IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

PHYTOCHEMICAL INVESTIGATION AND ANALYSIS OF BIOACTIVE COMPOUNDS IN MUSA PARADISIACA LEAVES

Praveen Kumar Dasari*, Punem Shanthi, Aduri Vasundhara, Ajmeera Vamshi Vinay, Kurram Sai Bhargavi, Munari Chikitha,

Department of Pharmaceutical Biotechnology,

Mother Teresa Pharmacy College, Kothuru, Sathupally 507303, Telangana, India.

ABSTRACT:

Phytochemical investigation was the scientific procedure to extract, examine, analyse and identifying different phytoconstituents present in various plant parts for the discovery of drugs, the active components could be further taken for investigation and research. The present research work is primarily aimed to carry out phytochemical investigation to detect the major classes of bioactive compounds presented in Musa paradisiaca leaves. Many solvents like Methanol, Ethanol, Acetone, Petroleum ether, Chloroform, Ethyl acetate, Cyclohexane and Acetic acid were used to screen the best solvent which can be used for extraction and to perform chromatographic technique of extracts. For thin layer chromatography, silica gel plates were used and mobile phase was Ethanol:Methanol:Chloroform in (1:1:1 ratio) for all extracts. Phytochemical investigation shown the presence of major components like Alkaloids, flavonoids, tannins, phenols, glycosides, saponins and steroids in Musa paradisiaca leaves. Among different solvent extracts, methanol extract shown high. Further thin layer chromatographic studies were done with the Musa paradisiaca leaf extract, observed different coloured bands of phytochemical compounds with different Rf values. In this methanol extract shown seven bands with Rf less than 1 for 24 hrs. Column chromatographic studies with methanolic extract was done to determine better separation, the mobile phase used was Ethanol:Methanol:Chloroform. The research work was concluded that the methanol extract of Musa paradisiaca leaves contain a higher amount bioactive compounds and methanol as a solvent shown effective separation of bioactive compounds, which can be used for future research on this plant.

KEYWORDS: Musa paradisiaca, Phytochemical investigation, Thin layer chromatography, Column chromatography

INTRODUCTION:

Plants consist of various number of chemical compounds known as phytoconstituents. Phytochemical compounds serve the plants by contributing secondary functions like, protecting the plants by act as defence mechanism, helps in plant growth, imparting colour, odour, and flavour to the plants. Medicinal plants are useful species of plant kingdom, according to the traditional medicinal practices and modern scientific research for the medicinal purposes to treat diseases and make human health more strength. These medicinal plants are treasury of ingredients that can be used in the synthesis and production of new drugs. Natural plant products and their derivatives exhibit less side effects and improved efficacy than other synthetic compounds [1,2].

Standardization of crude drugs extracted from plant plays a vital role in identification of the quality and purity of drugs. Plants products are very crucial in medicinal field; they have similar properties as conventional pharmaceutical drugs. Chromatographic techniques are playing an important role for the

separation, identification, and estimation of different bioactive compounds [3-5]. These compounds have various biological actions, such as antidepressant, urolithiasis, antibacterial, laxatives, anthelminthics, antihypertensive, antiulcerogenic etc. Hence, the analysis of bioactive compounds in plants would help in determining various biological activities which will be useful to mankind. The aim of this research work is phytochemical investigation of Musa paradisiaca leaves and analysis of bioactive compounds by thin layer chromatography and column chromatography.

MATERIAL AND METHODS

Collection of the Plant Material

Musa paradisiaca leaves were collected from local market, Sathupally, Khammam, Telangana.

Preparation of Musa paradisiaca leaves powder:

Musa paradisiaca leaves are collected and air dried because to prevent it from direct sunlight impact to minimize undesirable chemical reactions of plant metabolites. Dring was crucial to prevent the formation of artifacts because of microbial fermentation and subsequent degradation of the plant metabolites. In this present investigation, leaves are dried in shade and then powder with a mechanical grinder. The leaf powder was passes through sieve size 44 and stored in an airtight container for further studies.

Extraction of plant material:

Maceration: It is one of the extraction techniques in which coarse and powdered plant material is soaked in different solvents, 50g of the leaf powder was dissolved in 250ml of Methanol, Ethanol, Acetone, Petroleum ether, Chloroform, Ethyl acetate, Cyclohexane and Acetic acid respectively for overnight. After 24 hours, the solvents were filtered by through Whatman filter paper and was stored at 4^{o}C in black caped bottles for the further studies [6-9].

.

