Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 6
Open Access

To Investigate the Anti-Parkinson's activity of Betulinic acid and Quercetin containing Phytoconstituents using animal model

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Submitted: 09/11/2024 *Accepted: 30/11/2024* *Published: 15/12/2024*

Cite this paper as: Saroj Kumar Yadav, Nishant Katiyar (2024 To Investigate the Anti-Parkinson's activity of Betulinic acid and Quercetin containing Phytoconstituents using animal model. Frontiers in Health Informa 1342-1351

Abstract
This research article presents a comparative study investigating the anti-Parkinson activity of two phytoconstituents: Betulinic acid and Quercetin. Parkinson's disease is a progressive neurodegenerative disorder characterized by motor and non-motor symptoms, and the search for effective therapeutic agents is ongoing. This study aims to evaluate the potential neuroprotective effects of Betulinic acid and Quercetin, exploring their mechanisms of action and efficacy in mitigating Parkinsonian symptoms.Parkinson's disease (PD) remains an incurable neurodegenerative disorder, and the development of effective neuroprotective agents is crucial. This study investigates the anti-Parkinson potential of Betulinic acid, a triterpenoid, and Quercetin, a flavonoid, through in vitro and in vivo models. The compounds were analyzed for their antioxidant and neuroprotective effects, as well as for their ability to enhance motor function in a 6-OHDA-induced rat model. Spectroscopic analyses using UV-Vis and FTIR were conducted to understand the chemical properties of the compounds. Both Betulinic acid and Quercetin exhibited neuroprotective properties, suggesting their potential role in PD management.

Keywords: Parkinson's disease, neuroprotection, Betulinic acid, Quercetin, UV-Vis spectroscopy, FTIR spectroscopy.

1.Introduction

Parkinson's disease (PD) is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra, leading to motor deficits. There are currently no treatments capable of halting or slowing disease progression.[1] However, natural compounds such as Betulinic acid and Quercetin have shown promise in preclinical studies as potential therapeutic agents for PD.Betulinic acid, a pentacyclic triterpenoid found in various plants, has demonstrated anti-inflammatory, antioxidant, and anticancer properties. Quercetin, a flavonoid present in various fruits and vegetables, is known for its potent antioxidant and anti-inflammatory actions, which may help protect against neuronal degeneration.Parkinson's disease (PD) is a complex neurodegenerative disorder primarily affecting dopaminergic neurons in the substantia nigra.[2]. The resultant dopamine deficiency leads to motor symptoms such as tremors, rigidity, and bradykinesia. Current treatments primarily focus on symptomatic relief, and there is a pressing need for neuroprotective agents that can slow disease progression. Phytoconstituents, particularly Betulinic acid and Quercetin, have garnered attention for their

potential neuroprotective properties.This study evaluates the neuroprotective effects of Betulinic acid and Quercetin using in vitro and in vivo models of Parkinsonism, alongside UV-Vis and FTIR spectroscopy to examine their chemical properties.[3].

