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Research Article

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Isolation and identification of L-Dopa in the seeds of *Abrus precatorius*

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Abstract

In the present work, methanolic extract of red-black colored seeds and white colored seeds of *Abrus precatorius* were analyzed phytochemically for the presence of non-protein amino acid. Phytochemical studies revealed the presence of amino acid. By using chromatographic and spectroscopic techniques 3, 4-dihydroxyphenylalanine i.e., L-DOPA is identified in the seeds of *Abrus precatorius*. Levodopa is requires for the proper functioning of the brain. L-DOPA is used in the treatment of Parkinson's disease, which is a second most common neurodegenerative disease in the world. In red-black colored seed concentration of levodopa is 1.14gm $100gm^{-1}$ while in white colored seed concentration of levodopa is 0.99gm $100gm^{-1}$. It shows that red and black colored seeds have more concentration of levodopa in comparison to white colored seeds.

Keywords: *Abrus precatorius,* non-protein amino acid, levodopa, neuroprotective, etc.

Introduction

Since vedic period *Abrus precatorius* has been used for therapeutic purpose[1]. It belongs to family fabaceae is an indigenous plant found in Himalayan region to southern part of India. It is commonly known as Gunja. Its other names are Jequirity and Crabs eye in English. It is also known as Ratti. In Bengali it is known as Kunch, Koonch, Chunhali, in Gujarati as Gumchi, Chanothi, in Kanada as Gurugunji, in Kashmiri as Shangir, in Malayalam as Kunni, Gundumanai, in Persian as Chasani-Khurosa, in Punjabi as Mulati, in Tamil as Kunthamani, in USA as Precatory bean, in Nepal as Ratigedi, in Indonesia as Weglis. In old time its seeds are used by goldsmiths to weigh gold and silver as its seeds have uniform weight $1/10^{th}$ of gram. In traditional & folklore medicine its roots, seeds and leaves are used. The pharmacological studies have shown that *Abrus precatorius* possesses a number of biological activities such as anti- arthritic[2], anti-cancer[3], anti-fertility[4], antimicrobial[5], anti-bacterial[6], anti-diabetic[7], anti-oxidant, anti-inflammatory, antiseratonergic, nephroprotective, anti-insecticide, antiallergic[8], antimalarial[9] etc. Over the past 45 years, Levodopa, has remained the gold standard symptomatic replacement therapy for a progressive neurodegenerative disease i.e., Parkinsonism. Levodopa a precursor of the neurotransmitter dopamine is the most widely prescribed drug in the treatment of Parkinson's disease. People with Parkinson's disease have depleted levels of dopamine, which causes tremor, muscle stiffness or rigidity, slowness of movement (bradykinesia) and loss of balance. Dopamine cannot be administered directly because it does not cross the blood brain barrier readily, while its precursor levodopa is given orally and is easily absorbed through the bowel and converted into dopamine by decarboxylase. Then, levodopa is used to increase dopamine in the brain, which reduces

the symptoms of Parkinson's disease. Nevertheless, elevated levels of dopamine also cause adverse reactions such as nausea, vomiting and cardiac arrhythmias[10]. The main object of this study to extract and characterize non-protein amino acid compound in the seeds of *Abrus precatorius*.