Fig 01: Maceration Process

Fig 02: Different solvent extractions of Musa paradisiaca leaves

Phytochemical Investigation:

The phytochemical investigation determines the presence of different bioactive compounds possessing various therapeutic values. The different solvent extracts of Musa paradisiaca leaves were used for screening the presence of glycosides, alkaloids, flavonoids, steroids, coumarin, tannins, carbohydrate, saponins, phenol, protein, xanthoprotein, quinone, anthraquinone, sugar and terpenoids according to standard procedures.

Tests for alkaloids:

To 2 mL of leaf extract add 1 mL of Dragendorff's reagent if an orange red precipitate was observe, indicates the presence of alkaloids.

Tests for flavonoids

To 2 mL of leaf extract add three drops of sodium hydroxide. Initially, a deep yellow colour was observed but it gradually became colourless by the addition few drops of dilute hydrochloric acid, indicates the presence of flavonoids.

Test for phenolic compounds and tannins.

To 1 mL of leaf extract add 2 mL of 5% ferric chloride solution if dark blue colour was observed that indicates the presence of phenolic compounds and tannins.

To 0.5 mL of extract add 1 mL of lead tetra acetate solution if precipitation was formed indicates the presence of phenolic compounds and tannins.

Tests for glycosides

To 2 mL of leaf extract add 0.5 mL of glacial acetic acid and 2-3 drops of ferric chloride mix it, add 1 mL of concentrated H_2SO_4 on the walls of the test tube. If deep blue colour was observed at the junction of two solutions indicates the presence of cardiac glycosides.

Tests for saponins

To 5 mL of leaf extract add few drops of Na_2CO_3 solution in a test tube. After vigorous shaking, keep it as side for five minutes. If foam was observed indicates the presence of saponins.

Test for steroids.

Salkowski test: The leaf extract was treated with chloroform and concentrated H_2SO_4, if red colour observed, indicates the presence of steroids.

Chromatographic Analysis:

Chromatography is a separation method where the sample is combined within a mobile phase, which is pumped through a stationary phase. Depending on their polarity, they interact with the stationary phase and retarded to a greater or lesser extent. This tends to the separation of the different components present in the sample.

Thin Layer Chromatography

In thin-layer chromatography, the mixture of substances is separated into its components with the help of a glass plate which is coated with a very thin layer of adsorbent, such as silica gel. The sample mixture to be separated is applied as a small spot at 2 cm above one end of the plate. The plate is then placed in a closed jar containing a mobile phase, which rises the plate carrying sample components of the mixture to different heights.

Method:

On the thin-layer chromatography plates, drawn a line at 2 cm above one end of the plate. The leaf extract is applied on pre coated plate with the help of capillary tubes. The mobile phase moves upward through the stationary phase, the solvent moves up the thin plate soaked with the solvent by means of capillary action. This upward travelling rate depends on the polarity of the solvent. After this the TLC plates were air dried and observed under the ultraviolet light. The development of separated bands movement was expressed by its retention factor (R_f) values being calculated for different leaf extracts.

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Fig: 03 TLC of solvent extractions

Column Chromatography:

Column chromatography is separation technique, substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allows them to get separated in fractions. When the mobile phase along with the sample to be separated is introduced from the top of the column, the movement of the individual components of the sample mixture is at different rates. The components with lower adsorption and affinity to the stationary phase moves faster when compared to the greater adsorption and affinity with the stationary phase. The components that move quickly from stationary phase are removed first whereas the components that move slowly are eluted out last. This method is a type of adsorption chromatography technique [(10-14)].

Preparation of leaf extract for column chromatography:

The methanolic leaf extract concentrated by evaporation under the room temperature. The concentrated product was semi solid dark green in colour, viscous in consistency. The obtained product was preserved at 4°C.

Isolation Method with Column Chromatography:

Concentrated methanolic leaf extract was placed in column chromatography on silica gel packed and eluted with a mixture of chloroform, ethanol, methanol of increasing polarity to obtain fractions. The admixture was packed on a silica gel column and elution begins with 100% chloroform and increased with solvent polarity ethanol and methanol. Chloroform produced a colourless compound and followed by further purification with ethanol and methanol for the separation of bioactive compounds.

Fig: 04 Column Chromatography

RESULTS AND DISCUSSION:

The present research work was to carry out by phytochemical investigation to detect the major classes of bioactive compounds presented in Musa paradisiaca leaves. Many solvents like Methanol, Ethanol, Acetone, Petroleum ether, Chloroform, Ethyl acetate, Cyclohexane and Acetic acid were used to screen the best solvent which can be used for extraction and to perform chromatographic technique of extracts. These compounds have various biological actions, such as antidepressant, urolithiasis, antibacterial, laxatives, anthelminthics, antihypertensive, antiulcerogenic etc.

