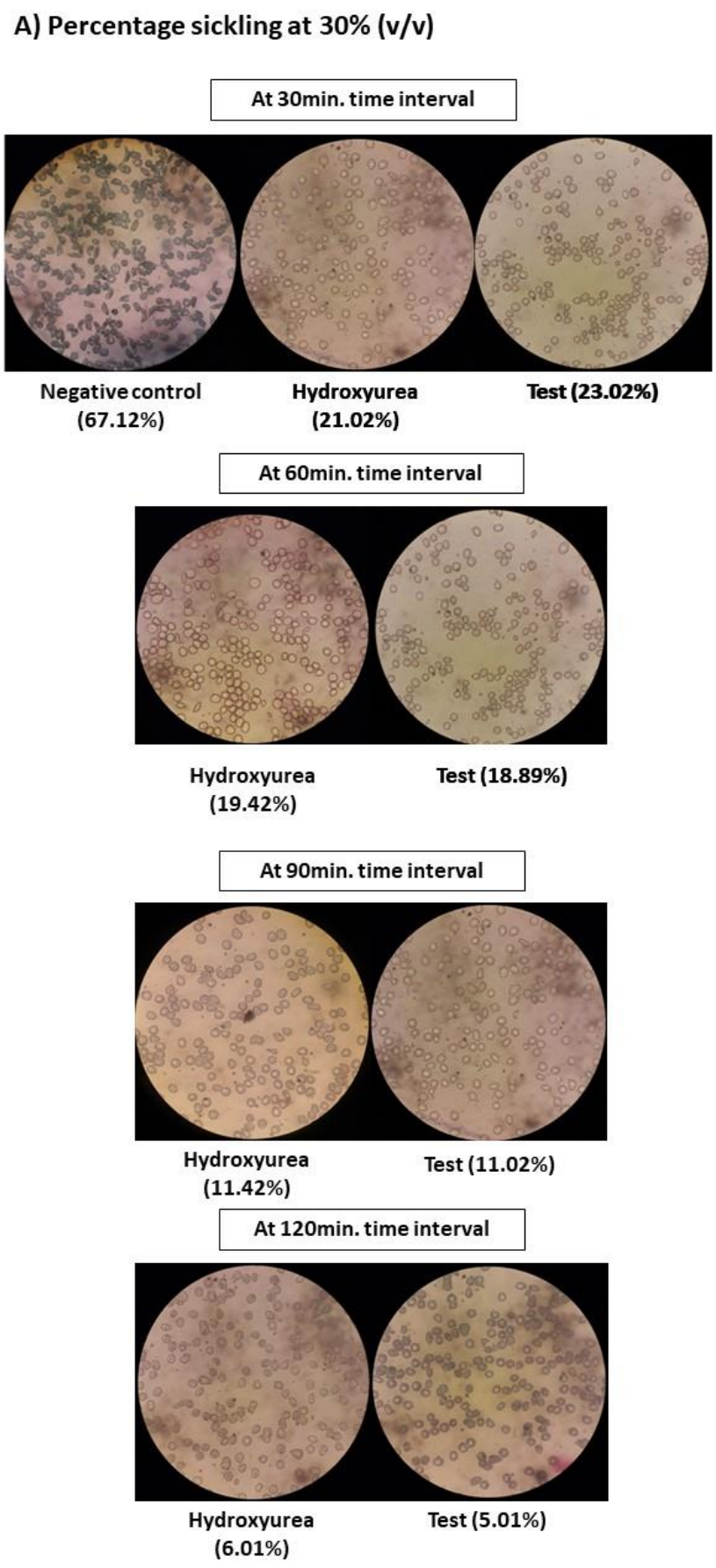
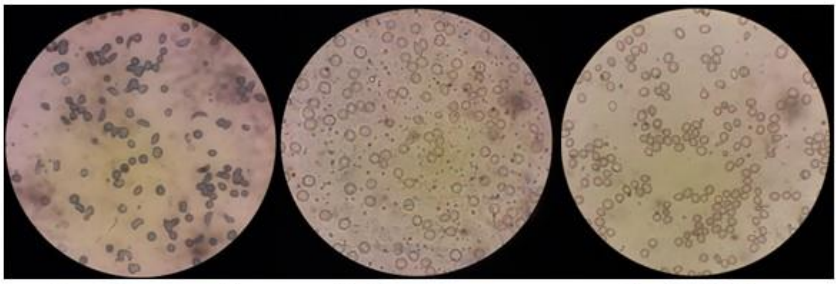
Supplementary Information

Figure S1.



