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Research Article

Preliminary Phytochemical Estimation and HPTLC Chemo-profiling of *Sickle senna* Leaves Extract

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ABSTRACT
Sickle senna is a widely distributed medicinal plant in India and other tropical countries. Different parts of the plants are reputed for their medicinal value. The extract of *Sickle senna* leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity. The present investigation was aimed at preliminary phytochemical evaluation and HPTLC chemo-profiling of hydroalcoholic (90 % Methanol) extract of Sickle senna leaves.

Key words: HPTLC, *Sickle senna,* Total glycosides, Total tannins.

1. INTRODUCTION

Sickle senna / Cassia tora Linn. (Family-Leguminoseae) is a well-known medicinal plant widely distributed in India and other tropical countries[1]. It is an annual undershrub and grows wild in waste land. It is commonly known as "Sicklepod." Various medicinal properties have been attributed to this plant in the traditional system of Indian medicine. Different parts of the plants are reputed for their medicinal value. Several anthraquinones have been isolated from the seeds of *Sickle senna*[2, 3]. Sennosides, which are well known for their medicinal importance, have been detected in the leaves of the plants[4]. The extracts of *Sickle senna* have been used as a remedy for various skin ailments, rheumatic disease and as laxatives [5, 6, 7]. The extract of *Sickle senna* leaves has been found to possess significant hepatoprotective activity and anti-inflammatory activity[8, 9]. The seeds of *Sickle senna* have been used in Chinese medicine as aperient, antiasthnic, diuretic agent and also improve the visual activity[10].
TLC and HPTLC techniques, now days, are important analytical tools for micro-analytical separation and determination of phytoconstituents. These chromatographic techniques offer the best method of recording the fingerprinting which can be reproduced anywhere provided the same conditions are maintained. The present investigation was aimed at preliminary phytochemical evaluation and HPTLC Chemo-profiling of *Sickle senna* leaves extract.

2. MATERIALS AND METHODS

2.1 Collection and Processing of Plant Material

The leaves of *Sickle senna* were collected from Kalyan region, Dist. Thane (Maharashtra) in the month of September and October. The leaves were shade dried. The dried leaves were powdered to obtain a coarse powder (40 #) and extracted with hydroalcoholic solvent (90% methanol) using percolation method. The extract obtained was evaporated to get dry residue. The dried extract was reconstituted with methanol to get final concentration of 10 mg/ml.

2.2 Preliminary Phytochemical Evaluation

2.2.1 Qualitative Evaluation

The leaves extract of *Sickle senna* was tested for presence of bioactive compounds according to standard procedures as described below:

Test for Cardiac Glycosides
1.0 g of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of concentrated sulphuric acid.

Test for Alkaloids
Two millitre of aqueous sample extract was measured using a measuring cylinder and equal volume of ethanol containing 3 % tartaric acid was added and shaken. Then few drops of marquin's reagent were
added into the mixture. The formation of precipitate any form indicated the presence of alkaloids.

Test for Saponins
In determining the presence orabsence of saponins, 0.5 g of the dried extract was placed in a test tube and 3ml of distilled water added and boiled for fifteen minutes. The content was filtered and the filtrate shaken vigorously.

Test for Tannins
Two millitres of sample aqueous extract was measured by measuring cylinder and equivalent volume of 2ml of 10% sodium chloride was added to the extract. The mixture was filtered and divided into two different test tubes. Four drops of lead acetate solution was added to one of the test tubes and four drops of ferric chloride to the other.

Test for Steroids and Triterpenoids
2.0 g of the dried aqueous extract was dissolved in 10 ml chloroform and filtered. 2 ml of filtrate was measured and placed in two different test tubes. Two drops of concentrated sulphuric acid was added to one of the test tubes and five drops of acetic anhydride followed by five drops of concentrated sulphuric acid were added to the other test tube for confirmation.

Test for Flavonoids
About 2 g of the powdered leaves were completely detanned with acetone. The residue was extracted in warm water after evaporating the acetone in a water bath. The mixture was filtered while still hot. The filtrate was cooled and used.

Test for Anthraquinones
About 0.5 g of the extract was taken and placed into a dry test tube and then 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate shaken with an equal volume of 100% ammonia solution.

2.2.2 Quantitative Estimation

The preliminary phytochemical screening indicated the presence of glycosides and flavonides in the hydroalcoholic extract of *Sickle senna* leaves. Hence, to estimate these constituents, following methods were employed. Quantitative phytochemical tests were carried out according to standard procedures[11].

Total glycosides
Coarsely powdered drug (10g) was macerated with 50ml of 70% alcohol for 1 hour. The solution was filtered and the filtrate retained. Strong solution of lead acetate was slowly added until the precipitation (of pigments, proteins and alkaloidal substances) was complete. The solution along with the precipitate was filtered through a buchner funnel and the filtrate was retained. To this 6.3% sodium sulphate solution was added to precipitate excess lead and filtered. The aqueous filtrate was evaporated to dryness on a water bath to constant weight.

Total tannins
Coarsely powdered drug (2gms) was shaken with 100ml water for 30 min. The extract was filtered and the residue was washed thoroughly with water. The filtrate was treated with lead acetate solution to precipitate the tannins as lead tannate. The precipitate was centrifuged, washed with water and suspended in ethanol. Hydrogen sulphide gas was passed to remove excess of lead. The solution was filtered, the precipitate was discarded and the filtrate was evaporated to dryness on a water bath to constant weight.

