



Properties Of Betula Utilis (Anti-diabetics And Anti-cancer) And Phytochemistry,

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Abstract

It is common known that the plant, when prepared in a medicinal form, has potential efficacy. A recent surge in attention in the use of birch bark as a possible therapy for cancer may be attributed to the high concentration of triterpenoids found in birch bark. These triterpenoids include betulinic acid and oleanolic acid, amongst others. The plant known as betula has a wide range of applications due to its adaptability and versatility. The D. Don tree, which is a member of the Betulaceae family and has a very long lifetime, has been referred to in Sanskrit for around four centuries as the Himalayan birch, bhojpatra, or Bhurja. This name comes from the fact that the D. Don tree is a member of the family Betulaceae. Cancer cells are the target of the anticancer drug known as betulinic acid, which is responsible for their death. As a direct result of this, it set itself apart from several other alternative therapies for cancer. It has been shown that several species of the genus B. utilis contain both phenolic and flavonoid compounds. In addition to detecting the ability of phytochemicals to attach themselves to cancer cells, the SRB test is used to examine whether or not phytochemicals had anticancer properties when tested in a range of different solvents.

Keywords: phytochemicals, anticancer medication , anti-diabetic, cancer cells.

Introduction

The Betula perform out functions tree is unique to the Himalayas and can also be found anywhere else on the planet. This is due to the high altitude, as well as its susceptibility to soil instabilities and/or significant snowfall. The stem bark of the B. utilis plant can be used to treat a wide number of symptoms and ailments, including the disinfection of the skin, the healing of wounds, bronchitis, leprosy, convulsions, and issues with the ear and blood. According to research that made use of pharmacological techniques, it has properties that allow it to be

helpful against pathogens, hyperglycemia, cancer, HIV, & antioxidants.

There are a variety of applications for the plant that is often referred to as betula. Some estimates place the age of the Betulaceae tree D. Don, also called as the Himalayan birch, bhojpatra, and Bhurja in Sanskrit, at a minimum of 400 years. Other names for this tree are birch of the Himalayas and bhurja. It is generally accepted among the medical community that all this plant has advantageous medicinal characteristics.

The *Betula utilis* tree may reach heights of up to 20 metres and is classified as a medium-sized species. This is a tree that has a bole that is not uniform in height and numerous branches. This plant is covered in fine hairs that have a velvety texture all over the stem, the young leaves, and the bracts. It has deciduous leaves which are oval in shape and irregularly serrate, and they are arranged in an alternative manner. Its bracts continue to be much bigger than the wings of the nut. It produces nuts that have lenticular wings and blooms during the months of May and June. The nuts are borne on a spike. It has bark that is either brilliant reddish-white or white. Its outer bark is made up of numerous smooth layers that can be peeled off and in flakes that run horizontally across the surface. The hue of its inner cortex is rather reddish. There is a species of betula called *Betula utilis*, and it has a lifetime of more over 400 years. Through the prevention of soil erosion and the establishment of a bio-shield for such remaining sub-alpine meadows and forests located underneath the treeline zone, it makes a contribution to the conservation of the fragile ecosystem that exists in the Himalayas. In contrast to its many positive effects on the environment, the *Betula utilis* tree has also been put to a wide variety of cultural and non-cultural applications across the Himalaya and abroad.