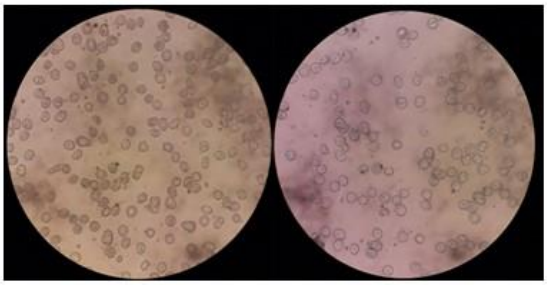
B) Percentage sickling at 60% (v/v)

At 30min. time interval



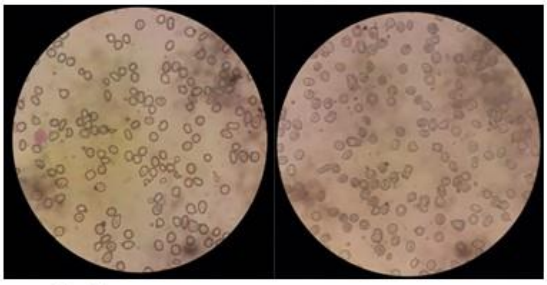
Negative control (87.2%) | Hydroxyurea (3.92%) | Test (3.02%)

At 60min. time interval



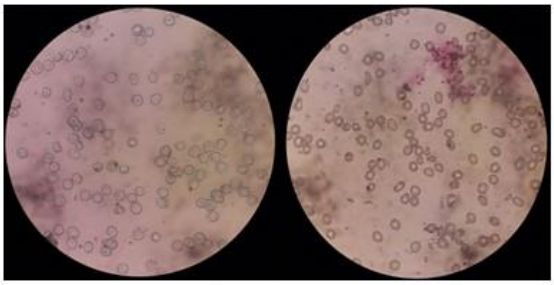
Hydroxyurea (4.42%) | Test (4.99%)

At 90min. time interval



Hydroxyurea (2.92%) | Test (2.81%)

At 120min. time interval



Hydroxyurea (0.98%) | Test (1.91%)

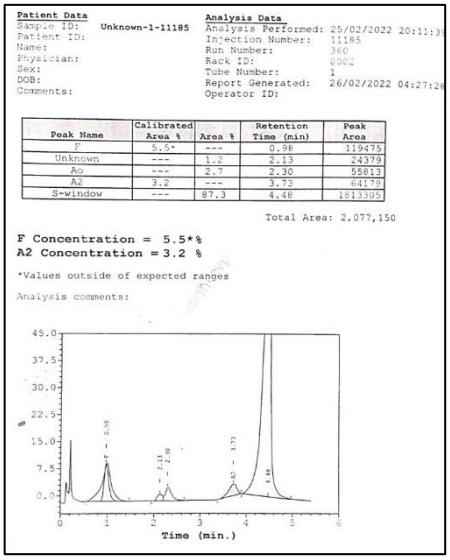
Figure S1. Microscopic Observation (40X) of percentage sickling at different dose and time duration of *L. plantarum* (CS) and hydroxyurea.

A) **Percentage sickling at 30% (v/v) culture supernatant and hydroxyurea at different time duration:** The percentage of sickling decreased in *L. plantarum* (CS) as well as hydroxyurea of 30%(v/v) at 30 min, 60 min, 90 min and 120 min of time intervals compared to the Negative control. The least percentage of sickling in *L. plantarum* (CS) was observed at 60 min which was comparable to Hydroxyurea (Drug Control).