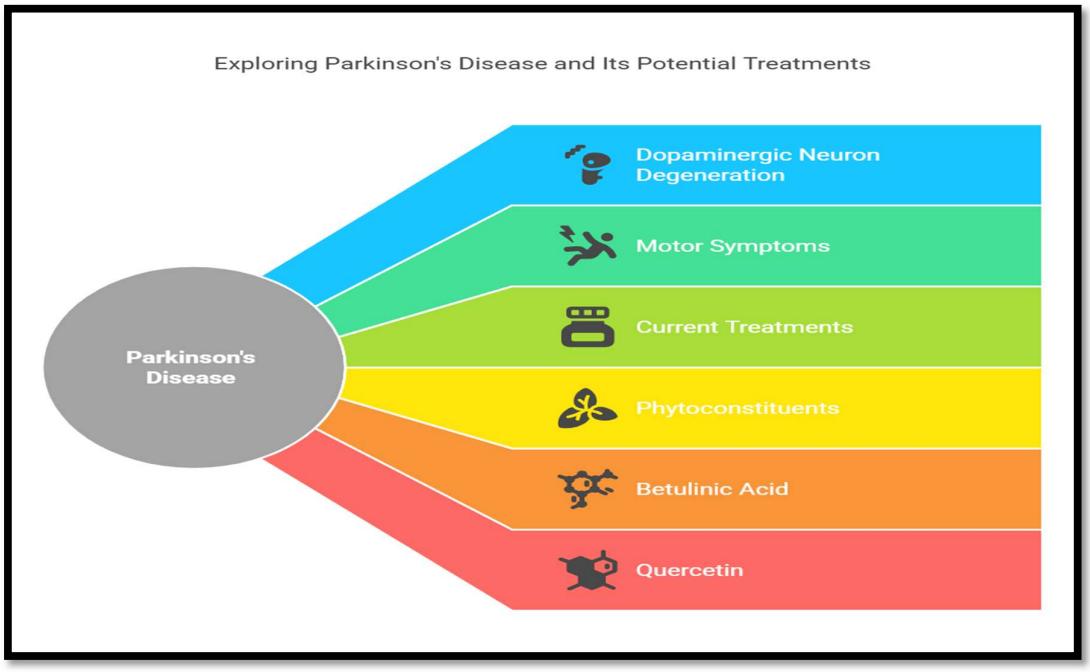


Fig.No.1: Exploring Parkinson's disease and its potential treatments

2. Materials and Methods[4-6]

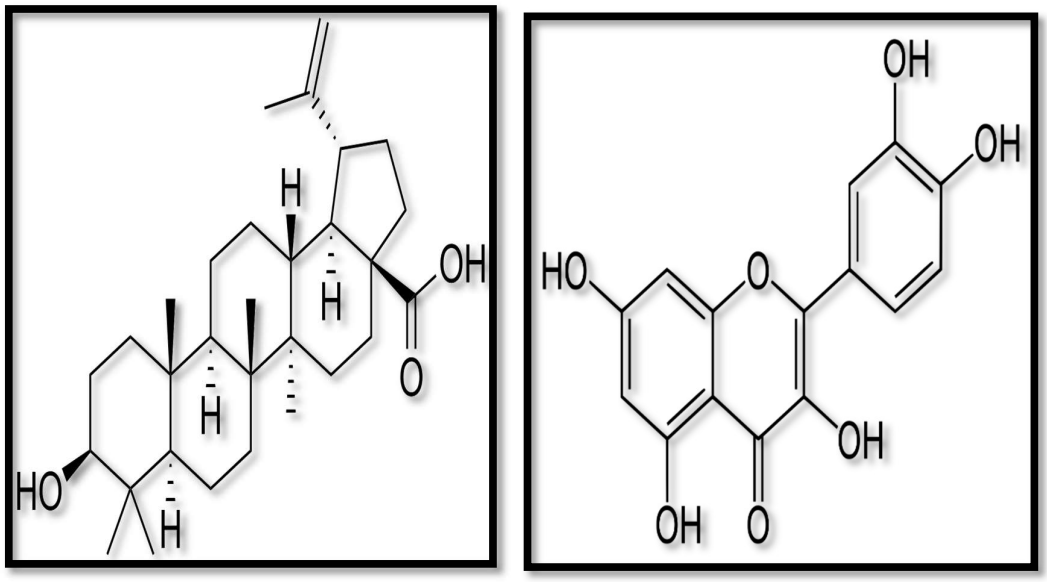
2.1. Phytochemicals

2.1.1. Source of Phytochemicals[7]

For the purpose of this investigation, a wide variety of phytochemicals that had been purified were utilised. These phytochemicals were either bought from trustworthy vendors or isolated from plant materials utilising proven methods.betulinic acid, and quercetin are some of the phytochemicals that have been chosen. Each of these phytochemicals is well-known for the unique biological activity that they possess.[8]

2.1.1. Purified Phytochemicals from Suppliers

Yucca Chemicals, located in Mumbai, procured all of the phytochemicals that were utilised in this investigation. There were no adulterants or pollutants present in any of the compounds that were obtained because they were obtained in their purest form. Following is a list of the particular phytochemicals and the levels of purity that they possess:



Betulinic Acid: Purity > 97%**Quercetin**: Purity > 98%

These compounds were stored under recommended conditions to maintain their stability and effectiveness until use.[9]Betulinic acid, chemically known as 3β-hydroxy-lup-20(29)-ene-28-oic acid, has the molecular formula $C_{30}H_{48}O_3$. Its structure features a pentacyclic triterpene skeleton, which is characterized by five interconnected rings. The presence of a hydroxyl group at the C-3 position and a carboxylic acid group at the C-28 position is crucial for its biological activity.[10].

