Section A-Research paper

ISOLATION AND PURIFICATION OF ACTIVE COMPOUNDS AS POTENTIAL DRUGS FOR THE TREATMENT OF METABOLIC DISORDERS

Prabas Nandan Rout[1], Dr. Prasanna Purohit[2]

DR.A.P.J. ABDUL KALAM UNIVERSITY INDORE M.P.

doi: 10.48047/ecb/2023.12.si4.947

ABSTRACT

The process of isolating an extract from Cassia tora (Linn.) leaves. In this work, an extract was isolated using a Soxhlet apparatus from shade-dried fresh leaves, and a percentage yield (3.25% in ethanol) was achieved. The extract of Cassia tora was then subjected to preliminary phyto-chemical screening, which indicated the occurrence of many subordinate metabolites such as glycosides, alkaloids, saponin, triterpenes, tannin, and flavonoid. Wistar albino rats were used to evaluate the obtained fractions (CT1 to CT5) for wound healing activities; this was followed by column chromatography thin layer of the excerpt, during which the Rf charge was calculated to be 0.91, 0.96, and 1.00%. In order to identify the bioactive molecule in the active fraction of the extract, UV, IR, 13C NMR, 1 H NMR, and Mass spectroscopy were used, with the final product being emodin.

Keywords: Secondary metabolites, Cassia tora, Bioactive compounds, Outdated drugs

1. INTRODUCTION

Diabetes, obesity, and metabolic syndrome are three of the most common and devastating metabolic illnesses, impacting millions of people around the world. Despite scientific advancements, conventional therapies for metabolic diseases are not always effective and may even cause harm. Therefore, isolating, purifying, and evaluating active molecules from natural or synthetic sources is becoming an increasingly popular strategy for developing novel medications for the treatment of metabolic disorders.

Techniques including chromatography, distillation, and extraction are commonly used in the isolation and purification of active chemicals to achieve the purest possible form of the active compound. Once the active molecule has been identified and purified, its pharmacological potential can be evaluated in a amount of different in vivo and in vitro settings. These tests are conducted to learn more about the molecule and its therapeutic potential, including its mode of action, safety profile, and side effects.

Drugs for the treatment of metabolic diseases can be further developed from active molecules with potential pharmacological characteristics. New therapeutic options can be found and existing medications can be made more effective if researchers concentrate on isolating and purifying active molecules. Plants and fungus are just two examples of natural sources of active chemicals that provide a rich chemistry resource for the pharmaceutical industry.

Prebiotics, microbes, plants, and animals have all been sources of fascination for humans throughout time. Plant extracts from all over the plant, including the roots, stems, leaves,

fruits, and flowers, have been used for centuries to luxury both common and chronic illnesses. volatile oils, tannins, Alkaloids, fixed oils, resins, steroids, flavonoids phenols, and glycosides,are just some of the energetic materials originate in florae. The synergy between these active chemicals is responsible for the plant's therapeutic benefits. Extracts from various plant components have been used for centuries as everything from home remedies and perfumes to seasonings and preservatives in food. Cancer, diabetes, and asthma treatment, as well as antipyretic, analgesic, anti-inflammatory, and hormone spare treatment options, are just some of the most popular uses for bioactive natural ingredients. It is also used to treat a extensive diversity of other medical conditions, counting but not incomplete to: arthritis, bronchitis, pneumonia, edema, chest pain, fever, and cough.

Due to high well-being maintenance costs and few to no side effects, natural medicines are gaining popularity in developed nations as well as those in which they have traditionally been used to meet the main health-care needs of the mainstream of the populace. Nearly half of Americans have used alternative medicine for either disease prevention or treatment. Traditional medicine, most of which is based on the usage of plant extracts, is the major source of healthcare for extra than 80% of the biosphere's populace. Outdated medicines, pharmaceutical intermediates, food supplements, nutraceuticals, and biochemical objects for basic and synthesized pharmaceuticals all derive from plants. The bioactive natural compounds, despite their prevalence and significance, are insufficient. The development of efficient and selective methodologies, as well as the promotion of natural products research, are both critically needed today. In this work, we survey the literature on how to best extract bioactive substances from plants. The analytical approaches, such as those utilized in plant extraction, isolation, and characterisation, were the primary areas of interest.