B) **Percentage sickling at 60% (v/v) culture supernatant and hydroxyurea at different time duration:** The percentage of sickling decreased in *L. plantarum* (CS) as well as hydroxyurea of 60%(v/v) at 30 min, 60 min, 90 min and 120 min of time intervals compared to the Negative control. The least percentage of sickling in *L. plantarum* (CS) was observed at 60 min which was comparable to Hydroxyurea (Drug Control).

Figure S2.

(A) Percentage increase in fetal hemoglobin at 60 min. interval



Patient Data
Sample ID: Unknown-1-11185
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 25/02/2022 20:11:3
Injection Number: 11185
Run Number: 360
Rack ID: 0002
Tube Number: 1
Report Generated: 26/02/2022 04:27:28
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	5.5*	---	0.98	119475
Unknown	---	1.2	2.13	24379
Ao	---	2.7	2.30	55813
A2	3.2	---	3.73	64179
S-window	---	87.3	4.48	1813305

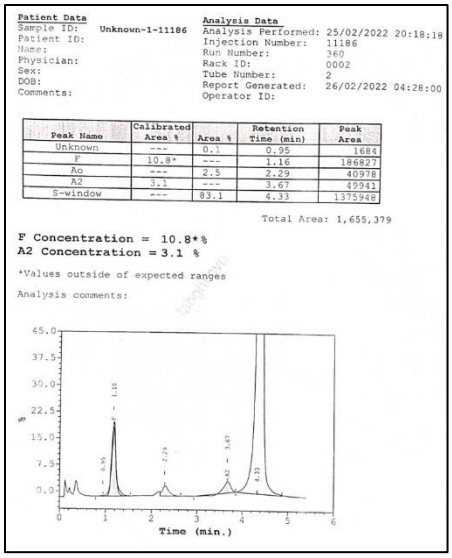
Total Area: 2,077,150

F Concentration = 5.5*%
A2 Concentration = 3.2 %

*Values outside of expected ranges

Analysis comments:

Negative



Patient Data
Sample ID: Unknown-1-11186
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 25/02/2022 20:18:18
Injection Number: 11186
Run Number: 360
Rack ID: 0002
Tube Number: 2
Report Generated: 26/02/2022 04:28:00
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.95	1684
F	10.8*	---	1.16	186827
Ao	---	2.5	2.29	40978
A2	3.1	---	3.67	49941
S-window	---	83.1	4.33	1375948

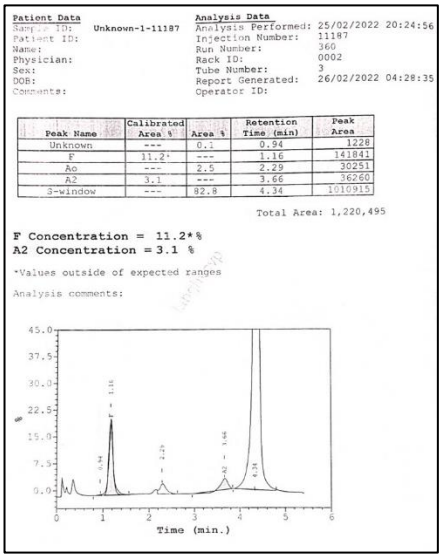
Total Area: 1,655,379

F Concentration = 10.8*%
A2 Concentration = 3.1 %

*Values outside of expected ranges

Analysis comments:

***L. plantarum* at 30% (v/v)**



Patient Data
Sample ID: Unknown-1-11187
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 25/02/2022 20:24:56
Injection Number: 11187
Run Number: 360
Rack ID: 0002
Tube Number: 3
Report Generated: 26/02/2022 04:28:35
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.94	1228
F	11.2*	---	1.16	141841
Ao	---	2.5	2.29	30251
A2	3.1	---	3.66	36260
S-window	---	82.8	4.34	1010915