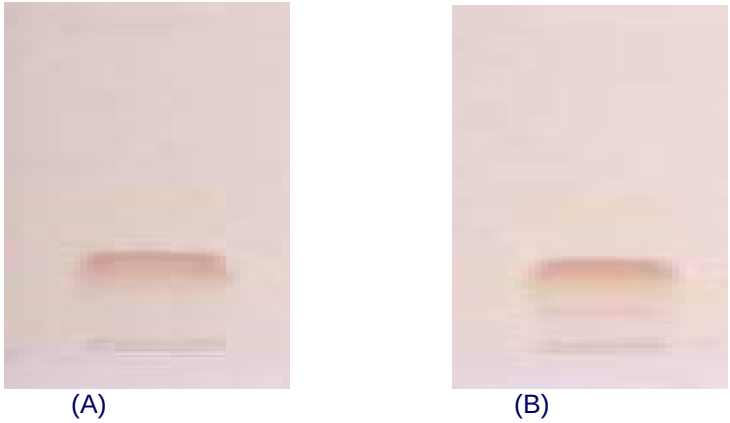
Materials and Methods

The EI- mass was recorded on Shimadju QP 2000 mass spectrometer. UV-spectra was recorded on Shimadju UV- 160 spectrophotometer. The IR spectrum was recorded on Perkin Elmer, spectrum100 instrument. The ^{1}HNMR spectrum was recorded on Bruker DRX 500 advance spectrometer. Seeds of *Abrus precatorius* varieties viz. red and black and white were collected from various wild locations in India. Seeds of both varieties are collected were powdered (separately). About 100gm seeds of both varieties were separately extracted with 400ml. of methanol about 6 hours at room temperature then it was filtered and this procedure was repeated twice (separately) . Both filtrate was collected and concentrated at 70-80^{0} C over water bath. At last obtained soft mass was used for fractionation. About 10gm of methanolic extract was taken in 250ml round bottom flask and reflux with 100ml hexane at 40^{0}C for 30 minutes and soluble fraction was separated and collected. Further hexane insoluble residue was reflux with 100 ml chloroform at 50^{0}C for 30 minutes and soluble fraction was separated and filtered. Residue insoluble in chloroform was reflux with 100ml. ethyl acetate for 30 minutes and filtered, then residue was reflux with 100ml. methyl alcohol for 20 minutes at 60^{0} C and soluble fraction was also separated. To methanol insoluble residue 100 ml. demineralized water was added and it was stirred gently[23]. Thus compound was settled down at the round bottom flask. Now water soluble fraction was decanted twice and collected on Whatman filter paper and rinsed with 10 ml. of methyl alcohol to increase the purity of compound then it was dry in oven.

Chromatographic conditions: Chromatography was performed on precoated silica gel TLC plate (10×10cm), application of standard and test sample at 25mm distance on the TLC plate. Then the plate was developed using optimized solvent system isopropanol: ethyl acetate: water: acetic acid 40:38:20:2 as a mobile phase in a glass chamber, previously saturated for 30 minutes .The Plate was developed up to 8cm and the average development time was 60 minutes. After development the plate was air dried for 10 minutes and sprayed with ninhydrin reagent (50mg of ninhydrin in 50ml of methanol).The plate was dried at 105^{o}C for 5minutes to enable the full color of the spot to develop (Table.1& Fig.1).

Table.1 R_f value of isolated compound in seeds of *Abrus precatorius*

Sample	R_f value	Color of the band
Red and black color seed	0.35	Brownish Pink
White color seed	0.35	Brownish Pink



(A) (B)

Fig.1 Red and black color seed (A) White color seed (B)

Quantitative estimation[24] of the concentration of the supernatant was by made by measuring the UV absorption at 283 nm. Extracts were examined by TLC on silica gel G using isopropanol: ethyl acetate: water: acetic acid 40:38:20:2 as eluent. Visualization was effected by spraying with 0.5% ninhydrin in butanol acetone 1:1 and heating at 110°C for 10 min. Levodopa was further identified by IR spectra and NMR spectra.

Chemical Identification:

(1) Add 1 ml. of concentrated nitric acid to 1ml. of the test sample. Heat the mixture and cool it. Slowly add sodium hydroxide solution until the mixture becomes alkaline and a color change is noted. The color changes from yellow to orange indicate the presence of an aromatic amino acid.

(2) To 5 mg add 1ml. of water, 1ml. of pyridine and 5mg of 4-nitrobenzyl chloride, mix and allow to stand for 3 minutes; a violet color is produced which changes to pale yellow on boiling. On shaking add 0.1ml. of sodium carbonate to this, the violet color reappears.

Levodopa, white crystalline powder, m.p. 285^{o}C, molecular formula $C_9H_{11}NO_4$, IR (KBr) V_{max} cm^{-1}: 3370 (N-H), 3205(OH-stretching), 3065(NH_3^+), 2700-2300 (broad), 1740 (C=O), 1655, 1570 (COO^-), 1495(C=C), 1400-1440(O-H bonding vibration), 1245(C-O stretching), 820,815 (Aromatic CH). ^{1}HNMR (500 MHz, H_2O) δppm=6.88 (*d*, 2H, *J*=, 2H, J=8.10), 6.81(*d*, 2H, *J*=1.93), 6.73(*dd*, 2H, J=8.10,1.82), 3.91(*dd*, 2H, *J*=7.76,5.09), 3.12(*d*, 2H, *J*=14.64,4.98), 2.97(*dd*, 2H, *J*=14.64,7.68).

