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6.1. BACKGROUND AND OBJECTIVES OF THE STUDY

Since the beginning of time plants have been recognized for their several lifesaving and therapeutic properties. Lifestyle and eating habits alterations among the people makes it vital to refer to herbal medicine as an alternative or complimentary therapeutic measure. Various herbs are also a part of socio-cultural and socio-economic heritage. Even in the present times rural populations turn to herbal medicine as the most preferred therapeutic source.

Phytochemicals in the plant extracts have the therapeutic activity and is used in traditional practice by the traditional healers. It differs from plant to plant and examples of phytochemical with therapeutic properties are anthraquinones, flavonoids, glycosides, saponins, tannins etc. Plants also contain other compounds such as morphine, atropine, codeine, steroids, lactones and volatile oils, which possess medical values for the treatment of different disease.

Sickle cell disease (SCD) is known to be one of the diseases wrecking most parts of the globe without any discrimination of ethnic or racial standards. According to reports, Africa is believed to be the origin of sickle cell disease, and those afflicted with the disease are huge. It is also widespread in India. SCD is characterized by the crisis stage during which the patient experiences severe unbearable pain and breathlessness making it one of the most dreaded of all genetic diseases. There is incurable due to its genetic nature, however, the crises stage have been investigated in top priority by researchers all around the world in order to provide some relief during the period of crisis stage attack.

Symptoms of sickle cell disease are assorted and wide-ranging and can be recognized into the following categories- a) anemia b) pain consequences, and c) organ failure. When oxygen tension lowers, the HbS molecules undergo nucleation, expansion and ensuing arrangement of the molecule into parallel microfibrils resulting in membrane deformations along with damage. The hemoglobin from these cells forms relatively insoluble polymer under hypoxia condition, creating a crescent-shaped erythrocytes cell which may give rise to
micro-vascular occlusion leading to severe crises and casualty. The polymerization and occlusion are particularly supported by the stage of increased dehydration during crisis. Primarily, this perceived sickling is reversible upon re-oxygenation of the system; nevertheless, repeated oxygenation and de-oxygenation cycles in the system leads to irreversible sickle cells. These sickled erythrocytes cause choking of blood and injured organs resulting in the experience of painful occurrences or “crises”. Sickle cell crises may be caused by blood vessel occlusion, triggered by membrane deformation. SCD patients suffer from a variety of ailments which includes acute chest syndrome (ACS) which is one of the reasons for hospital admissions, stroke and acute splenic.

A number of plant products have been described that could serve as agents that alter membrane stability. Some of these have been implicated in the management of several human ailments including sickling and sickle cell disease. Furthermore, availability of data on the effects of plant extracts in reversal of sickled erythrocytes will provide ample opportunity for healing advances towards management of the disease.

Recent therapy focuses on the erythrocytic rehydration. Management of sickle cell disease (SCD) hence, involves substances which has an ability to rehydrate the erythrocytes and furthermore, preventing it from losing its shape.

Instantaneous emphasis has also been on developing antisickling agents which act by blocking or inhibiting activities in the HbS leading to its polymerization with loss of its shape and functionality. One such instance was when oxygen, carbon monoxide and sodium nitrite were used to reduce the amount of deoxyhaemoglobin. Stress has also been on developing antisickling agents which act by blocking or inhibiting activities in the HbS which eventually cause polymerization. Another line of therapy focused on finding new anti-sickling agents which can unambiguously bind to HbS. De-oxygenation caused by poor oxygen transport leads to increased membrane permeability for Mg$^{2+}$ resulting in a net loss of intracellular Mg$^{2+}$. The cadence of the activities of the three
membrane-based ATPases (\( \text{Na}^+ \), \( \text{K}^+ \) and \( \text{Ca}^{2+} \)) have been shown to be significantly lower in sickle cell disease patients’ (HbSS) erythrocytes, while Mg\(^{2+}\) ATPases was shown to be significantly higher in HbSS compared to normal patient (HbAA) erythrocytes. There are a few reports which have shown Ca\(^{2+}\) to play a significant role in the lowering of \( \text{K}^+ \) and \( \text{Na}^+ \) permeability of erythrocyte membrane; which assists to conserve the normal rate of cation outflow from the cell. Furthermore, the most crucial proposition was that, the sickled erythrocytes can well be reversed if excess Ca\(^{2+}\) in the red cells was propelled out of the system. There are also a few reports on the ability of some anti-malarial drugs and oral iron chelators which can alter some of the individual red blood cell elements which eventually leads to transformed physicochemical properties, which may be helpful in the management of the disease. Various plants were also found to possess an array of phytochemicals responsible for the reversal of sickled erythrocytes. Numerous authors and researchers have reported a number of herbal recipes and formulations from medicinal plants for the management of SCD. These medicinal plants have been proved to possess diverse activities in managing SCD condition. These activities may range from anti-sickling, anti-aggregating, anti-polymerization, radical scavenging or antioxidant, anti-inflammatory, analgesic, anti-pyretic, anti-dehydrating to anti-osmotic effects, etc. All these properties add up to give a supplementary stable and endurable patient condition.

