

**PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF
RHIZOME METABOLITES FROM *CANNA INDICA* L. AND
CYPERUS ARTICULATUS L.**

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CENTER FOR RURAL DEVELOPMENT AND TECHNOLOGY

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RHIZOME METABOLITES FROM *CANNA INDICA* L. AND
CYPERUS ARTICULATUS L.**

by

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Dedicated to My Parents

Shri Abhimanyu Swain

Smt Manorama Swain

Certificate

This is to certify that the thesis entitled “**Phytochemistry and Biological Activities of Rhizome Metabolites from *Canna indica* L. and *Cyperus articulatus* L.**” submitted by **Mr. Ayusman Swain** to the Center for Rural Development and Technology, Indian Institute of Technology Delhi for the award of **DOCTOR OF PHYLOSOPHY** is a record of the bonafide research work carried out by him. Mr. Ayusman Swain has worked under our supervision for submission of this thesis which to our knowledge has reached requisite standard.

To the best of our knowledge the results in this thesis have not been submitted for any degree or diploma in any other Institute or University. He has fulfilled all the requirements for submission of his thesis.

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Abstract

The rhizome of *Canna indica* (family: Cannaceae) has been used as a traditional medicine source for treatments of fever, dropsy and dyspepsia. Similarly, leaf and seed extracts are used to treat analgesic and to cure earaches, respectively. The flower is said to cure eye diseases. In different regions of the world, it is consumed as a staple food as well as a source of starch. The rhizome has been used as a source of starch in food industries in many regions of the world. Here, the study of the rhizome of *C. indica* was aimed to determine its potential use as a functional food, a source of vitamins, nutrition and nutraceutical ingredients. Biomass and nutrient characterization showed the rhizome as a rich source of fiber (25.1%), starch (28.5%), protein (4.72%), and lipids (5.75%) with a total caloric value of 423 Kcal/100 g dw (dry weight). The rhizome also had a considerable amount of minerals and vitamins. Acetone extracts of rhizome showed significantly higher antioxidant properties. The IC₅₀ values with DPPH, ABTS⁺ and O₂⁻ radical were found to be 21.6, 23.2 and 169 µg/mL, respectively. The reducing properties (FRAP and CUPRAC) and DNA protection assay was correlated with total phenolic and flavonoid content of the rhizome extracts. Acetone and methanol extract showed protection against free radical-induced DNA and protein degradation. In food model system, acetone extract significantly decreased the bleaching of β-carotenoids and thiobarbituric acid reactive substance (TBARS) from ground pork meat. α-glucosidase activity was significantly inhibited by water extract (IC₅₀ 2.35 µg/mL) and acetone extract (IC₅₀ 27.1 µg/mL). HR-LCMS/MS analysis of different extracts showed the occurrence of different bioactive compounds such as rosmarinic acid, psoromic acid, usnic acid, isoeugenitol, ellagic acid, coumaric acid, phloionolic acid and swietenine. The results suggested that *C. indica* rhizome as a potential source of nutrients and metabolites for health benefit.