Table 1: Qualitative Phytochemical screening of Musa paradisiaca leaves.

Tests/Solvent Used	Methanol	Ethanol	Acetic acid	Acetone	Ethyl acetate	Chloroform	Cyclo hexane	Petroleum ether
Alkaloids	++	++	+	+	+	++	++	+
Flavanoides	++	++	++	++	-	++	+	+
Tannins	+	+	++	++	++	+	+	++
Phenols	++	++	++	++	+	++	++	+
Glycosides	++	++	++	++	++	++	++	+
Saponins	+	-	++	+	+	+	++	-
Steroids	++	++	+	++	++	++	+	-

Chromatographic purification: TLC

TLC of Methanol extract of Musa paradisiaca leaves with mobile phase Chloroform: Methanol: Ethanol (1:1:1) shown the presence of 7 compounds having Rf values of 0.42, 0.51, 0.58, 0.64, 0.87, 0.93, 0.97 respectively. With ethanol extract of shown 5 bands having Rf values of 0.44, 0.59, 0.69, 0.74, 0.79 respectively. With petroleum ether extract shows 5 bands having Rf values of 0.47, 0.59, 0.65, 0.76, 0.81 respectively. With Acetone extract shows 4 bands having Rf values of 0.67, 0.73, 0.79, 0.83 respectively. With Ethyl acetate extract shows 3 bands having Rf values of 0.60, 0.73, 0.88 respectively. Cyclohexane extracts shown 3 bands having Rf values 0.56, 0.67, 0.75 respectively.

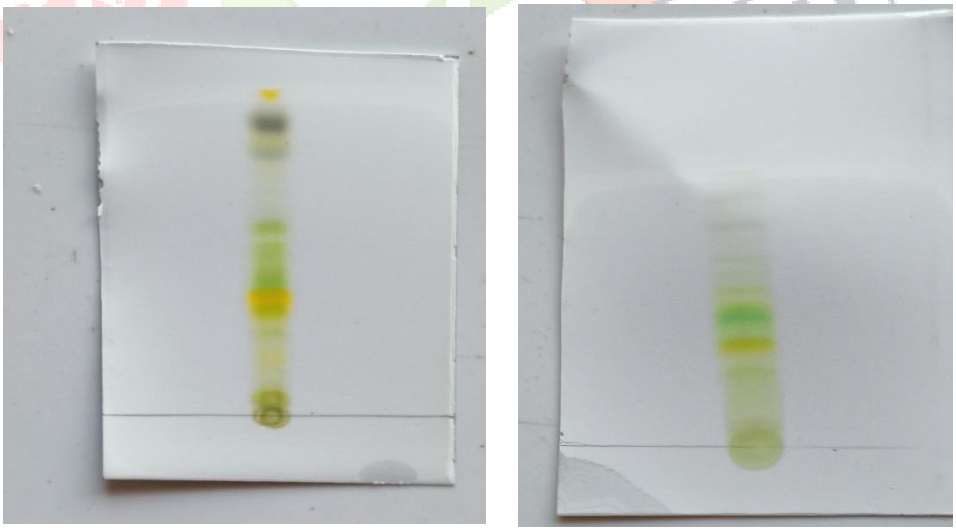


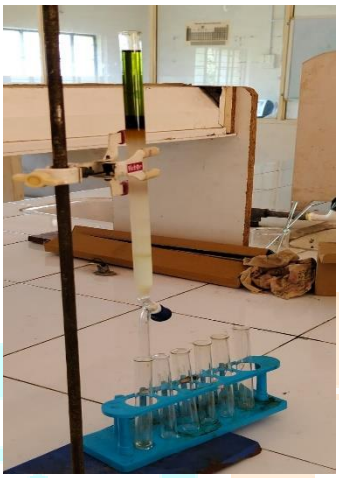
Fig: 05 (a) & (b) Separation of different phytochemical compounds

Chromatographic purification: Column chromatography

Isolation of compound was performed by column chromatography by using various solvents (chloroform, ethanol, methanol) of increasing order of polarity and 15 fractions were collected and phytochemical screening was performed for each fraction. The methanol fraction showed the better separation of bioactive compounds than chloroform and ethanol.

Table 2: Screening of Musa paradisiaca leaves by column chromatography.