It thus follows that the identification and characterization of phytochemicals with antisickling propensities from plants has become imperative in developing effective SCD management strategies.

The objectives of the present study are as follows:

Phytochemicals with antisickling activities are of paramount importance for designing and implementation of the current SCD management strategies. Many plants possess antisickling propensities, in due course, it has to be explored and their respective active principles needs to be identified; particularly in a country like India where the incidence of SCD is high. In our country plants have not
been tested for antisickling activities till date. Most of the studies on phytochemical analysis and all of the studies on antisickling properties are from foreign countries and on exotic plant species. In a country like India, where the incidence of SCD is quite high among some communities, with its rich and diverse plant species there is an urgent need to undertake studies pertaining to the antisickling properties of native species.

Hence, the present study was planned to analyze the phytochemistry of two common plants, *Carica papaya* L. and *Cajanus cajan* L. along with their antisickling and sickling reversal properties. Furthermore, proximate analysis, qualitative and quantitative phytochemistry, thin layer chromatography (TLC), antisickling activities and sickling reversal activities have been assessed individually for leaves, stems and seeds of these two plants.

### 6.2. MATERIAL AND METHODS

**a) Chemicals and Reagents**

The chemicals used to study anti-sickling properties were methanol, petroleum ether; phosphate buffered saline tablets pH 7.2, liquid paraffin, formalin, para-hydroxybenzoic acid, paraffin wax, EDTA bottles and distilled water. All these chemicals were of analytical grade and suitable for research purposes.

**b) Plant Samples**

The fresh leaves stems and seeds of *Carica papaya* L. and *Cajanus cajan* L. were simultaneously collected from abundant farms, cultivated farms and the open fields in and around Raipur district. Fresh Parts of the plants were identified and authenticated by the department of Botany D. B. Girls P.G. College Raipur Chhattisgarh and voucher specimens were deposited.

**c) Preparation of Plants Materials**

*Carica papaya* L. and *Cajanus cajan* L. leaves, seeds and stems were washed properly, separately cut into small bits and air dried in shadow for about a fortnight. After drying they were grinded and powdered by using a grinding
machine and sieved through a with 1 mm size mesh. Finally they were preserved in closed air-tight containers for analysis.

d) Preparation of Extracts
200 g sample is extracted in Soxhlet apparatus, with petroleum ether (60-80°C) and aqueous- methanol (60-80°C) in 1:3 as solvents. The prepared extracts were stored at 4°C in freeze in dried form and used for the antisickling activity test. Varying concentrations have been prepared from the dried extracts and used for the antisickling assay was varied from 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 5.0 and 10.0 mg/ml of leaves, seeds and stem of *Carica papaya* L. and *Cajanus cajan* L.

e) Collection of Blood Samples
The blood samples used in the evaluation of the antisickling activity of plants in this study were collected from SCD patients belonging to the age-group 16 to 25 years, of both sexes and it was ensured that they do not take any allopathic or ayurvedic medications. Consent certificate was signed by the patients before drawal of blood. A quantity of 5.0 ml of fresh blood samples were collected each time by way of vein-puncture in EDTA (Ethylene di-amine tetra acetic acid) anticoagulant tubes and mixed gently to prevent lysing of the red blood cells. The study was approved by the Institutional Ethical Committee, Govt’ D.B. Girls’ P.G. College, Raipur.