Table.2 (Amount of L-Dopa in *Abrus Precatorius*)

Component	*Abrus precatorius* (red and black colored seed coat)	*Abrus precatorius* (white colored seed coat)
L-DOPA (gm 100gm-^{1})	1.14 ± 0.08	0.99 ± 0.02

Results and Discussion

Both red and black colored seed and white colored seed of *Abrus precatorius* was studied. The compound was isolated as white crystalline powder. Mass spectra (Fig.2) of isolated compound show molecular ion m/z 197[M^+] corresponding to the molecular formula $C_9H_{11}NO_4$. The UV spectrum of this compound exhibited two major peaks in the region 221nm and 280.5nm which indicates the presence of carboxylic group and amine group.

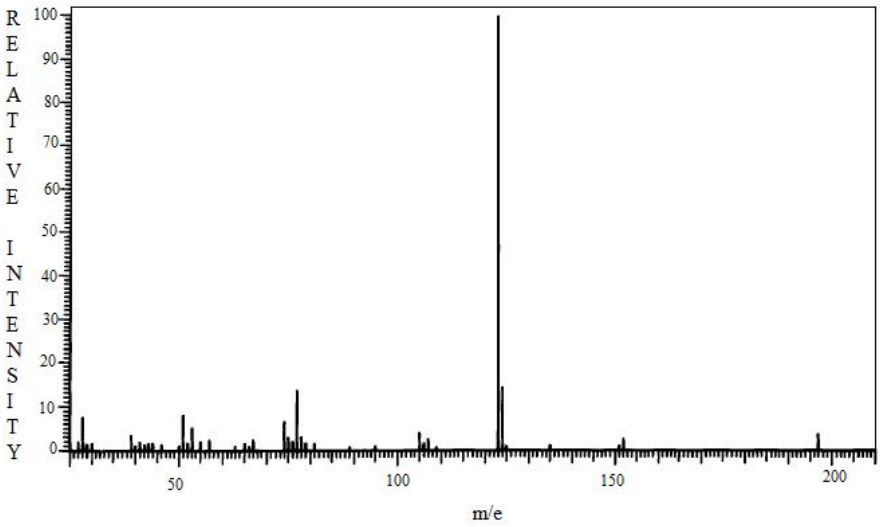


Fig.2 Mass Spectrum of Levodopa

The IR spectrum (Fig.3) of levodopa shows many intense, sharp absorption peaks that was due to the presence of different functional groups in the molecules i.e., the carboxylic acid, hydroxyl groups, primary amine, benzene ring. The peaks at 3370 cm^{-1} due to N-H while at 3205 cm^{-1} due to OH-stretching. Peak at 3065 cm^{-1} and broad spectra 2700-2300cm^{-1} corresponds to NH_3^+. Peak at 1740cm^{-1} shows C=O bond while at 1655cm^{-1} and 1570cm^{-1} corresponds to carboxylic group. The band at 1495cm^{-1} due to C=C bond in the benzene ring. Absorpiton peak at 1400-1440 cm^{-1} (broad band) due to O-H bending vibration, 1245 cm^{-1} shows C-O stretching, 820 cm^{-1} and 815cm^{-1} due to aromatic CH out of the plane bending of two adjacent free H's.