Solvents Used	Alkaloids	Flavonoids	Tannins	Phenols	Glycosides	Saponins	Steroids
Methanol	+	+	+	+	+	+	+
Ethanol	+	+	+	-	+	+	-
Chloroform	+	+	+	-	+	-	-



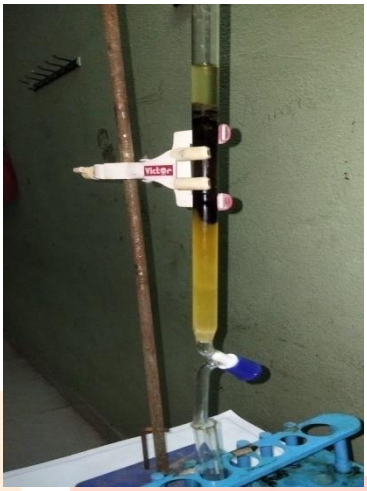


Fig:06 (a) & (b) Separation of different phytochemical compounds

CONCLUSION:

Phytochemical investigation of present research shown the presence of major components like Alkaloids, flavonoids, tannins, phenols, glycosides, saponins and steroids in Musa paradisiaca leaves by using different solvents. The TLC technique shown that Musa paradisiaca leaves are rich sources of bioactive compounds. These findings suggested that Musa paradisiaca leaves have a potential source of natural antioxidants which have great importance as therapeutic agents for many diseases. Further investigation done by using column chromatography among different solvents (methanol: ethanol: chloroform) methanolic leaf extract of Musa paradisiaca have shown important phytochemicals. The methanol extract of Musa paradisiaca leaves shown the presence of alkaloids, flavonoids, tannins, phenols, glycosides, saponins and steroids. Future studies needed to determine individual phytochemical compounds and test for their biological activities such as antidepressant, urolithiasis, antibacterial, laxatives, anthelminthics, antihypertensive, antiulcerogenic etc

ACKNOWLEDGEMENT:

The authors are thankful to the management of Mother Teresa Pharmacy College, Kothuru, Sathupally, Khammam for providing the necessary facilities to carry out the research work.

REFERENCES:

1. Ingle KP, Deshmukh AG, Padole DA, Dudhare MS, Moharil MP, Khelurkar VC. 2017. Phytochemicals: Extraction methods, identification, and detection of bioactive compounds from plant extracts. J Pharmacogn Phytochem. 6:32–36.
2. Azwanida NN. 2015. A review on the extraction methods use in medicinal plants, principle, strength, and limitation. Med Aromat Plants. 4:196.
3. Marston A. 2011. Thin-layer chromatography with biological detection in phytochemistry. J. Chromatogr. A. 1218(19):2676–2683

4. Homans AL, Fuchs A. 1970. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. J. Chromatogr. 51:327–329
5. Kenyon AS., Flinn PE., Layloff TP. 1995. Rapid screening of pharmaceuticals by thin-layer chromatography: Analysis of essential drugs by visual methods. J. AOAC Int. 78:41–49.
6. Rungsung W, Ratha KK, Dutta S, Dixit AK, Hazra J. 2015. Secondary metabolites of plants in drugs discovery. World J Pharm Res. 4:604–613.
7. World Health Organization (WHO). Traditional Medicine. 2008, WHO Fact Sheet No.134. Geneva.
8. Ahlam Rashed, 2019. Moringa Oleifera Methanol, Ethanol, Water and Ethyl Acetate Extracts; Saudi journal of medical and pharmaceutical sciences; 5(11); 817-820.
9. B. K. Sharma; 2011. Instrumental methods of chemical analysis; Goel publishing house; 27; 3-20, 96-112, 241-267.
10. Archana A. Bele, Anubha Khale; 2011. An Overview on Thin Layer Chromatography; International Journal of pharmaceutical Sciences and Research; 2(2); 256-267.
11. Hannah R. Klein, William J. Mader; 1971. Thin Layer Chromatography Identification of Sulfonamides; Journal of Pharmaceutical Sciences; 60(3); 448-450.
12. R. Devika, Justine Koilpillai; 2015. Column Chromatographic Separation of Bioactive Compounds from Tagetes Erecta Linn; International journal of Pharmaceutical Sciences and Research; 6(2); 762-766.
13. Anju T, Divya A, Sri Varsha V, Nandini R, Prasanna Bhavani K, Bhavani Y, Varma V and Satyanarayana T. 2023. Phytochemical Screening and Separation of Bioactive Compounds from Azadirachta Indica leaves By TLC And Column Chromatographic Techniques. World Journal of Pharmaceutical Research. 12(4); 2080-2090.
14. Li Wenying, Ye Cuiping, Feng Jie; 2006. Influence of column chromatography and Soxhlet Extraction on the Composition of Coal Pyridine-Soluble; Chinese Journal of Analytical Chemistry; 34(7); 905-910.