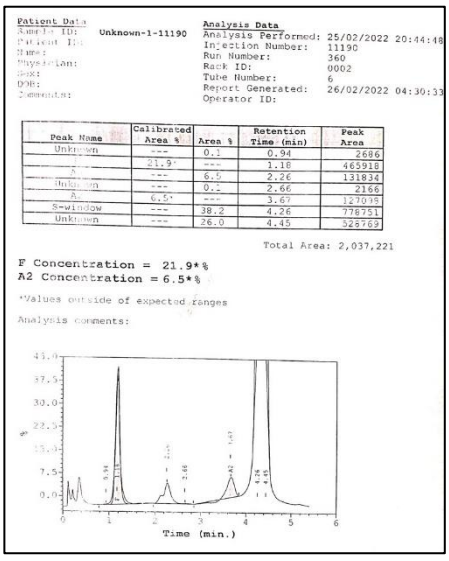
Total Area: 1,220,495

F Concentration = 11.2*%
A2 Concentration = 3.1 %

*Values outside of expected ranges

Analysis comments:

***L. plantarum* at 60% (v/v)**



Patient Data
Sample ID: Unknown-1-11190
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 25/02/2022 20:44:48
Injection Number: 11190
Run Number: 360
Rack ID: 0002
Tube Number: 6
Report Generated: 26/02/2022 04:30:33
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.94	2686
[illegible]	21.9*	---	1.18	465918
[illegible]	---	6.5	2.26	131834
Unknown	---	0.1	2.66	2166
A2	6.5*	---	3.67	127035
S-window	---	38.2	4.26	778751
Unknown	---	26.0	4.45	528769

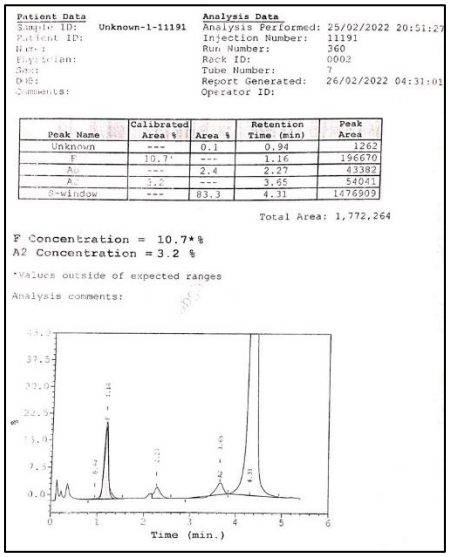
Total Area: 2,037,221

F Concentration = 21.9*%
A2 Concentration = 6.5*%

*Values outside of expected ranges

Analysis comments:

Hydroxyurea at 30% (v/v)



Patient Data
Sample ID: Unknown-1-11191
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 25/02/2022 20:51:27
Injection Number: 11191
Run Number: 360
Rack ID: 0002
Tube Number: 7
Report Generated: 26/02/2022 04:31:01
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.94	1262
F	10.7*	---	1.16	196670
Ao	---	2.4	2.27	43382
A2	3.2	---	3.65	54041
S-window	---	83.3	4.31	1476909

Total Area: 1,772,264

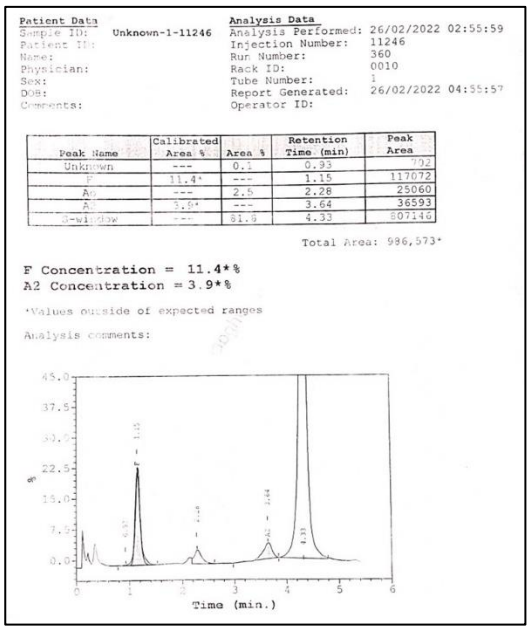
F Concentration = 10.7*%
A2 Concentration = 3.2 %

*Values outside of expected ranges

Analysis comments:

Hydroxyurea at 60% (v/v)