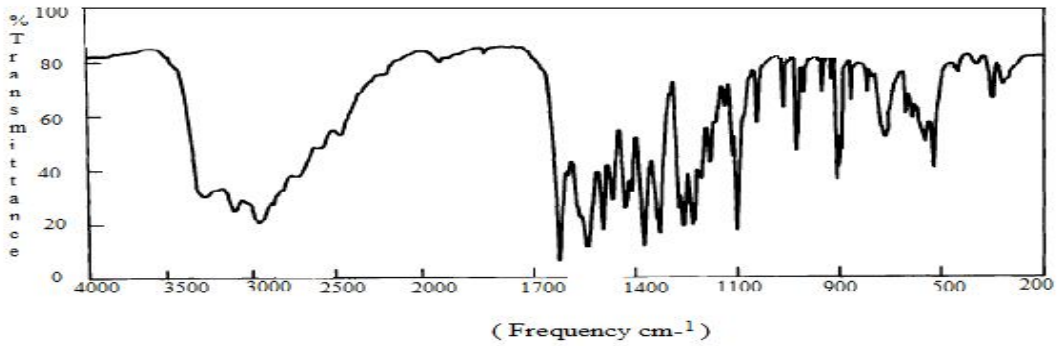


Fig.3 IR of Levodopa

The ^{1}HNMR showed that the two aromatic protons appeared as a duplet at δ6.88ppm and δ 6.81ppm while one aromatic proton appeared as double duplet at δ6.73ppm. Two methylene protons appeared as double duplet at δ3.12ppm and δ2.97ppm while one methylidine proton appears as double duplet at δ3.91ppm.

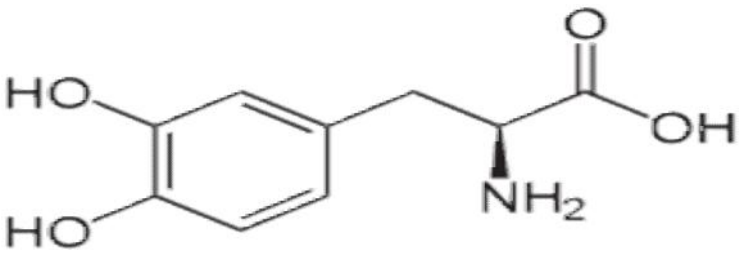


Fig.4. L-3, 4-dihydroxyphenylalanine

Quantitative study (Table.2) of seeds of both varieties shows that red and black color variety contains 1.14gm per 100gm of levodopa while white color variety contains 0.99gm per 100gm of levodopa. It indicates that red and black color variety contains more concentration of levodopa. When compared [25-26] with other concentration of levodopa is low. L-Dopa (3, 4-dihydroxy L- phenylalanine) is an intermediate in biosynthesis of dopamine. To increase dopamine levels for the treatment of Parkinson's disease[11], levodopa is used as a prodrug because it has ability to cross blood- brain barrier where as dopamine itself cannot. In the biosynthesis of brain catecholamines the initial enzymatic reaction involves the formation of the catechol amino acid L- dihydroxy phenylalanine (Levodoopa) from tyrosine. Once levodopa has entered the central nervous system it is metabolized to dopamine by aromatic L- amino acid decarboxylase.

Levodpa is a precursor of dopamine which is an important neurotransmitter, used for the medication of neural disorders such as Parkinson's disease. Through enzymatic reaction catalysed by dopadecarboxylase, after administration, levodopa is converted into dopamine. Parkinson's disease affects a part of brain called the "basal ganglia", which controls movement. Cells in the basal ganglia begin to degenerate as a result of the condition, and lose of their ability to produce a neurotransmitter (a chemical that carries messages between brain and nerve cells) called dopamine. As dopamine levels drop, the production of another neurotransmitter, called acetylcholine, increases. The balance between these two is critical, because they have opposite effects; acetylcholine stimulating muscle contraction, and dopamine damping it down. When the balance shifts in favour of acetylcholine, muscles become rigid with increasing jerky movements which are difficult to control this is often accompanied by tremors in hands. Whilst conventional drugs can be administered to control symptoms, they have a range of unpleasant side effects and cannot limit its progression. During the early stages of the disease, conventional drugs, called anticholinergics, can reduce symptoms of muscle rigidity and excess salivation by blocking the action of acetylcholine. However, they can cause dry mouth, constipation, anxiety, drowsiness and blurred vision. Whereas, direct administration of L-DOPA also has dangerous side-effects, including nausea, internal bleeding, palpitations, dizziness and depression because of it is being converted to dopamine before it reaches to brain. The protective effects of L-Dopa on small bowel injuries, gastrointestinal diseases, ulcers, diabetes and oxidative stress were proven scientifically [12]. Levodopa has been earlier reported in various plants[13--15].

Conclusion

From the physical, chemical and spectral[16-22] characteristics, compound extracted and isolated is identified as levodopa (Fig.4). So, this study confirms levodopa in the seeds of *Abrus precatorius* which is used in the treatment of Parkinson's disease. The concentration of levodopa is more in red and black color species. Thus, seeds of *Abrus precatorius* may be the source of extraction of levodopa which is the precursor of dopamine.

Conflict of Interest Statement

We decline that we have no conflict of interest.

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