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PHYTOCHEMICAL ANALYSIS OF LEAF EXTRACT OF CASSIA ALATA USING HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

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Abstract: Cassia alata, commonly known as the ringworm bush or Senna alata, is a medicinal plant widely recognized for its therapeutic potential. This study aimed to perform a comprehensive phytochemical analysis of the leaf extract of Cassia alata using High-Performance Thin-Layer Chromatography (HPTLC) and identify the presence of bioactive compounds. The leaves of Cassia alata were collected, air-dried, powdered, and subjected to sequential extraction using appropriate solvents. The resulting extracts were concentrated, and the dried residue was dissolved in suitable solvents for HPTLC analysis. Silica gel-coated plates were used as the stationary phase, while different mobile phases were employed to achieve optimal separation and resolution of compounds. Preliminary phytochemical screening of the leaf extract revealed the presence of proteins, amino acids, sugars, monosachharides, alkaloids, tanins, sterols, saponins. HPTLC analysis provided detailed information about the phytochemical composition of the leaf extract by comparing the chromatographic profiles with standard compounds and applying suitable detection methods. The HPTLC analysis of Cassia alata leaf extract identified several bioactive compounds, including flavonoids such as quercetin, kaempferol, and their derivatives. These flavonoids are known for their antioxidant, anti-inflammatory, and antimicrobial properties. Additionally, other compounds, such as phenolic acids, alkaloids, and terpenes, were also detected in the leaf extract, suggesting their potential therapeutic activities.

Index Terms *- Cassia, alata, HPTLC, Phytochmicals*

INTRODUCTION

Cassia alata, commonly known as the ringworm bush or Senna alata, is a tropical medicinal plant belonging to the family Fabaceae. It is native to Central and South America but is now widely distributed in tropical and subtropical regions across the globe. The plant is recognized for its extensive traditional uses in various indigenous medical systems.

In traditional medicine, Cassia alata has been employed to treat a diverse range of ailments. The leaves, bark, and roots of the plant are utilized for their medicinal properties. The most common traditional uses of Cassia alata include the treatment of skin infections, fungal infections, gastrointestinal disorders, inflammation, and wound healing.

Scientific investigations have been conducted to validate the traditional uses of Cassia alata and explore its potential therapeutic benefits. Phytochemical analysis of the plant has identified several bioactive compounds, such as alkaloids, flavonoids, phenolic compounds, tannins, saponins, and terpenoids. These phytochemical constituents contribute to the various pharmacological activities exhibited by Cassia alata, including antimicrobial, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer properties.

The antimicrobial activity of Cassia alata has been demonstrated against a wide range of pathogenic bacteria, fungi, and parasites. Studies have highlighted its effectiveness against Staphylococcus aureus, Escherichia coli, Candida albicans, and Trichophyton species, among others. The presence of bioactive compounds such as chrysophanol, emodin, kaempferol, and quercetin has been associated with the antimicrobial activity of Cassia alata.

Moreover, Cassia alata exhibits notable antioxidant properties, which can help neutralize free radicals and reduce oxidative stress. The antioxidant activity is attributed to the presence of flavonoids and phenolic compounds in the plant extract. These antioxidants have shown potential in preventing or mitigating various diseases associated with oxidative damage, including cardiovascular disorders, neurodegenerative diseases, and cancer.

The anti-inflammatory activity of Cassia alata has also been investigated. The plant extract has demonstrated inhibitory effects on inflammatory mediators, such as cytokines and enzymes, thereby exhibiting potential in the treatment of inflammatory conditions. Additionally, Cassia alata extract has shown promising results in wound healing studies, promoting the regeneration of skin tissue and accelerating the closure of wounds.

The pharmacological potential of Cassia alata has attracted the attention of researchers and has led to further investigations, including preclinical and clinical studies, to validate its therapeutic efficacy and safety. However, more research is needed to explore the mechanisms of action and isolate specific compounds responsible for its medicinal properties.