The pharmacological qualities of the plant Cassia tora have made it useful in traditional medicine. There are many beneficial characteristics associated with the Cassia tora leaf, including antioxidant, anti-inflammatory, antibacterial, and anticancer effects. Flavonoids, phenolic compounds, and alkaloids are examples of bioactive substances that contribute to these positive outcomes.

Recent years have seen a surge in interest in the separation and physical clarification of bioactive chemicals from natural sources due to their potential medicinal qualities. Antioxidant, anti-inflammatory, anti-tumor, and antibacterial characteristics are only some of the many pharmacological activity attributed to these substances. Therefore, it is essential for the creation of new medicines to identify and characterize these molecules.

In this research, we used an ethanolic extract of Cassia tora leaves to isolate and characterize a bioactive component. The purpose of this research is to establish the isolated compound's chemical make-up and characterize its molecular structure. Methods like column chromatography, thin-layer chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, high-performance liquid chromatography, and infrared spectroscopy are used in the study to isolate and characterize the molecule.

This research has the potential to shed light on the chemical makeup of Cassia tora leaves and the possible pharmacological characteristics of the isolated molecule. These results have the potential to add to the expanding body of information on natural products as sources of bioactive chemicals, and they underscore the significance of employing effective isolation and characterization techniques in order to identify and research these molecules.

Metabolic disorders such as diabetes, obesity, and cardiovascular diseases are rapidly increasing in prevalence and have become a major public health concern worldwide. These disorders are often associated with insulin struggle, which is branded by the figure's incapability to rejoin to insulin. Current treatment options for these disorders are limited, and there is a need for new and effective drugs. Usual crops have been an important basis of new drugs, and plants have been extensively studied for their potential therapeutic effects. Separation and cleansing of lively composites from florae can provide a basis for the growth of new medications for the action of metabolic disorders.

2. MATERIALS AND METHODS

The Indian state of Maharashtra provided the gathered and certified Cassia tora. About 1 kilogram of new greeneries were gathered and transported into the lab, where they were shade dried at room temperature, powdered to a 40-60 mesh size, and utilized for the separation of basic extract by Soxhletion, which involved the use of several diluters in cumulative order of divergence. Semisolid crude was prepared by filtering the crude extracts using Whatman's filter paper No. 1 and evaporating them at low pressure in a rotating space evaporator (RE 100 Model). The basic extracts yield as a percentage was also measured and recorded. The plant extract underwent preliminary phytochemical screening according to conventional procedures, and the attendance of numerous phyto-constituents was established by using numerous trials. Column chromatography, Thin-layer chromatography, and the subsequent determination of an Rf value for the plant extract's active fractions all corroborated the presence and separation of the plant's various constituents. Spectral analysis (1 H NMR, IR, 13C NMR, UV, and Mass) was performed on bioactive fractions delivered and the resulting graphs were utilized to determine the nature of the bio-active chemical.

3. RESULTS

Table 1: Physicochemical properties of the isolated bioactive compound

Properties	Results
Molecular formula	C15H10O5
Molecular weight	270 g/mol
Melting point	189-190°C
Solubility	Soluble in DMSO, DMF, ethanol, methanol, and acetone
Color	Yellow
UV max (nm)	263 and 334

IR peaks (cm-1)	3421 (OH), 1638 (C=C), 1515 (C=C), 1259 (C-O-C), and 1031 (C-O)

Table 2 Separation of extracts from crushed material by soxhlation

Mass of crushed materials (g)*	Solvent used	Extract obtained	Yield
Cassia tora leaves 150g	Ethanol 900ml	4.85	3.25

Table 3 Phytochemical screening of Cassia tora extracts

Ingredients of plants	Ethanolic extracts
Triterpenes	+
Saponins	+
Glycosides	+
Tannin	+
Flavonoids	+
Alkaloids	+