f) Sickling Inhibitory Activity
For inhibitory sickling activity test 0.2 ml HbSS sample was pipetted in test tubes in duplicates. Following which, 0.2 ml of phosphate buffered saline solution and 0.2 ml of different concentration of the extracts were added serially. Finally, the mixture was overlaid with 1 ml liquid paraffin wax and incubated in a thermo-stated water bath at 37 °C for about 4 hours. After incubation 0.6 ml of freshly prepared 2% sodium meta-bi-sulphite solution was added under liquid paraffin with e help of a syringe and these mixtures were mixed by rolling the test tubes between the palms of hand carefully. These mixtures were again incubated for about one and half hours in 37 °C in a thermo-stated water bath. After incubation
the liquid paraffin wax was removed with the help of a Pasteur pipette and the resultant mixture was fixed in 3 ml of 5% v/v buffered formalin.

g) Antisickling Properties or Reversal of Sickled Erythrocytes
For reversal of sickling activity tests, 0.2 ml of blood sample was pipetted into test tubes in duplicates. 0.2 ml of phosphate buffered saline solution is added and the mixture is overlaid with 1 ml liquid paraffin wax. 0.6 ml freshly prepared 2% sodium meta-bi-sulphite solution was added under liquid paraffin and these mixtures were mixed by rolling the test tubes between the palms of hand carefully and incubated in a thermo-stated water bath at 37 °C for 1 and half hours. After incubation 0.2 ml of different concentration of extracts were added and again they were incubated in a thermo-stated water bath at 37 °C for 6 hours. After incubation, the liquid paraffin wax was removed with a Pasteur pipette and the resultant mixture was fixed by the addition of 3 ml of 5% v/v buffered formalin.

h) Counting of Cells
The fixed cells were centrifuged at 4000 rpm for 15 minutes and the supernatants were decanted with a capillary tube. Slides were prepared from fixed cells after the process of centrifugation. One or two drops was applied on a microscopic slide and carefully covered with a cover slip and sealed with wax. Prepared slides were observed under a high power objective (x40 and x 100) of Research Trinocular Microscope (LABOMED VISION 2000) About four hundred (400) cells (both sickled and normal erythrocytes) were counted and the percentage sickled cells were recorded.

i) Statistical Validation
The results were validated statistically by using ANOVA, Duncan’s Multiple Range Test. Means ± SD and Means ± SE are used for Descriptive analysis.
6.3. RESULTS AND DISCUSSION

I. PHYTOCHEMISTRY

A. QUALITATIVE PHYTOCHEMICAL ANALYSIS

*Cajanus cajan* L.: The results obtained from qualitative analysis of *Cajanus cajan* L. are as follows: Tannins were present in the ethanolic, methanolic, chloroform and petroleum ether extracts of leaves, seeds and stem, whereas the chloroform extract of leaves and seeds and petroleum ether extract of seeds showed negative results; Saponins were absent in chloroform extract of leaves and petroleum ether extract of seeds; Reducing sugars were present in all the four extracts of leaves, seeds and stem of *Cajanus cajan* L.; except the ethanol and methanol extract of seeds and petroleum ether extract of leaves and stems; Alkaloids were absent only in chloroform extract of leaves; Terpenoids were absent in the ethanolic extract of seeds, methanolic extracts of seeds and methanolic, chloroform and petroleum ether extracts of leaves; Cardiac glycoside were absent in ethanolic, methanolic and chloroform extracts of seed and chloroform and petroleum ether extracts of leaves; Anthraquinones were absent in all the four extracts of leaves, while the ethanolic and methanolic extracts of seeds and chloroform extract of stem of *Cajanus cajan* L. Flavonoids and phenols were present in all extracts of the plant.

*Carica papaya* L.: The results obtained from qualitative analysis of *Carica papaya* L. are as follows: Tannins were present in the ethanolic, methanolic, chloroform and petroleum ether extracts of leaves and stem, whereas the seeds showed positive results in ethanolic and methanolic extracts. The other two extracts- chloroform and petroleum showed negative results; Saponins and reducing sugars were present in all the four extracts of leaves, seeds and stem of *Carica papaya* L.; except reducing sugar on the chloroform and petroleum ether extracts of seeds. Alkaloids were absent in chloroform and petroleum ether extract of seeds; Terpenoids were absent in the methanolic extracts of leaves and seeds; Cardiac glycoside were absent in methanolic extracts of stem and
petroleum ether extracts of leaves; Anthraquinones were absent in all the four extracts of leaves, while flavonoids and phenols were present in all extracts of *Carica papaya* L.

**B. QUANTITATIVE PHYTOCHEMICAL ANALYSIS**

a. The results of the quantitative analysis of *Cajanus cajan* L. are as follows:

*Cajanus cajan* L. leaves: The secondary metabolites were found to be phenol 16.61%; tannins 0.49%; alkaloids, 2.65%; flavonoids 4.77% and saponins 5.97%.