2.3 HPTLC Chemo-profiling of Leaf Extract

Before use, precoated silca gel TLC (E. Merck, Darmstadt, Germany) plates 60GF254 (20cmx10cm with 0.2mm thickness)were prewashed by dipping the plates in methanol and the solvent was allowed to overrun the plate followed by drying in fume hood. The plates were activated at 105°C for 5 min prior to chromatography. Sample solutions were applied onto the plates with TLC sampler Linomat IV (CAMAG). A constant application rate of 80 nLs-1 was employed with a band width of 3.0 mm and distance between two bands was 6.2mm. The plates were developed in 20×10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) containing 25 mL of mobile phase as mixture Ethyl acetate: Methanol: Water. (10:1.35:1,v/v/v).The optimized chamber saturation time for mobile phase was 10 minutes at room temperature (25 ± 2°C). The length of chromatogram run was up to 80mm from the point of application (10mm).After development; chromatographic plates were dried for 5 minutes in a current of air with the help of a hair dryer in normal mode. The chromatographic conditions are summarized in table-1.

Table 1: Methodology for HPTLC Chemo-profiling of Sickle senna leaves extract

Chromatographic condition	
Stationary phase	Precoated silica gel 60 GF 254 TLC plates (Merck).
Mobile phase	Ethyl acetate: Methanol: Water. (10:1.35:1)
Sample application	Crude extract - 10µl (2 Tracks)
Application mode	CAMAG LINOMAT IV
Development	CAMAG Twin through chamber.
Saturation time	10 min.
Development time	30 min. developed up to 50mm
Detection	At 254 and 366nm (CAMAG TLC Scanner 3).

3. RESULTS AND DISCUSSION

The preliminary phytochemical screening indicated the presence of glycosides and flavonoids in the hydroalcoholic extract of *Sickle senna* leaves. The results of quantitative phytochemical estimation are summarized in table 2.

Table-2: Results of Quantitative Phytochemical Estimation

Serial No.	Phytoconstituents	Results*
1.	Total glycoside	13%w/w
2.	Total tannin	1.6%w/w

***** Average reading of 3 determinations

Finger printing of leaves extract showed separation of several constituents detected at 366 and 254 nm. The HPTLC chromatograms of extract obtained at 254 nm and 366 nm are represented in figure 1 and 2. When the developed TLC plate was sprayed with 5% alcoholic KOH, an intense red fluorescence was observed under UV (366nm) at retention factor (RF) value 0.7 which was found to be similar to standard retention factor (RF) value reported for Chrysophanol. The constituent may be Chrysophanol, an anthaquinone glycoside.

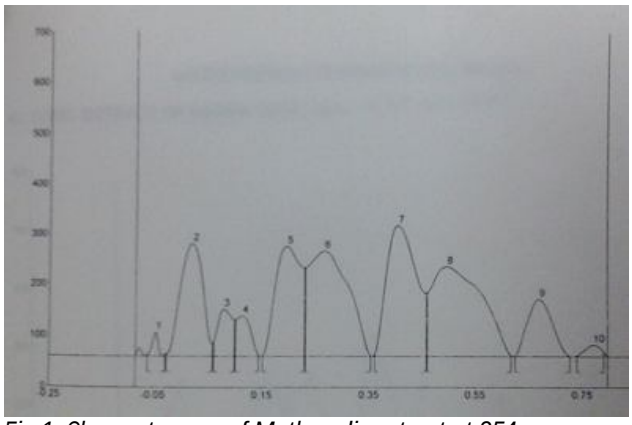

Fig.1: Chromatogram of Methanolic extract at 254nm

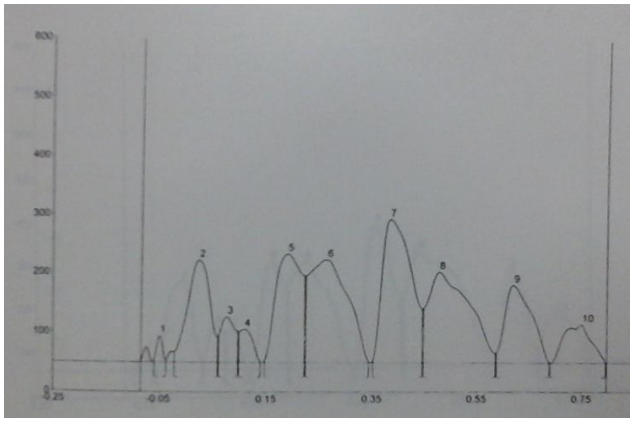

Fig.2: *Chromatogram of Methanolic extract at 366nm*

4. CONCLUSION

The preliminary phytochemical screening indicated the presence of glycosides and flavonides in the hydroalcoholic extract of *Sickle senna* leaves. The methanolic extract of *Sickle senna* leaves were reported to possess antioxidant activity probably because of the presence of several phenolic glycosides and flavanol glycoside (kaempherol- 3-diglucoside). Oxygen radicals are generated as a by-product of cyclo-oxygenase and lipo-oxygenase pathway and involved in inflammatory reactions. Activity of cyclo-oxygenase results in conversion of arachidonic acid to protacyclin and proinflammatory stable prostaglandin. Flavanoids are anti-inflammatory due to interaction with arachidonic acid. The anti-inflammatory activity of *Sickle senna* leaves extract may be due topresence of these flavanoids and phenolic glycosides.

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