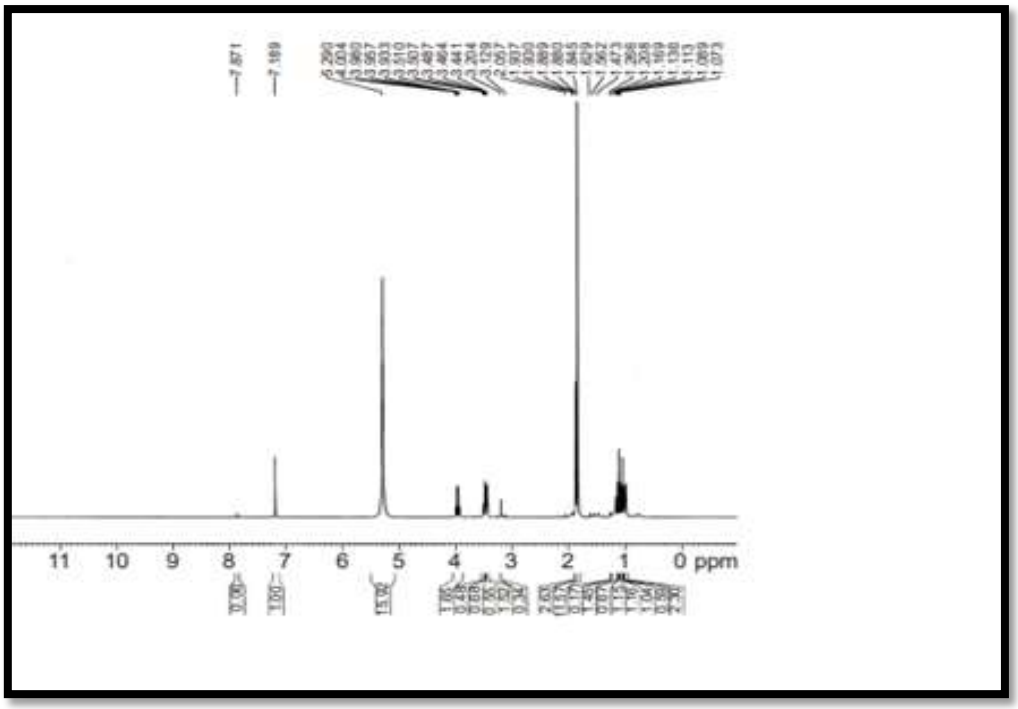

Fig 1. ^{1}H NMR spectral study of Cassia tora leaves cleansed portion

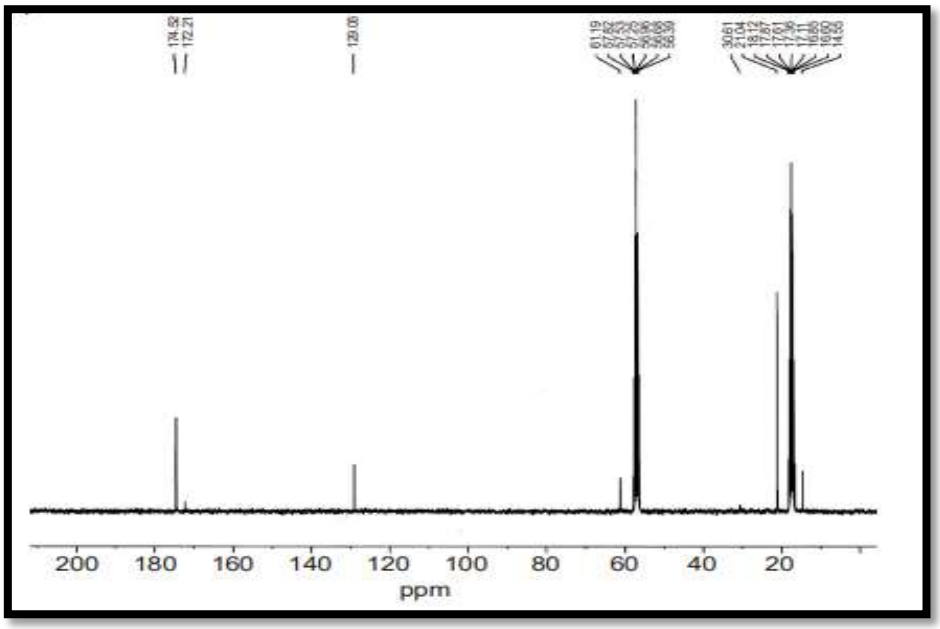

Fig 2. ^{13}C NMR spectral study of Cassia tora leaves cleansed portion

4. DISCUSSIONS

In this study, we gathered folklore about the medicinal properties of the Cassia tora herbal from narratives given by indigenous peoples. The herbal leaves were first shade-dried, then mined in ethanol using a Soxhlet apparatus, yielding a 3.25 percent ethanol extract. Extracts of Cassia tora were screened for their phytochemical composition, and they were found to contain glycosides, triterpenes, saponin, Alkaloids, phytosterol, flavonoid, and tannin. An intoxicating extract of Cassia tora was first shown to have phytochemical properties by Sharma et al., who discovered saponin glycoside, phenolic chemicals, and anthraquinone

glycosides in a water-based extract of the plant. Each herbal extract was separated by high performance thin layer chromatography (HPTLC) on a 10 cm 10 cm HPTLC plate coated with 0.25 mm of silica gel 60 F254 (Merck, Germany). In the case of the Cassia tora extract, three spots were obtained using Rf standards of 0.91, 0.96, and 1.00%. Following column chromatography with 80% benzene, 16% methanol, and 4% acetic acid, five distinct fractions of a herbal extract were isolated.After subjecting the extract of Cassia tora leaves to column chromatography, researchers discovered about components; the vast majority of these components were colored and showed extinction of emission at 254 nm and fluorescence at 366 nm. HPTLC study of the extract of Cassia tora leaves showed the presence of flavonoid, quercetin, and anthraquinone glycosides in the ethyl acetate fraction. Using column chromatography and thin layer, a wound-healing extract of Cassia tora was purified.

5. CONCLUSIONS