Materials and method

The leaf extract of Cassia alata was prepared by Soxhlet method in three different solvents, methanol, hexane and acetone. Fresh and healthy leaves were collected from neighbouring Panna district. The collected leaves were sorted and washed thoroughly with water and pat dried to release held water. Clean leaves were subjected to air dry in cardboard tray under shaded, moisture free and well-ventilated area. It took 6 days for leaves to be crisp dried.

Once the leaves were dry, it was ground to fine powder in mixer-grinder. Powder was sieved to obtain uniform texture. Leaf powder was stored in polythene bag for further use.

Soxhlet extraction was performed using acetone, hexane and methanol as solvent separately. 100 gm of leaf powder was weighed and placed in thimble. Thimble was placed in Soxhlet. 250 ml of solvent was poured through condenser passing onto sample and collected in bottom flask. Process was repeated for all three solvents. Thermostat bearing heating mantle was set at 580C for methanol, 630C for hexane and 510C for acetone. Soxhlet was run for 8 hrs for all solvents.

Leaf extract was then concentrated using rotary vacuum evaporator till thick slurry and then air dried. Final extract was mixed in DMSO (50 ml) for each solvent and subsequently final volume was raised to 100ml by adding double distilled water. Final extract was kept in air tight glass jars.

Preliminary constituent detection test for proteins, amino acids, sugars, monosachharides, alkaloids, tanins, sterols, saponins were performed by chemical tests as described by Harborne and discussed by Rangarajan (2014), slight modification in process were made.

Test for proteins and amino acid

a. Ninhydrin Test : To 1ml of extract few drops of Ninhydrin reagent was added and heated in a boiling water bath. A purple blue colour indicates the presence of proteins.
b. Biuret Test : To 1ml of extract, equal volume of 5% NaOH solution and copper sulphate solution added. A blue colour indicates the presence of proteins.

Test for carbohydrates

Benedict's Test : To 0.5ml of the filtrate, 0.5ml of Benedict's reagen was added. The mixture was heated on a boiling water bath for 2 minutes. A characteristic red colour precipitate indicates the presence of sugar.

Test for saponins

Froath test : To 0.05ml of filtrate, added 5ml of distilled water and shaken vigorously for a stable persistence froath. Froathing which persisted on warming indicates the presence of saponins

Test for alkaloids : To a few ml of filtrate, a few drops of Mayer's reagent was added by the side of the tube. A creamy white precipitate indicates the presence of alkaloids.

Test For flavanoids test : To 5ml of the extract, 5-10 drops of dilute HCl and small piece of magnesium chloride was added and the solution was boiled for a few minutes. Appearance of reddish pink colour or ditry brown colour indicates the presence of flavanoids

Test for phytosterols

a. **Libermann-Burchard's test :** To 2ml of the filtrate, 2ml of acetic anhydride was added. Concentrated sulphuric acid was added along the sides of the test tube. A colour change from violet to blue indicates the presence of phytosterols.

Test for tannins

a. Ferric Chloride test : To 2ml of extract, few drops of 5% ferric chloride solution was added. The appearance of violet colour indicates the presence of tannins.

Test for terpenoids

Salkowski Test : 5ml of the extract was mixed with 2ml of chloroform and concentracted sulphuric acid was added to form a layer. A reddish-brown colour indicates the presence of terpenoids.

Test for polyphenols : 10ml of plant extract was heated for 30 minutes in a water bath. 1ml of 1% FeCl3 was added to the mixture followed by the addition of 1% potassium ferricyanide. The mixture was filtered and formation of green-blue colour indicates the presence of polyphenols.

High Performance Thin- Layer Chromatography of the test solutions of samples A, B and C were carried out on Silica Gel 60 F254 precoated plates (0.2 mm thickness; from Merck India Limited Mumbai). A TLC applicator from Camag Linomat-5 (Camag Switzerland 140443) was used for band application and photo documentation unit (Camag Reprostar-3: 140604) was used for documentation of chromatographic fingerprints.