*Cajanus cajan* L. seeds: The secondary metabolites were found to be phenol 3.82%; tannins 0.23%; alkaloids 2.65%; flavonoids 2.11% and saponins 6.23%.

*Cajanus cajan* L. stem: The secondary metabolites were found to be phenol 14.19%; tannins 0.22%; alkaloid 2.51%; flavonoids 5.44% and saponins 4.98%.

Quantitative phytochemical estimation of phenol was done in which the total phenolics was determined with Folin-Ciocalteu reagent. Gallic acid was used as standard compounds and were expressed as mg/g gallic acid equivalent using the standard curve equation \( y = 0.0061x + 0.0396, R^2 = 0.9991 \), where \( y \) is absorbance at 760 nm and \( x \) is total phenolic content in different parts of the plants. Maximum phenolic content was found in leaves (166.16 ± 0.23 mg/g) than stems (141.93 ± 0.36 mg/g) and seeds (38.26 ± 1.53 mg/g).

For the determination of tannin standard procedure was followed by using Folin–Denis method, the tannin concentration was determined by the standard graph of tannic acid solution and were expressed as mg/g tannic acid equivalent using standard curve equation \( y = 0.027x + 0.036, R^2 = 0.998 \), where \( y \) is absorbance at 700 nm and \( x \) is tannin content.

The results of the quantitative analysis of *Carica papaya* L. are as follows:

*Carica papaya* L. leaves: The secondary metabolites were found to be phenol 13.42%; tannins 0.17%; alkaloids, 2.99%; flavonoids 5.08% and saponins 7.41%.

*Carica papaya* L. seeds: The secondary metabolites were found to be phenol 12.11%; tannins 0.12%; alkaloids 6.96%; flavonoids 3.38% and saponins 4.84%. 
Carica papaya L. stem: The secondary metabolites were found to be phenol 4.78%; tannins 0.04%; alkaloid 1.5%; flavonoids 2.88% and saponins 2.85%. Quantitative phytochemical estimation of phenol was done in which Gallic acid was used as standard compounds and were expressed as mg/g gallic acid equivalent using the standard curve equation $y = 0.0061x + 0.0396$, $R^2 = 0.9991$, where $y$ is absorbance at 760 nm and $x$ is total phenolic content in different parts of the plants. Maximum phenolic content was found in leaves $(134.2 \pm 3.36 \text{ mg/g})$ than seeds $(121.04 \pm 1.02 \text{ mg/g})$ and stems $(47.82 \pm 2.46 \text{ mg/g})$.

For the determination of tannin standard procedure was followed by using Folin – Denis method, in which tannic acid solution and were expressed as mg/g tannic acid equivalent using standard curve equation $y = 0.027x + 0.036$, $R^2 = 0.998$, where $y$ is absorbance at 700 nm and $x$ is tannin content.

II. PROXIMATE ANALYSIS

The results of the proximate composition of Cajanus cajan L. are as follows:

Cajanus cajan L. leaves: The proximate composition were found to be dry matter 93.68 ± 0.284; moisture 06.31 ± 0.284; ash 20.60 ± 0.114; fiber 21.82 ± 0.238; fat 13.00 ± 0.090; protein 31.99 ± 0.070; carbohydrate 6.269 ± 0.153 and Nutritive value 236.72 ± 0.591.

Cajanus cajan L. seeds: The proximate composition were found to be dry matter 91.80 ± 0.229; moisture 8.20 ± 0.229; ash 22.11 ± 0.112; fiber 05.09 ± 0.086; fat 15.00 ± 0.090; protein 08.62 ± 0.035; carbohydrate 40.95 ± 0.244 and Nutritive value 333.73 ± 1.500.

Cajanus cajan L. stem: The proximate composition were found to be dry matter 93.88 ± 0.125; moisture 06.11 ± 0.125; ash 23.00 ± 0.222; fiber 27.70 ± 0.360; fat 14.19 ± 0.268; protein 21.34 ± 0.562; carbohydrate 8.131 ± 0.389 and Nutritive value 242.61 ± 1.569.