Cyperus articulatus L. is a perennial plant distributed in tropical and subtropical region and its rhizome has been used as traditional medicine in different regions to treat some common diseases such as malaria, epilepsy and dysentery. The rhizome contains essential oil which is of pharmacological and economic interest and has antimicrobial properties. The plant was mostly studied for its essential oil but its other biological properties and metabolite profile were least explored. Possible utilization of rhizome extracts of *C. articulatus* as a source of natural drug ingredients to manage diseases like oxidative stress, diabetes and Alzheimer was the main objective of the present study. *In vitro* biological activities such as antioxidant property, application of antioxidants in food and biological model systems, enzyme (α -amylase, α -glucosidase, acetylcholinesterase) inhibitory activity was studied for the rhizome extracts. The metabolite analysis was done by HR-LCMS/MS. Among different solvent extracts, acetone extract showed potent activity as antioxidant and enzyme inhibitors followed by ethyl acetate extract. The IC_{50} values for scavenging of radicals such as DPPH, $ABTS^{\cdot+}$ and $O_2^{\cdot-}$ are found to be 12.2, 16.3 and 83.2 $\mu\text{g/mL}$, respectively. Similarly, IC_{50} values for reducing property (FRAP, 156 $\mu\text{gGAE/mg}$ extract and CUPRAC, 407 $\mu\text{g BHAe/mg}$ extract), Metal chelating (44.8 $\mu\text{g EDTAE/mg}$ extract), and total antioxidant activity (311 $\mu\text{g AE/mg}$ extract) was found significantly lower than other extracts studied. Further, acetone extract protected DNA, protein, inhibit β -carotene bleaching and reduce TBARs value during meat oxidation. It also showed high inhibition potential against α -glucosidase (IC_{50} , 9.05 $\mu\text{g/mL}$) and acetylcholinesterase enzyme (IC_{50} , 25.2 $\mu\text{g/mL}$) thus envisaging the rhizome as a potential source of drug ingredients against Diabetes as well as Alzheimer's diseases. In HR-LCMS/MS major compounds identified were quercetrin, dihydroquercetin, mycophenolic acid, embelin, meptazinol, c16-sphinganine, deoxyelephantopin, phytosphingosine, colforsin, stearic acid derivatives and venpocentine.

Further studies were conducted with respect their enzyme inhibitory properties focusing on α -glucosidase. Various class of compounds were separated using different chromatographic technics and bioactive fractions of both the rhizome extracts were analyzed with LC-MS and IR Kinetics and molecular docking studies were performed to establish structure activity relationship. Among the two bioactive fractions of *C. indica* rhizome, acetone extract recorded competitive type of inhibition and LC-MS/MS analysis revealed the presence of phenolics type metabolites in the active fraction whereas water extract of *C. indica* rhizome recorded noncompetitive type of inhibition which was dominated by metals as analyzed through ICP-MS. In the case of *C. articulatus*, acetone extract had two active fractions recording competitive inhibition. LC-MS/MS analysis revealed the presence of phenolics like quercetrin, dihydropuercetin, embelin and mycophenolic acid in one active fraction and hydroxy fatty acid derivatives in other fraction. The enzyme inhibition activities of the identified metabolites were also supported by molecular docking studies. The study reveals the *C. indica* and *C. articulatus* rhizome as a source of many important health beneficial metabolites and suggests its various food, medicinal and pharmaceutical usability.