Procedure: Applied 6 µl each of the test solutions as 8 mm bands and develop the plates separately in a solvent system toluene: ethyl acetate (7: 3) to a distance of 9.5 cm. Dry the developed plates in air and examined under at 254 nm and at 366 nm before derivatization. Derivatized the plates using 5% Methanolic-sulphuric acid reagent and heating at 1050c till the bands are clearly visible and examined the plate under 366nm & UV light. The Rf values and colours of the bands obtained were recorded.

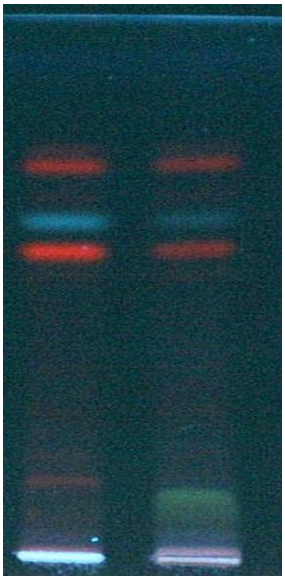
RESULTS AND DISCUSSION

Primary chemical test for examining presence of different components is summarised in table 1.

Sl. No.	Test	For detection of	Acetone	Hexane	Methanol
1	Biuret test	Protein	-	+	+
2	Salkowski	Sterols	-	+	-
3	Wagner's	Alkaloids	-	+	+
4	Ferric chloride	Tanins	-	-	-
5	Ninhydrin	Amino acid	-	-	-
6	Molisch	Sugar	+	+	+
7	Barford	Monosachharides	+	+	+
8	Foam test	Sapnins	+	+	+
9	Shimoda	Flavones	-	-	+
10	Borntrager's	Glycosides	-	-	+

Table1: Presence or absence of bioactive compounds (legends: - (not present) + (present)

HPTLC fingerprints profile of the test solutions are depicted in (Figures. A, B & C) indicates the presence of different types of phytochemicals. Development of fingerprint profile would serve as a reference standard of the authentic sample. The TLC plate was examined under 254nm, 366nm, after derivatization 366nm. The Rf values and colours of the bands obtained were recorded. It shows major spots and the Rf values and colours of the bands obtained were recorded and given in tables.



A B

Fig. I, 366nm before derivatization

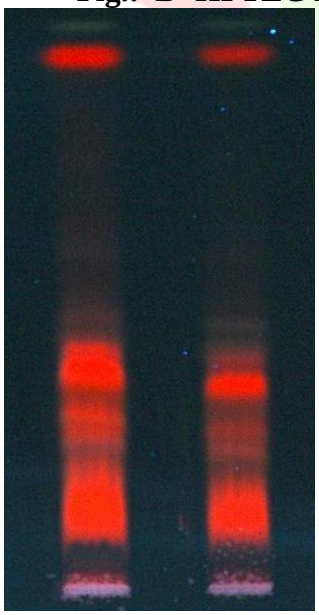
A B

Fig.2- 366nm after derivatization

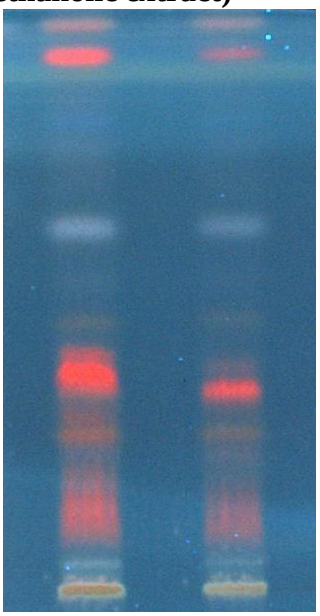
Table-1: Rf values of HPTLC fingerprints profile of Sample A-(Acetone extract)

R_f values	366nm before derivatization	366nm after derivatization
$R_{f\,1}$	0.10 (pink)	0.08 (brown)
$R_{f\,2}$	0.60 (red)	0.30 (whitish brown)
$R_{f\,3}$	0.65 (sky blue)	0.45 (whitish brown)
$R_{f\,4}$	0.72 (red)	0.60 (red)
$R_{f\,5}$	-	0.65 (brown)
$R_{f\,6}$	-	0.72 (pink)