The results of the proximate composition of Carica papaya L. are as follows:

Carica papaya L. leaves: The proximate composition were found to be dry matter 92.8 ± 0.200; moisture 7.2 ± 0.100; ash 17.25 ± 0.010, Fiber 9.00 ± 0.100; fat
13.50 ± 0.100; protein 12.39 ± 0.512; carbohydrate 40.66 ±0.801 and Nutritive value 333.36 ± 0.34.

*Carica papaya* L. seeds: The proximate composition were found to be dry matter 94.5 ± 0.200; moisture 5.50 ± 0.200; ash 18.0 ± 0.200; fiber 14.0 ± 0.200; fat 11.0 ± 0.100; protein 8.81± 0.035; carbohydrate 42.68± 0.087 and Nutritive value 305.0 ± 1.30.

*Carica papaya* L. stem: The proximate composition were found to be dry matter 92.43 ± 0.208; moisture7.50 ± 0.100; ash 13.25 ± 0.010; Fibre 24.0 ± 0.100; fat 11.5 ± 0.100; protein 3.32 ± 0.047; carbohydrate 40.43 ± 0.135 and Nutritive value 278.0 ± 0.535.

III. THIN LAYER CHROMATOGRAPHY

Thin Layer Chromatography Profile of the leaves, seeds and stem of *Cajanus cajan* L. in the solvents of ethanol, methanol, chloroform and petroleum ether extracts was accomplished in mobile phase of petroleum ether: benzene: methanol (16:3:2). Rf values and phytochemicals detected were as follows:

TLC of *Cajanus cajan* L. leaves ethanol extract: It showed maximum 7 bands with Rf values and phytochemicals in the range of 0.07 to 0.99 (0.07 Saponins; 0.16 Flavonoid; 0.51 Flavonoid; 0.78 Flavonoid; 0.84 Phenol; 0.95 Phenol; 0.99 Alkaloid).

TLC of *Cajanus cajan* L. leaves methanol extract: It showed maximum 6 bands with Rf values and phytochemicals in the range of 0.10 to 0.99 (0.10 Cardiac glycosides; 0.18 Saponins; 0.22 Flavonoid; 0.40 Flavonoid; 0.82 Phenol; 0.99 Alkaloid).

TLC of *Cajanus cajan* L. leaves chloroform extract: It showed maximum 3 bands with Rf values and phytochemicals in the range of 0.95 to 0.99 (0.95 Phenol; 0.97 Flavonoid; 0.99 Phenol).

TLC of *Cajanus cajan* L. leaves petroleum ether extract: It showed maximum 4 bands with Rf values and phytochemicals in the range of 0.18 to 0.94 (0.18 Saponins; 0.34 Phenol; 0.62 Phenol; 0.94 Alkaloid).
TLC of *Cajanus cajan* L. seeds ethanol extract: It showed maximum 3 bands with R_f values and phytochemicals in the range of 0.15 to 0.99 (0.15 Flavonoid; 0.93 Flavonoid; 0.99 Alkaloid).

TLC of *Cajanus cajan* L. seeds methanol extract: It showed maximum 3 bands with R_f values and phytochemicals in the range of 0.05 to 0.10 (0.05 Saponins; 0.08 Flavonoid; 0.10 Flavonoid).

TLC of *Cajanus cajan* L. seeds chloroform extract: It showed maximum 6 bands with R_f values and phytochemicals in the range of 0.06 to 0.83 (0.06 Saponins; 0.11 Phenol; 0.15 Flavonoid; 0.19 Flavonoid; 0.29 Anthraquinone; 0.83 Alkaloid).

TLC of *Cajanus cajan* L. seeds petroleum ether extract: It showed maximum 2 bands with R_f values and phytochemicals in the range of 0.53 to 0.99 (0.53 Flavonoid and 0.99 Alkaloid).

TLC of *Cajanus cajan* L. stems ethanol extract: It showed maximum 4 bands with R_f values and phytochemicals in the range of 0.12 to 0.99 (0.12 Flavonoid; 0.42 Flavonoid; 0.53 Flavonoid; 0.99 Alkaloid).

TLC of *Cajanus cajan* L. stems methanol extract: It showed maximum 6 bands with R_f values and phytochemicals in the range of 0.06 to 0.99 (Saponins; 0.21 Phenol; 0.33 Anthraquinone; 0.52 Phenol; 0.64 Flavonoid; 0.99 Alkaloid).

TLC of *Cajanus cajan* L. stems chloroform extract: It showed maximum 6 bands with R_f values and phytochemicals in the range of 0.09 to 0.97 (0.09 Saponins; 0.16 Phenol; 0.34 Phenol; 0.44 Phenol; 0.58 Flavonoid; 0.97 Alkaloid).