Ecological Distribution

This species' distribution spans from Afghanistan (Nuristan and Safed Koh) and Northern Pakistan in the west, across the Himalaya in India, and into Sichuan in the east and Hebei province in the north of China via the western Chinese Heng Duan mountains in northwestern Yunnan and southern Yunnan. In other words, its westernmost point of occurrence is in Afghanistan (Nuristan and Safed Koh). In other words, it may be discovered all the way from the westernmost regions of Afghanistan (Nuristan and Safed Koh) and Northern Pakistan to the easternmost regions of Sichuan and Hebei province in China. It is not known what happened to it in the remote parts of northern Japan or in the neighbouring Amur-Ussuri area, which is situated on the continent of Asia. It is more common to refer to *Betula utilis* as bhojpatra in India; however, this tree is known by a variety of names in those other languages, including bhrja in Sanskrit, bhurjamaram in Tamil, bhujapatram in Malayalam, bhujapatri in Telugu, and bhuyapathra in Kannada. Other names for this tree include bhojp. The name Bhojpatra is the one that is used the majority of the time in India. People in India often use the word Bhojpatra to refer to this thing. It is possible to find it growing at elevations ranging from 750 to 1,400 metres (2,400 to 4,300 feet) above mean sea level in the province of Hebei in northern China, which is part of its geographic distribution, which runs from Nuristan in Afghanistan to the province of Hebei in China. These elevations range from 2,400 feet to 4,300 feet above mean sea level. It is native to Afghanistan, Bhutan, China, India, Nepal, Pakistan, Kazakhstan, Kyrgyzstan, Tajikistan & Uzbekistan. The Indian states of Arunachal Pradesh, Darjiling, Himachal Pradesh, Jammu and Kashmir, Sikkim, and Uttaranchal are the only places in the world where you may find this particular species. You won't be able to find it anywhere else. The *Betula utilis* birch is the most common species of birch that one is likely to encounter in the Indian Himalaya. This species lives in the highest part of the natural treeline zone, which stretches over the United Kingdom from an altitude of 3300–2200 metres in the north-west to an altitude of 3800–2200 metres in the north-east. It is possible to come across substantial stands of this plant on the slopes and ravines that get shade from the direction of the north. It is the only species of angiosperm tree found in the Himalayas that has wide leaves, and as a result of its existence, it is responsible for the dominance of a huge sub-alpine zone. At lower altitudes (below 3,400 metres), where it is sometimes found, its distribution is more dispersed than at higher elevations. However, at higher elevations it develops dense and gregarious stands, either in pure masses or commonly alongside the undergrowth of *Rhododendron campanulatum*, *Abies pindrow*, *Abies spectabilis*, and occasionally Junipers. These stands can be found either in pure masses or commonly alongside these other species. These stands may be found either in isolated masses or more often in association with the undergrowth. During the winter months, open, exposed places that are blanketed in snow for durations of between five and six months at a time are typical habitats for the *Betula utilis* tree. The only species of birch that can be found in Jammu and Kashmir as well as the North-West Himalaya is the *B. utilis* D. Don, which was described and given its name by botanist David Don in his book "Prodromus Florae Nepalensis," based on specimens that were gathered by Nathaniel Wallich in Nepal in the year 1820. The *B. utilis* D. Don can be found in Jammu and Kashmir as well as the North-West Himalaya Jammu and Kashmir as well as the North-West Himalaya are both home to the *B. utilis* D. Don.

Because of extensive deforestation and overexploitation of *Betula utilis* trees for a number of uses, habitat has been lost or decreased in many of its original groves across the Himalayas. This has occurred as a result of widespread deforestation. Because of this, the species has been completely wiped off. The Himalayan birch in Kashmir is one of the sixty-three (63) species which are regarded to be in a near-threatened position, as stated by the ENVIS Centre of Conservation of Medicinal Plants, FRLHT, Bangalore (NT). The IUCN/SSC Global Trees Specialist Group, on the other hand, reviewed this species and came to the conclusion that it presents "Least Concern," despite the fact that population is diminishing. The J&K Forest Act of 1930, as well as any later revisions to that statute, are the legal authorities that have jurisdiction over Kashmir's birch forests. There is no recognition of any legal rights; nonetheless, in accordance with the Kashmir Forest Notice, habitations that are within a 5-kilometer radius of a forest border are allowed certain concessions in lieu of necessary insurance for the preservation of the forest. Even though the selected forest stands are available for grazing, with the exception of the areas that have been restricted for the sake of conservation, unrestricted grazing has been a significant factor in the decline of these forest regions as well as the lack of regeneration that has occurred there. The widespread felling of broad-leaved trees, particularly birch, for the purpose of providing feed and fuel is against the law but is nonetheless common practise.

Phenology

In the same manner that deciduous trees, birches start to get their leaves in the late spring (April to May). The leaves range in shape from ovate-acuminate to oblong, and they are tapering, elliptic, glossy dark green, & irregularly serrate. In the autumn, the leaves turn yellow. Birches often produce flowers between both the ages of ten and fifteen years. Birch trees that have reached maturity have an abundance of both male and female catkins. The monoecious flowers of these trees bloom from May to June & yield fruit from June to July. Staminate catkins are generated with in late summer or the fall after the catkins had spent the whole winter in a latent state and greatly lengthened themselves over the duration of the winter. The pistillate catkins, that has the look of cones and have scales that tightly overlap one another, were born terminal on short spur-like lateral roots and emerge along the leaves. These catkins have had the look of cones and have scales that tightly overlap one another. Additionally, the scales that make up the pistillate catkins closely overlap one another. When the female catkins, which are also referred to as strobilus, have reached their full maturity, either late in the summer or early in the fall, they turn brown and become woody. When they are mature, the seeds will change colour, changing from a brownish greenish tint to a lighter brown or tan colour. This indicates that they have reached maturity. From late autumn until the next spring, the seeds are at their most delicate and lightest, making it possible for them to glide through the air. Even if the seeds may begin to scatter in the late summer, the quality of the seeds which are shed early can be of a lower standard. The wind is a crucial factor in the process of dispersion and has the capacity to carry seeds up to 80 metres distant from the mother tree along the surface of the snow. This may happen when the wind blows over snow. Even though there are a great number of seeds in the soil of the forest, it does not change the fact that those seeds only have a limited period of time to survive. The vast majority of seeds have lost their capacity to create healthy

progeny after two to three years have passed. It was discovered that an exceptionally rare occurrence of an excessive number of seeds was responsible for the degeneration of the crown and the stunted growth of the parent trees. [Further citation in required] It was found out that the seeds from the yellow birch that were thrown away in August were not viable at all. It was not until September that the first significant discharge of viable seeds took place, and it was not until October when the maximum number of viable seeds were discharged.