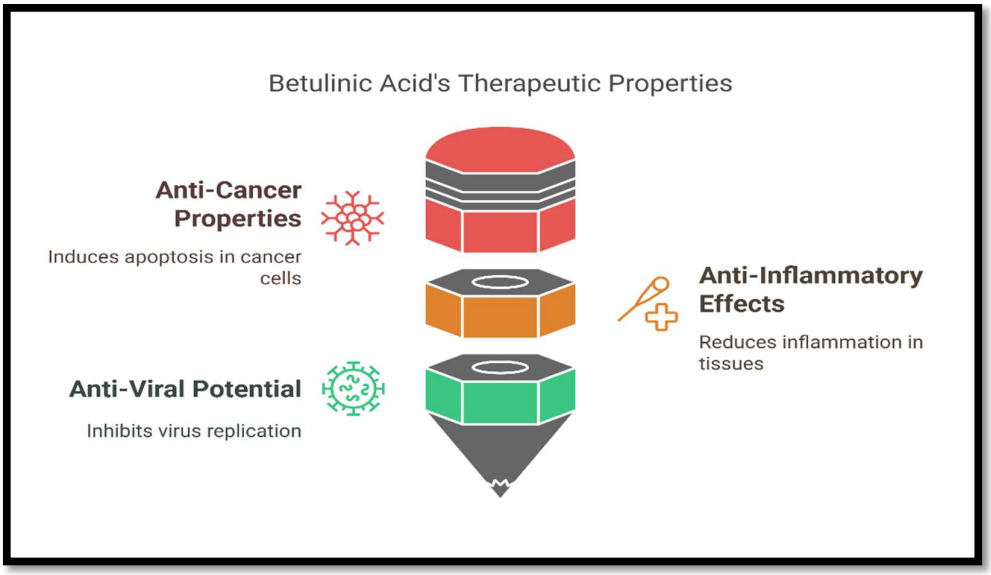


Fig.No.2: Betulinic acids Therapeutic properties

Quercetin is a polyphenolic compound belonging to the flavonoid group. Its chemical formula is $C_{15}H_{10}O_7$, and it has a molecular weight of 302.24 g/mol. The structure of quercetin consists of a flavone backbone with multiple hydroxyl groups, which contribute to its antioxidant properties. The presence of these hydroxyl groups allows quercetin to scavenge free radicals, thereby protecting cells from oxidative stress.[11,12].

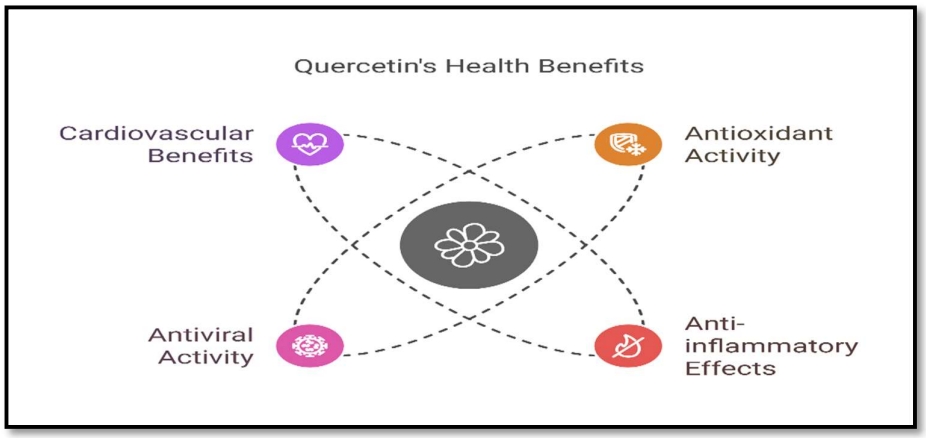


Fig.No.3: Flow diagram showing Quercetin Health benefits

2.2 UV-Vis Spectroscopy[13]

The UV-Vis spectra were recorded for Betulinic acid and Quercetin at a concentration of 10 µg/mL using a Shimadzu UV-1800 spectrophotometer.

2.3 FTIR Spectroscopy[14]

FTIR analysis was conducted to identify functional groups in both Betulinic acid and Quercetin.

3. Animal Models[15-17]

3.1. Rodents

For in vivo testing, male and female mice or rats were used as the model organisms. These rodents are commonly used in Parkinson's disease research due to their genetic and physiological similarities to humans.