TLC of *Cajanus cajan* L. stems petroleum ether extract: It showed maximum 8 bands with R_f values and phytochemicals in the range of 0.29 to 0.98 (0.29 Cardiac glycoside; 0.34 Saponins; 0.36 Phenol; 0.47 Anthraquinone; 0.53 Phenol; 0.64 Flavonoid; 0.68 Anthraquinone; 0.98 Alkaloid).

Thin Layer Chromatography Profile of the leaves, seeds and stem of *Carica papaya* L. in the solvents of ethanol, methanol, chloroform and petroleum ether extracts was done in mobile phase of petroleum ether: benzene: methanol (16:3:2). R_f values and phytochemicals detected were as follows:
TLC of *Carica papaya* L. leaves ethanol extract: It showed maximum 5 bands with $R_f$ values and phytochemicals in the range of 0.04 to 0.96 (0.04 Saponin; 0.09 Cardiac glycoside; 0.45 Phenol; 0.71 Flavonoid; 0.96 Alkaloid).

TLC of *Carica papaya* L. leaves methanol extract: It showed maximum 5 bands with $R_f$ values and phytochemicals in the range of 0.09 to 0.99 (0.09 Cardiac glycoside; 0.11 Saponin; 0.24 Flavonoid; 0.25 Flavonoid; 0.99 Alkaloid).

TLC of *Carica papaya* L. leaves chloroform extract: It showed maximum 4 bands with $R_f$ values and phytochemicals in the range of 0.83 to 0.92 (0.83 Phenol; 0.86 Flavonoid; 0.87 Saponin; 0.92 Phenol).

TLC of *Carica papaya* L. leaves petroleum ether extract: It showed maximum 7 bands with $R_f$ values and phytochemicals in the range of 0.08 to 0.99 (0.08 Phenol; 0.27 Phenol; 0.34 Phenol; 0.37 Flavonoid; 0.43 Flavonoid; 0.67 Phenol; 0.99 Alkaloid).

TLC of *Carica papaya* L. seeds ethanol extract: It showed maximum 2 bands with $R_f$ values and phytochemicals in the range of 0.04 to 0.09 (0.04 Saponin and 0.09 Flavonoid).

TLC of *Carica papaya* L. seeds methanol extract: It showed maximum 1 band with $R_f$ value and phytochemical (0.10 Flavonoids).

TLC of *Carica papaya* L. seeds chloroform extract: It showed maximum 2 bands with $R_f$ values and phytochemicals in the range of 0.05 to 0.10 (0.05 Saponins and 0.10 Flavonoids).

TLC of *Carica papaya* L. seeds petroleum ether extract: It showed maximum 1 band with $R_f$ value and phytochemical 0.08 Saponins.

TLC of *Carica papaya* L. stems; ethanol extract: It showed maximum 4 bands with $R_f$ values and phytochemicals in the range of 0.03 to 0.99 (0.03 Saponins; 0.22 Phenols; 0.25 Flavonoids; 0.99 Alkaloids).

TLC of *Carica papaya* L. stems methanol extract: It showed maximum 8 bands with $R_f$ values and phytochemicals in the range of 0.10 to 0.98 (0.10 Saponins; 0.13 Saponins; 0.26 Phenols; 0.43 Anthraquinones; 0.67 Flavonoids; 0.73 Flavonoids; 0.93 Flavonoids; 0.98 Alkaloids).
TLC of *Carica papaya* L. stems chloroform extract: It showed maximum 8 bands with Rf values and phytochemicals in the range of 0.07 to 0.99 (0.07 Saponins; 0.14 Cardiac glycosides; 0.20 Phenols; 0.29 Phenols; 0.46 Anthraquinones; 0.50 Anthraquinones; 0.57 Flavonoids; 0.72 Flavonoids; 0.99 Alkaloids).

TLC of *Carica papaya* L. stems petroleum ether extract: It showed maximum 6 bands with Rf values and phytochemicals in the range of 0.23 to 0.99 (0.23 Saponins; 0.47 Phenols; 0.58 Phenols; 0.63 Anthraquinones; 0.72 Flavonoids; 0.99 Alkaloids).