Betulinic acid & oleanolic acid are two examples of the types of triterpenoids that may be found in birch bark, and they have recently prompted renewed interest in the anticancer qualities of the bark. A breakthrough anti-cancer medicine known as apoptosis, which originates from betulinic acid, is responsible for the death of cancer cells. As a direct result of this, it differs from several other kinds of anticancer treatment. There is evidence that some species of *B. utilis* contain phenolics and flavonoids.

Researchers in the past have used high-performance thin layer chromatography¹⁶ in order to analyse the components that make up *B. utilis*. This approach is not sensitive enough and does not have a high enough resolution to be able to accurately measure the component parts. As a result, its capacity for doing so is restricted. Because it is the most cutting-edge and cutting-edge chromatography technology that is presently available, this research makes use of the ultra-high-performance liquid chromatography (UHPLC) approach. The achievement of the highest quality work is intended to be the outcome of this project. The fact that the UHPLC-QqQLIT mass spectrometer has a grade sensitivity that is higher than that of other mass spectrometers is what distinguishes it from the other models on the market. Because of its sensitivity, it is simple to identify analytes even when they are present in very low concentrations. This is a significant advantage. This investigation is using the multiple reaction monitoring (MRM) technique since it is capable of accurately and sensitively analysing a significant number of analytes all at the same time, and it also has the capability to do so.

Medical Values

- *Betula utilis* is one of most important tree species which is used in alternative therapy systems that are used in India. These systems are employed in the country of India. India is home to many communities that uphold and follow these philosophies. In the therapy of the three doshas that are comprised by tridosha, namely vata (air), pitta (phlegm), & kaph, it is used (cough). This herbal treatment may be

obtained in a variety of forms, such as an infusion, a powder, a paste, and a decoction, amongst others.

- Birch has also yielded a novel triterpenoid known as karachic rustics, which is fragrant and possesses antibacterial effects. Betulin demonstrates anticancer action by inhibiting the development of malignant melanoma and liver and lung cancer.
- The bark of the tree includes betulin and other essential oils with therapeutic effects.
- Because of its astringent properties, the bark of the stem can be utilised as a styptic, for washing of wounds, and in the treatment of leprosy. Additionally, it is used in the treatment of obesity.
- Using the tree's bark, leaves, and resin as a treatment for ailments such as rheumatism, bone fractures, joint discomfort, gout, malaria, swellings, asthma, and purification of the blood are just some of the things that may be accomplished.
- The resin has been used as a contraceptives as well as a remedy for burns and other external wounds and conditions.
- A paste that is made from the resin that is taken from bark is used to cure boils.
- The bark is used to cure earaches as well as renal and bladder conditions.
- Boiling bark in water is used to treat jaundice and as ear drops to alleviate earache.
- Insanity, epilepsy, and hysteria are treated with this substance because it is believed to include an effective therapeutic agent for psychiatric diseases.
- Betula bark can also be used to prepare a purportedly blood-purifying beverage.
- Birch tar is an astringent material which is used in topical treatments of skin problems such as eczema and psoriasis. Other skin ailments that may be treated with birch tar include: The procedure of burning the tree bark results in the bark being reduced to ashes, and also the paste that is generated from of the ashes then is applied to severe wounds and injuries that have been sustained by animals.
- Birch leaf tea is beneficial for the treatment of gastrointestinal issues, including diarrhoea, constipation, and the common cold. The tea is made by steeping birch leaves. Birch leaves are used to make this herbal beverage.
- In addition, betula leaves are used topically to the scalp in order to cure both dandruff and hair loss.
- The conventional use of *Betula utilis* is investigated by scientists, which led to the finding that the plant had bactericidal and antibacterial characteristics. In addition, it has been established that the bark has high levels of antioxidant activity. Furthermore, the bark contains a higher proportion of palmitic, linoleic, & oleic acids, all of which have use in the fields of nutraceuticals and medicine correspondingly. *Betula utilis* is the source of six different types of triterpenes, including betulin, betulinic acid, lupeol, oleanolic acid, ursolic acid, & -amyrin. Ursolic acid is the seventh kind of triterpene that may be found in *Betula utilis*. Ursolic acid is the sole chemical that has been found to exhibit a tumour cell-specific cytotoxic activity, suggesting its therapeutic promise in the battle against

breast cancer. This action has been shown to be very effective.