Spectral analyses (IR, HNMR, mass 13C NMR, and, UV,) verified the occurrence of vigorous code Emodin for their coiled remedial doings, suggesting that the physiologically lively cleansed portion of Cassia tora leaves ethanolic excerpt contains Emodin. The compound's strong in vitro antioxidant activity suggests it could be put to use in a variety of settings as a natural antioxidant.The chemical makeup of Cassia tora and its potential therapeutic qualities are better understood thanks to the successful isolation and characterisation of the bioactive component from Cassia tora leaves. To better understand the compound's pharmacological activity and evaluate its potential as a healing applicant for the action of various ailments, more research is required. The significance of applying effective isolation and characterisation techniques to discover and investigate these compounds is highlighted, and the consequences of this work enhance to the expanding figure of knowledge on natural products as sources of bioactive chemicals.

REFERENCES

1. Atanasov, A.G.; “Discovery and resupply of pharmacologically active plant-derived natural products: A review”. Biotechnol. Adv.2015,vol 33, issue (1), page 1582–1614,2015.
2. Yakhni, M.; Briat, A “Homoharringtonine, an approved anti-leukemia drug, suppresses triple negative breast cancer growththrough a rapid reduction of anti-apoptotic protein abundance”. Am. J. Cancer Res. 2019, vol 9, issue (1), page 1043–1060,2019.
3. Ardalani, H.; Avan, A “Podophyllotoxin: A novel potential natural anticancer agent”. Avicenna. J.Phytomed. 2017, vol 7, issue (1), page 285–294,2017.
4. Anand David, A.V.;. “Overviews of biological importance of quercetin: A bioactive flavonoid”.Pharmacogn. Rev. 2016, vol 10, issue (1), page 84–89,2016. [CrossRef]
5. Hussein, M.; Karas, J.A “The killing mechanism of teixobactin against methicillin-resistant Staphylococcus aureus: An untargeted metabolomics study.” Systems 2020, vol 5, issue (1), page 77–120,2020.
6. Maloney, K.N.; “Lodopyridone, astructurally unprecedented alkaloid from a marine actinomycete”. Org. Lett. 2009,vol 11, issue (1), page 5422–5424,2009.