(B) Percentage increase in fetal hemoglobin at 120 min. interval



Patient Data
Sample ID: Unknown-1-11246
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 26/02/2022 02:55:59
Injection Number: 11246
Run Number: 360
Rack ID: 0010
Tube Number: 1
Report Generated: 26/02/2022 04:55:57
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.93	792
F	11.4*	---	1.15	117072
A2	---	2.5	2.28	25060
A	3.8*	---	3.64	36593
S-window	---	81.6	4.33	807146

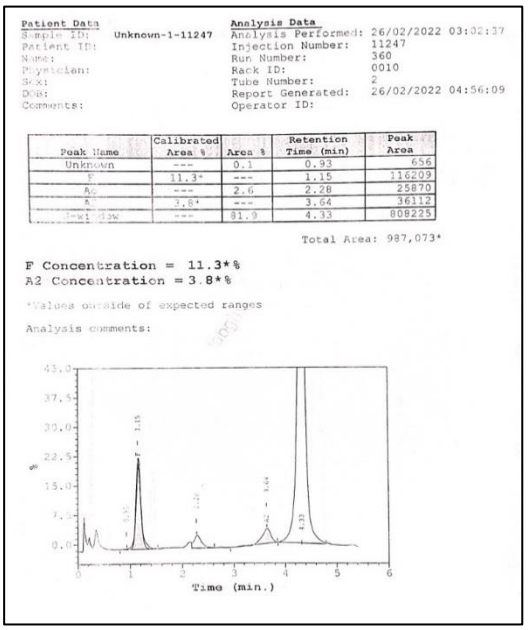
Total Area: 986,573*

F Concentration = 11.4*%
A2 Concentration = 3.9*%

*Values outside of expected ranges

Analysis comments:

***L. plantarum* at 30% (v/v)**



Patient Data
Sample ID: Unknown-1-11247
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 26/02/2022 03:02:37
Injection Number: 11247
Run Number: 360
Rack ID: 0010
Tube Number: 2
Report Generated: 26/02/2022 04:56:09
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.93	656
F	11.3*	---	1.15	116209
A2	---	2.6	2.28	25870
A	3.8*	---	3.64	36112
S-window	---	81.9	4.33	809225

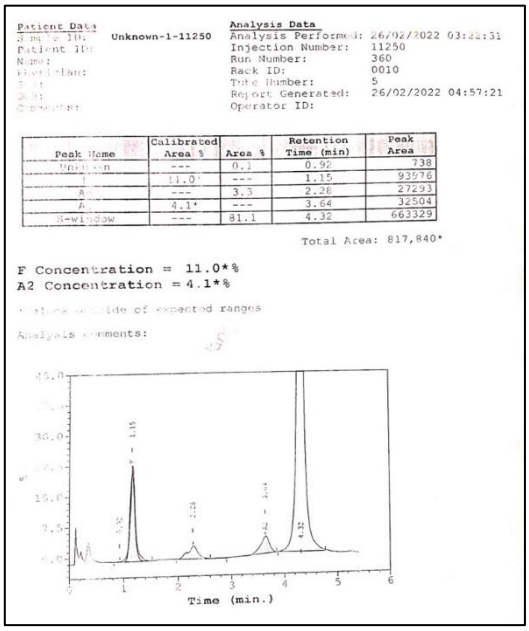
Total Area: 987,073*

F Concentration = 11.3*%
A2 Concentration = 3.8*%

*Values outside of expected ranges

Analysis comments:

***L. plantarum* at 60% (v/v)**



Patient Data
Sample ID: Unknown-1-11250
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 26/02/2022 03:22:31
Injection Number: 11250
Run Number: 360
Rack ID: 0010
Tube Number: 5
Report Generated: 26/02/2022 04:57:21
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.92	738
F	11.0*	---	1.15	93576
A2	---	3.3	2.28	27293
A	4.1*	---	3.64	32504
S-window	---	81.1	4.32	663329