सार

Canna indica (परिवार: Cannaceae) के प्रकंद का उपयोग बुखार, झोंप्सी और अपच के उपचार के लिए एक पारंपरिक औषधि स्रोत के रूप में किया गया है। इसी तरह, पत्ती और बीज के अर्क का उपयोग एनाल्जेसिक के इलाज के लिए किया जाता है और क्रमशः कान का दर्द ठीक करने के लिए। फूल को नेत्र रोगों को ठीक करने वाला कहा जाता है। दुनिया के विभिन्न क्षेत्रों में, इसे मुख्य भोजन के साथ-साथ स्टार्च के स्रोत के रूप में भी सेवन किया जाता है। प्रकंद का उपयोग दुनिया के कई क्षेत्रों में खाद्य उद्योगों में स्टार्च के स्रोत के रूप में किया गया है। यहाँ, सी। इंडिका के प्रकंद के अध्ययन का उद्देश्य कार्यात्मक भोजन, विटामिन, पोषण और पोषक तत्वों के स्रोत के रूप में इसके संभावित उपयोग को निर्धारित करना था। बायोमास और पोषक तत्व लक्षण वर्णन ने रेशे को फाइबर के एक समृद्ध स्रोत (25.1%), स्टार्च (28.5%), प्रोटीन (4.72%), और लिपिड (5.75%) के रूप में 423 किलो कैलोरी/100 ग्राम dw (सूखी) के कुल कैलोरी मान के साथ दिखाया। वजन)। प्रकंद में खनिज और विटामिन भी काफी मात्रा में थे। प्रकंद के एसीटोन के अर्क ने काफी अधिक एंटीऑक्सीडेंट गुण दिखाए। DPPH, ABTS+ के साथ IC₅₀ का मान। और O²⁻ मूलक क्रमशः 21.6, 23.2 और 169 µg/mL पाए गए। कम करने वाले गुण (FRAP और CUPRAC) और डीएनए सुरक्षा परख को प्रकंद अर्क की कुल फेनोलिक और फ्लेवोनोइड सामग्री के साथ सहसंबद्ध किया गया था। एसीटोन और मेथनॉल अर्क ने मुक्त कणों से प्रेरित डीएनए और प्रोटीन के क्षरण के खिलाफ सुरक्षा दिखाई। खाद्य मॉडल प्रणाली में, जमीन के सूअर के मांस से एसीटोन निकालने ने decreased-कैरोटेनॉइड और थायोबार्बिट्यूरिक एसिड प्रतिक्रियाशील पदार्थ (टीबीएआरएस) के विरंजन में काफी कमी आई। α-glucosidase गतिविधि पानी के अर्क (IC₅₀ 2.35 µg/mL) और एसीटोन निकालने (IC₅₀ 27.1 µg/mL) द्वारा काफी बाधित थी। विभिन्न अर्क के एचआर-एलसीएमएस / एमएस विश्लेषण ने विभिन्न बायोएक्टिव यौगिकों जैसे कि रोसमारिनिक एसिड, सोरोमिक एसिड, यूनिक एसिड, आइसोयूजेनिटोल, एलाजिक एसिड, कौमारिक एसिड, फाइटोनोलिक एसिड और स्वेतेनिन की घटना को दिखाया। परिणामों ने सुझाव दिया कि स्वास्थ्य लाभ के लिए पोषक तत्वों और चयापचयों के संभावित स्रोत के रूप में सी। इंडिका प्रकंद।

साइपरस आर्टिकुलैटस एल। उष्णकटिबंधीय और उपोष्णकटिबंधीय क्षेत्र में वितरित एक बारहमासी पौधा है और इसके प्रकंद को मलेरिया, मिर्गी और पेचिश जैसे कुछ सामान्य रोगों के इलाज के लिए विभिन्न क्षेत्रों में पारंपरिक चिकित्सा के रूप में इस्तेमाल किया गया है। प्रकंद में आवश्यक तेल होता है जो औषधीय और आर्थिक हित का होता है और इसमें रोगाणुरोधी गुण होते हैं। संयंत्र को इसके आवश्यक तेल के लिए ज्यादातर अध्ययन किया गया था लेकिन इसके अन्य जैविक गुणों और मेटाबोलाइट प्रोफाइल को कम से कम पता लगाया गया था। ऑक्सीडेटिव तनाव, मधुमेह और अल्जाइमर जैसी बीमारियों का प्रबंधन करने के लिए प्राकृतिक दवा सामग्री के स्रोत के रूप में सी। आर्टिकुलैटस के प्रकंद अर्क का संभावित उपयोग वर्तमान अध्ययन का मुख्य उद्देश्य था। एंटीऑक्सिडेंट संपत्ति, भोजन और जैविक मॉडल प्रणालियों में एंटीऑक्सिडेंट के अनुप्रयोग, एंजाइम (α-amylase, α-glucosidase, acetylcholinesterase) निरोधात्मक गतिविधि के रूप में इन विट्रो जैविक गतिविधियों में निरोधात्मक अर्क के लिए निरोधात्मक गतिविधि का अध्ययन किया गया था। मेटाबोलाइट विश्लेषण एचआर-एलसीएमएस / एमएस द्वारा किया गया था। विभिन्न विलायक के अर्क के बीच, एसीटोन निकालने में एथिल एसीटेट निकालने के बाद एंटीऑक्सिडेंट और एंजाइम