Fig.: B- HPTLC Fingerprints Profile of Sample-B (Methanolic extract)



A B



A B

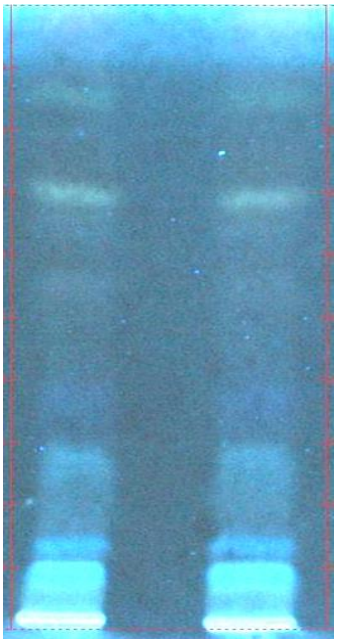
Table-2: R_f values of HPTLC fingerprints profile of Sample B-(Methanolic extract)

R_f values	366nm before derivatization	366nm after derivatization	UV light after derivatization
$R_{f\,1}$	0.08 (pink)	0.08 (brown)	0.10 (brown)
$R_{f\,2}$	0.10 (red)	0.10 (red)	0.35 (brown)
$R_{f\,3}$	0.20 (red)	0.20 (red)	0.40 (green)
$R_{f\,4}$	0.30 (red)	0.30 (red)	0.50 (brown)
$R_{f\,5}$	0.40 (red)	0.40 (red)	0.60 (brown)
$R_{f\,6}$	0.90 (red)	0.70 (brown)	0.92 (brown)
$R_{f\,7}$	-	0.90 (red)	-
$R_{f\,8}$	-	0.92 (red)	-

Fig.: C- HPTLC Fingerprints Profile of Sample-C (Hexane extract)

A B

366nm before derivatization



A B

366nm after derivatization

Table-3: R_f values of HPTLC fingerprints profile of Sample C-(Hexane extract)

R_f values	366nm before derivatization	366nm after derivatization
$R_{f\,1}$	0.08 (blue)	0.10 (sky blue)
$R_{f\,2}$	0.10 (brown)	0.12 (sky blue)
$R_{f\,3}$	0.12 (sky blue)	0.30(sky blue)
$R_{f\,4}$	0.84 (sky blue)	0.60 (sky blue)
$R_{f\,5}$	0.90 (red)	-
$R_{f\,6}$	-	-

Conclusion

Phytochemicals are compounds found in plants that often have biological activity and can be analyzed using various chromatographic techniques, including HPTLC (High-Performance Thin-Layer Chromatography). The visibility of phytochemicals at specific UV wavelengths, such as 254 nm and 366 nm, depends on the compounds' absorption characteristics.

Phytochemicals Visible at 254 nm:

Phenolic Compounds: Phenolic compounds, such as flavonoids and polyphenols, often exhibit strong UV absorption at 254 nm due to the presence of conjugated pi bonds in their structures. (Harborne, J. B., 1998)

Alkaloids: Some alkaloids, such as caffeine and nicotine, contain conjugated systems that can absorb UV light at 254 nm. (Cordell, G. A., 2012)

Phytochemicals Visible at 366 nm:

Carotenoids: Carotenoids, responsible for the colors in many fruits and vegetables (e.g., beta-carotene, lutein), often have absorption peaks in the UV spectrum around 366 nm. (Britton, G. (1995)

Flavonoids: While flavonoids can absorb UV light at 254 nm, some specific flavonoids may have absorption peaks around 366 nm. (Havsteen, B. H.,2002)

Coumarins: Certain coumarins can be detected at 366 nm due to their UV-absorbing properties. (Berhow, M. A., & Vaughn, S. F., 1999)

It's important to note that the specific absorption characteristics can vary among compounds within these classes, and the detection wavelength may also depend on the mobile and stationary phase used in the chromatography, as well as the derivatization techniques employed.

Presence of various phytochemicals makes Cassia alata leaf extract a potential subject for further research of isolating individual products for therapeutic and other control use.

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