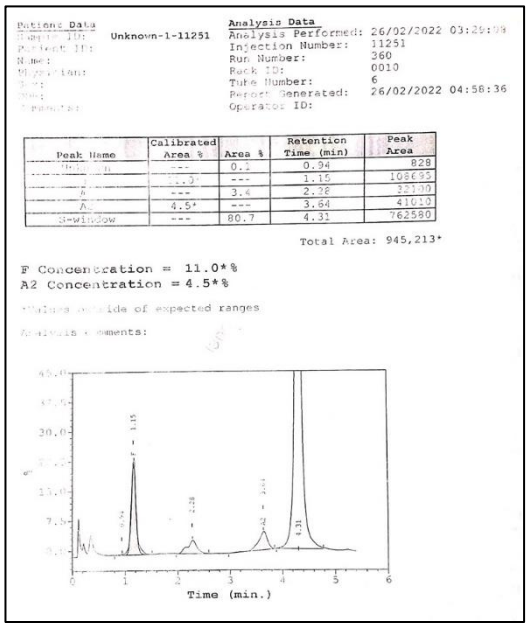
Total Area: 817,840*

F Concentration = 11.0*%
A2 Concentration = 4.1*%

*Values outside of expected ranges

Analysis comments:

Hydroxyurea at 30% (v/v)



Patient Data
Sample ID: Unknown-1-11251
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 26/02/2022 03:29:09
Injection Number: 11251
Run Number: 360
Rack ID: 0010
Tube Number: 6
Report Generated: 26/02/2022 04:58:36
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.1	0.94	828
F	11.0*	---	1.15	108695
A2	---	3.4	2.28	32100
A	4.5*	---	3.64	41010
S-window	---	80.7	4.31	762580

Total Area: 945,213*

F Concentration = 11.0*%
A2 Concentration = 4.5*%

*Values outside of expected ranges

Analysis comments:

Hydroxyurea at 60% (v/v)

Figure S2. The representative HPLC reports for the percentage increase in fetal hemoglobin (HbF%) upon treatment with *L. plantarum* (CS) at different time intervals.

Percentage increase in fetal hemoglobin at 60 min. interval: The percentage of fetal haemoglobin (Fetal Hemoglobin Concentration denoted as F%) increased in *L. plantarum* (CS) as well as

hydroxyurea of 30% (v/v) & 60% (v/v) concentration at 60 min of time intervals compared to the Negative control.

Percentage increase in fetal hemoglobin at 120 min. interval: The percentage of fetal haemoglobin (Fetal Hemoglobin Concentration denoted as F%) increased in *L. plantarum* (CS) as well as hydroxyurea of 30% (v/v) & 60% (v/v) concentration at 120 min of time intervals compared to the Negative control.

Table S1. **Percentage of sickling at 30% v/v & 60% v/v of *L. plantarum* (CS) and Hydroxyurea.**

Concentration of *L. plantarum* (CS) and Hydroxyurea	**Percentage Sickling at different time intervals (%) (Mean ± SEM) (N=15)**			
30 mg/ml	**30 min**	**60 min**	**90 min**	**120 min**
Negative Control	56.80 ± 6.250			
L. plantarum (CS)	9.066 ± 1.740	7.753 ± 7.703	7.717 ± 2.617	7.133 ± 2.212
Hydroxyurea	9.803 ± 2.018	7.795 ± 2.100	8.134 ± 3.516	5.344 ± 2.160
p* value	p<0.0001	p<0.0001	p<0.0001	p<0.0001
60 mg/ml				
L. plantarum (CS)	5.892 ± 1.278	6.846 ± 2.344	8.053 ± 2.465	6.828 ± 2.224
Hydroxyurea	8.910 ± 2.917	5.819 ± 1.436	6.4 ± 2.694	4.741 ± 1.170
p* value	p<0.0001	p<0.0001	p<0.0001	p<0.0001

*p values refer to comparison made between negative control (normal saline) and *L. plantarum* (CS) at different intervals.

Table S2. Percentage of Fetal Hemoglobin Concentration at 30% v/v & 60% v/v of *L. plantarum* (CS) and Hydroxyurea.