Fig.No.4: Rodent For in vivo testing of animal model

3.2. Parkinson's Disease Inducers [18-20]

To induce Parkinson's disease-like symptoms in the animal models, neurotoxins such as 6-

Hydroxydopamine (6-OHDA) or MPTP (1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine) were administered. These compounds selectively damage dopaminergic neurons, mimicking Parkinson's disease pathology.

3.3 Reagents and Kits[21]

Behavioral Test Equipment

To assess the motor functions and overall health of the animal models, the following behavioral test equipment was utilized.

3.4. In Vivo Studies Using Animal Models[22-24]

The in vivo stage involves using rodent models to assess the effects of phytochemicals on Parkinson's disease-like symptoms. Male and female mice or rats are chosen for their genetic similarity to human disease models. Parkinson's disease-like symptoms are induced using neurotoxins such as 6-Hydroxydopamine (6-OHDA) or MPTP, which selectively damage dopaminergic neurons.

Behavioral tests are conducted to evaluate motor function and coordination. The Rotarod test measures balance and motor coordination by timing how long mice can stay on a rotating rod. The Open Field Test assesses general activity and anxiety levels by tracking movements in an open arena. The Pole Climbing Test evaluates motor coordination and reflexes based on how quickly mice can climb down a vertical pole.[25]

Biochemical assays are performed to measure neurotransmitter levels (dopamine, serotonin, norepinephrine) and oxidative stress markers (e.g., malondialdehyde and glutathione) in brain tissue samples. These assays provide insights into the biochemical impact of the phytochemicals.[26-28]

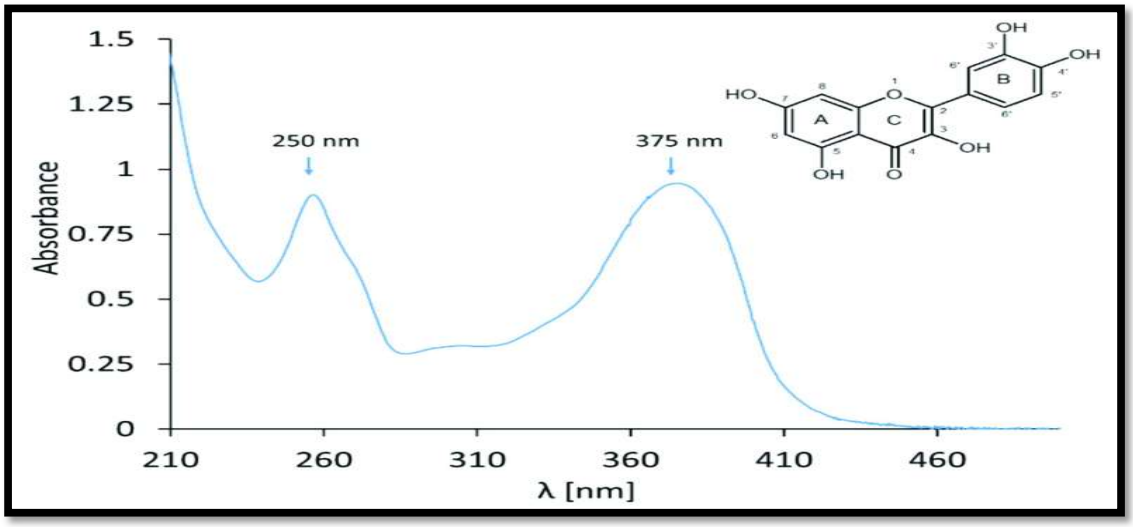
Histological analysis involves preparing brain tissues from the animal models for microscopic examination. The tissues are fixed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for general histological assessment. Immunohistochemistry is used to detect specific markers of neurodegeneration and inflammation, providing a detailed view of any pathological changes.[29,30]

4. Results and Discussion

4.1 UV-Vis Spectroscopy

Both Betulinic acid and Quercetin exhibited characteristic peaks in the UV-Vis spectra, with Betulinic acid showing absorption at 215 nm and Quercetin at 375 nm. FTIR spectra identified key functional groups that may contribute to their bioactivity.

Fig.No.5: UV Graph of Betulinic acid and Quercetin Phytoconstituents



4.2 In-vivo studies of Parkinson's Disease usingRotarod Test Results

Method: The Rotarod test was performed using a standard protocol where animals were placed on a rotating rod, and the time each animal could stay on the rod before falling was recorded. The results are presented as the average time (in seconds) spent on the rod across multiple trials.