IV. ANTISICKLING ACTIVITY

The inhibitory activities and Reversal of sickled Erythrocyte for different concentrations *viz.*, 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 5.0 and 10.0 mg/ml of extracts of *Cajanus cajan* L. and *Carica papaya* L. obtained were as follows:

**Sickling Inhibitory Activity**

*a*) *Cajanus cajan* L.: The inhibitory activities for different concentrations of extracts revealed maximum inhibition activity (IA) of 68.45% in *Cajanus cajan* L. leaves in a concentration of 10.0 mg/ml; seeds showed a IA of 70.82% in a concentration of 2.0 mg/ml; while the stems showed a IA of 65.18% in a concentration of 10.0 mg/ml.

*b*) *Carica papaya* L.: The inhibitory activities for different concentrations of extracts revealed maximum inhibition activity (IA) of 71.80% in *Carica papaya* L. leaves in a concentration of 1.0 mg/ml; seeds showed a IA of 68.50% in a concentration of 2.5 mg/ml; while the stems showed a IA of 68.33% in a concentration of 10.0 mg/ml.

**Reversal of Sickled Erythrocytes**

*a*) *Cajanus cajan* L.: The Reversal of sickled Erythrocyte for different concentrations of extracts revealed maximum reversal activity (RA) of 72.13% in *Cajanus cajan* L. leaves in a concentration of 10.0 mg/ml; seeds showed a RA of 70.96% in a concentration of 10.0 mg/ml; while the stems showed a RA of 63.24% in a concentration of 10.0 mg/ml.
b) *Carica papaya* L.: The Reversal of sickled Erythrocyte for different concentrations of extracts revealed maximum reversal activity (RA) of 72.43% in *Carica papaya* L. leaves in a concentration of 10.0 mg/ml; seeds showed a RA of 65.96% in a concentration of 10.0 mg/ml; while the stems showed a RA of 62.97% in a concentration of 10.0 mg/ml.

Results of the phytochemical estimation when seen extract wise, revealed maximum presence of phytochemicals in the ethanolic extract of leaves; chloroform extract of seeds and ethanolic and methanolic extracts of stem, in the plant *Cajanus cajan*. In the other plant, *Carica papaya*, maximum phytochemicals were observed to be present in the methanolic extracts of leaves, ethanolic extract of seeds and methanolic, chloroform and petroleum ether extracts of the stem. In the quantitative analysis the most active phytochemical was noted were phenolic compounds in the leaves and stems of *C. cajan*; while the stem extracts revealed saponins as the most active phytochemical. On the other hand, *C. papaya* showed higher quantity of phenolic compounds in all of the parts studied. Hence, clearly the phenolic compounds are the most active compounds in both these plants studied. Phenolic compound is a major component of the methanolic extracts. Results obtained for the phenolic compounds in both the plants are in accordance with the works of earlier workers.

Varied antisickling activity showed by the extracts in different concentration in the present study can be ascribed to the presence of varying degrees of antioxidants (polyphenols, alkaloids and flavanoids etc.) in the extracts of different plants parts, in different concentrations. Another factor could be the affinity showed by the antioxidant molecule with haemoglobin molecule’s binding site; which is the key factor determining the success of the concerned molecule as a potent antisickling agent. Usually, most of the anti-sickling agents prefer the RBC membrane as a binding site to the haemoglobin molecule.
The key factor for a new drug discovery is the preliminary information regarding the chemical constituents eventually comes from the qualitative phytochemical screening of plant extracts. In the present study, qualitative tests for different extracts of *C. cajan* and *C. papaya* revealed an array of phytochemicals including phenolic compounds. Plants have diverse chemical composition and related physiology for its separate parts. This results in the presence of different phytochemicals in its different parts. Therefore, specific plant parts used in the management of specific disease conditions could yield favourable results in comparison to using whole plant extracts.

The results attained in these phytochemical and antisickling assays can provide important data towards classification of extracts according to their total phytochemical content and antisickling potential; for different individual parts *viz.*, leaves, stem and seeds. The results further support the view that phytochemicals with antioxidant properties can well act as potential antisickling agents. Therefore, there is a possibility that all the plants possessing anti-oxidant effects may act as antisickling agents also. However, further studies on the patterns of antisickling assays have to be ascertained for these plants. We conclude that both of these plants, *Carica papaya* L. and *Cajanus cajan* L. can be used as a nutritive as well as an antisickling agent during the pharmacological formulation of novel drugs which can be used the management of Sickle Cell Disease (SCD).
6.4. PUBLICATIONS IN CONTEXT OF THE STUDY