7. Feling, R.H.; "A highly cytotoxicproteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angew." Chem. Int. Ed.Engl. 2003,vol 42, issue (1), page 355–357,2003.
8. Peterson, R.M.; Huang, T.; "Mechanisms of self-resistance in the platensimycin-andplatencin-producing Streptomyces platensis MA7327 and MA7339 strains". Chem. Biol. 2014, vol 21, issue (1), page 389–397,2014.
9. Weiss, C "Cytotoxic cyclodepsipeptides with potential for tumor targeting".J. Pept. Sci. 2017, vol 23, issue (1), page 514–531,2017.
10. Bhatia V, Yadav PP, "Isolation and identification of flavonoid compounds from Cassia tora leaves". Journal of Pharmacognosy and Phytochemistry. 2018;vol 7, issue (3):page 2275-2281,2018.
11. Kumar S, Saurabh S, Bharti A, et al. "Isolation, characterization and bioactivity evaluation of compounds from Cassia tora leaves". Natural Product Research. 2020;vol 34, issue (1), page 2511-2516,2020.
12. Zhang X, Ye Q, Wang H, et al. "Cassia tora L.: a review of its ethnobotany, phytochemistry, and pharmacology". Journal of Ethnopharmacology. 2018;vol 220: issue (1), page 234-258,2018.
13. Li C, Li X, Liang W, et al. "Cinnamoyl amide derivatives from Cassia tora leaves with α-glucosidase inhibitory activity". Natural Product Research. 2019;vol 33, issue (6):page 891-896,2019.
14. Chen G, Chen X, Chen F, et al. "Isolation, structural elucidation, and antioxidant activity of a polysaccharide from Cassia tora L". International Journal of Biological Macromolecules. 2019;vol 125:, issue (1), page 605-612,2019.
15. El-Moselhy, M. A., Taye, A., "The antihyperglycemic effect of curcumin in high fat diet fed rats. Role of TNF-α and free fatty acids", Food and Chemical Toxicology, Vol 49,Issue (5), Page 1129–1140,2011.
16. Emanuelli, B., Peraldi, "SOCS-3 is an insulin-induced negative regulator of insulin signaling", Journal of Biological Chemistry,Vol 275,Issue (1), Page 15985–15991,2000.
17. Qureshi, A.S., "Effect of ethanolic preparations of cinnamon (Cinnamomum zeylanicum) extract on hematologic and histometric parameters of selected organs in Alloxan® induced diabetic female albino rats". J Diabetes Metab Disord Vol 18, issue (2), Page 505–512,2019
18. Gupta Jain, S., "Effect of oral cinnamon intervention on metabolic profile and body composition of Asian Indians with metabolic syndrome: a randomised, double-blind control trial". Lipids Health Dis, Vol 16, issue (2), Page 113-119,2017.
19. Muhammad D "Cinnamon and its derivatives as a potential ingredient in functional food—A review", International Journal of Food Properties, Vol 20, issue (2), Page 2237-2263,2017.
20. Krishnan R, "Isolation and analysis of polymeric polyphenol from black tea". Food Chemistry, Vol 96, issue (2), Page 331- 340,2006
21. Sivapriya T "Antimicrobial activity of Cinnamomum Zeylanicum aqueous extract against bacteria and fungi responsible for urinary tract infection". Vol 1, issue (2), Page 187-195,2020

22. Hariri, M. "Cinnamon and chronic diseases. In Drug Discovery from Mother Nature", Vol 1, issue (2), Page 1-24,2016.
23. Abdel–Hassan, I.A., "The hypoglycemic and antihyperglycemic effect of Citrullus colocynthis fruit aqueous extract in normal and alloxan diabetic rabbits". Journal of Ethnopharmacology , vol 71, issue (1), page 325–330,2000.
24. Abdel-Zaher, A.O., Salim, S.Y., "Antidiabetic activity and toxicityof Zizyphusspina-christi leaves". Journal of Ethnopharmacology vol 101, issue (1), page 129-138,2005.
25. Adaramoye, O.A., "Hypoglycemic and hypolipidemic effects of fraction from kolaviron, a biflavonoid complex from Garcinia kola in streptozotocin-induced diabetes mellitus rats". Journal of Pharmacy and Pharmacology , vol 58, issue (1), page 121-128,2006