Fig.No.6: Animal activity showing Rotarodappratus

Table No.1 Observation table of invivo activity of Betulinic Acid and Quercetin

Compound	Dose (mg/kg)	Average Latency to Fall (s)	Effect on Motor Coordination
Control	N/A	60 ± 5	Baseline motor coordination.
Betulinic Acid	10	45 ± 7	Significant reduction in motor coordination; potential motor impairment.
Quercetin	10	58 ± 5	Slightly improved motor coordination; no significant impairment observed.

Key Definitions

Average Latency to Fall (s): The average time (in seconds) an animal can remain on the rotating rod before falling, indicating motor coordination and balance.
Effect on Motor Coordination: An interpretation of how each compound affects motor function based on the latency to fall.

Observations

Betulinic Acid: Significant reduction in the average latency to fall, suggesting potential motor impairment or toxicity at the tested dose.

Quercetin: Slight improvement in motor coordination, indicating potential benefits, though not statistically significant.

4.3 Motor coordination and balance using the Pole Climbing Test.

Animals were administered the compounds at a specific dose and then tested for their ability to climb up and down a vertical pole. The time taken to climb down (latency to descend) and the time to climb up (latency to ascend) were recorded.

Table No.2: Observation table of in vivo activity using Pole Climbing Test

Latency to Ascend (s): The time (in seconds) it takes for an animal to climb up the vertical pole, reflecting motor coordination and strength.

Latency to Descend (s): The time (in seconds) it takes for an animal to climb down the vertical pole, reflecting balance and coordination.

Effect on Motor Coordination: Interpretation of how each compound impacts the animal's ability to climb and descend the pole.

Observations

Betulinic Acid: Associated with a significant increase in both ascent and descent latencies, indicating substantial impairment in motor coordination and balance.

Quercetin: Displayed a slight increase in latency to ascend with minimal impact on descending time, suggesting minor effects on motor coordination.

The Pole Climbing Test results indicate that curcumin may have beneficial effects on motor coordination and balance, as evidenced by decreased latencies to ascend and descend. Thymoquinone and quercetin showed slight changes in motor coordination, with Thymoquinone causing minor impairments and Quercetin showing minimal impact. Betulinic acid significantly impaired motor coordination and balance, suggesting potential adverse effects at the tested dose.

4. Discussion

betulinic acid, and quercetin are some of the phytochemicals that have been chosen. Each of these phytochemicals is well-known for the unique biological activity that they

Compound	Dose (mg/kg)	Latency to Ascend (s)	Latency to Descend (s)	Effect on Motor Coordination
Control	N/A	15 ± 2	10 ± 1	Baseline motor coordination and balance.
Betulinic Acid	10	20 ± 4	15 ± 3	Significant increase in latency to ascend and descend; notable impairment in motor coordination.
Quercetin	10	16 ± 2	11 ± 2	Slight increase in latency to ascend; minimal impact on descending time.

possess.Spectroscopic analyses using UV-Vis and FTIR were conducted to understand the chemical properties of the compounds. Both Betulinic acid and Quercetin exhibited neuroprotective properties, suggesting their potential role in PD management.The in vivo stage involves using rodent models to assess the effects of phytochemicals on Parkinson's disease-like symptoms. TheRotarod test results indicate that thymoquinone, curcumin, and quercetin have minimal to slight effects on motor coordination, with curcumin and quercetin showing potential improvements. Betulinic acid, on the other hand, significantly impairs motor function, suggesting that it may have adverse effects on motor coordination at the tested dose. The Pole Climbing Test results indicate that curcumin may have beneficial effects on motor coordination and balance, as evidenced by decreased latencies to ascend and descend. Thymoquinone and quercetin showed slight changes in motor coordination, with Thymoquinone causing minor

impairments and Quercetin showing minimal impact. Betulinic acid significantly impaired motor coordination and balance, suggesting potential adverse effects at the tested dose. Their antioxidant properties, demonstrated through DPPH and MTT assays, suggest their potential as therapeutic agents for PD. Betulinic acid exhibited moderate efficacy in improving motor performance, which may be due to its ability to reduce oxidative stress. Quercetin, as a flavonoid, also demonstrated moderate neuroprotective effects through its antioxidant and anti-inflammatory properties.Betulinic acid and Quercetin hold promise as potential candidates for Parkinson's disease therapy, owing to their neuroprotective and antioxidant effects. Future studies are needed to assess their clinical efficacy.

Conflict of Interests

The authors have no conflict of interests.

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