अवरोधकों के रूप में शक्तिशाली गतिविधि दिखाई गई। DP50, ABTS + जैसे रेडिकल के मैला ढोने के लिए IC₅₀ का मान। और O²⁻ क्रमशः 12.2, 16.3 और 83.2 µg/mL पाए जाते हैं। इसी तरह, संपत्ति को कम करने के लिए IC₅₀ का मान (FRAP, 156 µgGAE/mg extract और CUPRAC, 407 µg BHAE/mg एक्सट्रैक्ट), मेटल केलेटिंग (44.8 µg EDTAE/mg एक्सट्रैक्ट), और कुल एंटीऑक्सीडेंट गतिविधि (311 µg AE/mg एक्सट्रैक्ट) काफी कम पाया गया। अन्य अर्क का अध्ययन किया। इसके अलावा, एसीटोन संरक्षित डीएनए, प्रोटीन को निकालता है, β-कैरोटीन विरंजन को रोकता है और ऑक्सीकरण के दौरान टीबीएआरएस मान को कम करता है। इसने α-glucosidase (IC₅₀, 9.05 µg/mL) और एसिटाइलकोलिनेस्टरेज़ एंजाइम (IC₅₀, 25.2 µg/mL) के खिलाफ उच्च अवरोधक क्षमता दिखाई। इस प्रकार यह मधुमेह के साथ-साथ अल्जाइमर रोगों के लिए एक संभावित स्रोत के रूप में राइज़ोम की परिकल्पना करता है। एचआर-एलसीएमएस / एमएस प्रमुख यौगिकों में पहचाने गए क्वेरसेट्रिन, डायहाइड्रोक्वैरेटिन, मायकोफेनोलिक एसिड, एम्बेलिन, मीप्टाजिनोल, सी 16-स्फिंगनिन, डीऑक्साइलेफेनोफिन, फाइटोस्फिंगोसिन, कोलफोरसिन, स्टीयरिक एसिड डेरिवेटिव और रेवेनपॉइटिन थे।

आगे के अध्ययन α-glucosidase पर ध्यान केंद्रित करने वाले उनके एंजाइम निरोधात्मक गुणों के संबंध में किए गए थे। संरचना के गतिविधि संबंध को स्थापित करने के लिए LCMS और IR कैनेटीक्स और आणविक डॉकिंग अध्ययनों के साथ अलग-अलग क्रोमैटोग्राफिक टेकनीक और बायो-फ्रैक्चर दोनों का उपयोग करके यौगिकों के विभिन्न वर्ग का विश्लेषण किया गया। सी। इंडिका प्रकंद के दो बायोएक्टिव अंशों के बीच, एसीटोन एक्सट्रैक्ट ने प्रतिस्पर्धी प्रकार के निषेध और LC-MS/MS विश्लेषण को सक्रिय अंश में फेनोलिक्स टाइप मेटाबोलाइट्स की उपस्थिति का पता लगाया, जबकि सी। इंडिका राइज़ोम के पानी के अर्क ने गैर-कैपेसिटिव प्रकार का निषेध दर्ज किया जो आईसीपी-एमएस के माध्यम से विश्लेषण के रूप में धातुओं का वर्चस्व था। सी। आर्टिकुलिटस के मामले में, एसीटोन के अर्क में दो सक्रिय अंश थे जो प्रतिस्पर्धी अवरोध को दर्ज करते थे। एलसी-एमएस / एमएस विश्लेषण ने क्वेरसेट्रिन, डायहाइड्रोपुरसेटिन, एम्बेलिन और एक सक्रिय अंश में माइकोफेनोलिक एसिड और दूसरे अंश में हाइड्रोक्सी फेटी एसिड डेरिवेटिव जैसे फेनोलिक्स की उपस्थिति का पता चला। आणविक डॉकिंग अध्ययन द्वारा पहचान किए गए चयापचयों के एंजाइम निषेध गतिविधियों का भी समर्थन किया गया था। अध्ययन कई महत्वपूर्ण स्वास्थ्य लाभकारी चयापचयों के स्रोत के रूप में सी। इंडिका और सी। आर्टिकुलिटस प्रकंद को प्रकट करता है और इसके विभिन्न भोजन, औषधीय और औषधीय प्रयोज्यता का सुझाव देता है।

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