Concentration of *L. plantarum* (CS) and Hydroxyurea	**Percentage of Fetal Hemoglobin Concentration at different time intervals (%)** **(Mean ± SEM) (N=10)**	
30% (v/v)	**60 min**	**120 min**
Negative Control	9.438 ± 1.368	
L. plantarum (CS)	22.98 ± 5.301	23.83 ± 5.771
Hydroxyurea	23.43 ± 5.916	23.18 ± 5.591
p* value	p<0.0001	p<0.0001
60% (v/v)		
Test Extract	26.40 ± 4.150	23.18 ± 5.591
Hydroxyurea	23.35 ± 5.827	23.10 ± 5.474
p* value	p<0.0001	p<0.0001

*p values refer to comparison made between negative control (normal saline) and *L. plantarum* (CS) at different intervals.

Table S3. Comparison of anti-sickling & HbF inducing activity of *L. plantarum* (CS) between erythrocytes of severe and less severe groups of SCD patients.

Treatment (30%v/v for 120 min.)	**(A) Percentage Sickling (Mean ± SEM)**		**p=value**	**Significant Difference (p<0.05)**
	Severe (N=6)	**Less Severe (N=9)**		
Negative Control (Normal Saline)	(65.32 ± 2.592)	(59.34 ± 1.317)	0.100[a]	No
***L. plantarum* (CS)**	(17.89± 3.890)	(15.47± 3.174)	0.635[b]	No
Hydroxyurea	(21.33± 3.751)	(29.67± 3.851)	0.146[c]	No
	(B) HbF Percentage (HbF%) (Mean ± SEM)			
Negative Control (Normal Saline)	(6.260± 0.647)	(8.838± 0.60)	0.019[a]	Yes
***L. plantarum* (CS)**	(17.42± 3.713)	(19.93± 4.797)	0.685[b]	No
Hydroxyurea	(18.14± 3.488)	(21.15± 4.545)	0.608[c]	No

'N' represents number of SCD Patients,

[a] Severe patients vs. Less severe patients for Negative control,

[b] Severe patients vs. Less severe patients for *L. plantarum* (CS),

[c] Severe patients vs. Less severe patients for Hydroxyurea.

Genotyping of *rs334* (A/T; M34058) polymorphism for identification of homozygous Sickle cell disease patients

Genomic DNA was extracted from SCD patients' blood samples to confirm the HbSS genotype. 300µl of venous blood was used to isolate genomic DNA using the QIAamp DNA Blood Kit (Qiagen, Germany). Following extraction, DNA was kept for further examination at 20°C, and 0.8% agarose gel electrophoresis was used to determine its purity. The rs334 (A/T) polymorphism of the β-globin gene was genotyped using allele-specific-polymerase chain reaction (AS-PCR). (GenBank accession no. M34058), as previously described.[25] The following wild-type primer set was utilized for amplification of 517 bp fragment: WT-AS [5′-ATG GTG CAC CTG ACT CCT GA-3′] and WT-CP517 [5′-CCC CTT CCT ATG ACA TGA ACT-3′]; and for 267 bp fragment amplification (SCD) following mutant primer set was used: MUT-AS [5′-CAG TAA CGG CAG ACT TCT CCA-3′] and MUT-CP267 [5′-GGG TTT GAA GTC CAA CTC CTA-3′]. By using 2.0% agarose gel electrophoresis, the three genotypes of the rs334 (A/T) polymorphism were determined: AA homozygous (Healthy individual), AT heterozygous (Carrier), and TT homozygous (SCD patient).

The 25 µl of the PCR reaction mixture contained 15 µl nuclease free water, 3 µl (50 ng) genomic DNA, 2.5 µl 2.5 mM dNTPs (Takara, Japan), 0.4 µL (2.5 U/µL) Taq Polymerase (Takara, Japan), 2.5 µl 10X PCR buffer and 1 µl of 10 µM corresponding forward and reverse primers (Eurofins, Luxembourg). The following protocol was used for performing the amplification using the Bio-Rad T100 Thermal Cycler: 95°C for two minutes (initial denaturation), 30 cycles of 95°C for 30 sec. (denaturation), 60°C for 30 sec. (primer annealing), and 72°C for 35 sec. (extension). All amplified products were evaluated by 2.0% agarose gel electrophoresis.