Literature Review

(Selzer et al., 2000) Both by itself and in combination with irradiation, betulinic acid's actions on malignant melanoma cells are studied for their potential therapeutic applications. Betulinic acid was very effective and maintained its ability to inhibit the proliferation and colony formation of each and every human tumour cell line. The impact on growth suppression was larger when colony-forming assays were conducted using betulinic acid & ionising radiation as the agents of investigation. According to this research, betulinic acid was also responsible for the death of human melanoma cells.

(Zuco et al., 2002) investigation on the cytotoxic effects of betulinic acid & doxorubicin on melanoma celllines in addition to those of other types of tumour celllines (also an anticancer drug). In addition, a large number of different cell lines with a range of different p53 statuses were investigated. It was discovered via in vitro research that betulinic acid effectively inhibited the development of malignant cell lines generated from melanomas, ovarian carcinomas, cervical carcinomas, and lung cancers of both small and non-small cell types. These tumours were all kinds of lung cancer. All of the lines that were tested showed signs of an antiproliferative impact within the dosage range that was investigated. (1.5-4.5mg/ml).

(Fulda and Debatin 2005) Betulinic acid is a relatively new cytotoxic medicine that has had promising effects in the treatment of neuroectodermal tumours. These include Ewing's sarcoma, neuroblastoma, medulloblastoma, and glioblastoma, in addition to glioblastoma. Children and young adults are also at risk for developing these tumours. Recent research opened the path for the discovery of this insight, which was a revelation. It was demonstrated that betulinic acid had significant anticancer action in primary tumour cell cultures across the board for neuroblastoma, medulloblastoma, and the vast majority of glioblastoma patients. The ex vivo ED50 in neuroblastoma cells was only 3-15 mg/ml, and the cells were resistant to CD95 or doxorubicin-induced apoptosis. Using cell cultures that were produced directly from actual tumours, this was successfully shown. In light of these results, the use of betulinic acid in the course of the therapy of neuroectodermal tumours in vivo could prove to be beneficial.

(Mishra et al., 2016) Researchers examined the bark of the *Betula utilis* tree in the hopes of identifying a bioactive component that may be used to combat cancer. In order to fractionate the *Betula utilis* Bark (BUB), which had previously been extracted using methanol, the researchers made use of a number of other solvents, including as hexane, ethylacetate, chloroform, & water. Additionally, the researchers also used water. Ethylacetate was revealed to be the anticancer component with the most potential after undergoing testing in vitro against nine different cancer cell lines. These tests showed that ethylacetate was the most effective. After having been recovered from the BUB extract using the process of ethyl acetate, these six triterpenes, which included betulin, -amyrin, lupeol, and -oleanolic acid (UA), were detected by spectroscopy in order to ascertain their structures. Some of the triterpenes included in this analysis include: The structures were able to be determined as a result of this. After that, we were able to categorise them based on the structures that they had

because of how they were constructed. Because of this discovery, for the first time ever, UA was discovered to be present in *Betula utilis* in addition to -amyrin. This was a very significant discovery. It was shown via in vitro study that the effect of isolated triterpenes on breast cancer cells was more significant than its influence on non-tumorigenic breast epithelial cell lines. This was the case when comparing the two types of cell lines. After analysing the differences and similarities between the two kinds of cells, this was shown to be the case (MCF 10A). The cancer cell-specific apoptotic activity of UA was predominantly responsible for the higher levels of DR4, DR5, as well as PARP cleavage in MCF-7 cells. This occurred within the context of the extrinsic apoptosis pathway. This was done in contrast to the MCF-10A cells, which did not produce tumours. The ability of UA to decrease the production of reactive oxygen species (ROS) and its ability to disrupt the potential of the mitochondrial membrane are both factors that contribute to the anti-cancer impact of UA. In addition to this, there is evidence that UA may slow the development of breast cancer. In conclusion, we found a novel source of UA that showed strong tumour cell specific cytotoxic effects. This prompted us to hypothesise that it may be employed in the treatment of breast cancer.

(Pandey et al., 2020) *Caenorhabditis elegans* were given a treatment called *B. utilis* ethanolic extract (BUE), that was shown to extend the worms' longevity and reduce the toxicity of amyloid. The researchers were able to estimate the lengths of the worms' lifespans by putting them through a series of stressful laboratory settings (including oxidative and high heat). The consequences of ageing were proven to be slowed down by BUE, as was indicated by age-related biomarkers in the study. This point was driven home by the fact of BUE was able to slow it down. Additionally, GFP-tagged mutants and strains were used in this research in order to get an understanding of the molecular mechanism which is responsible for the favourable effects that BUE supplementation produces. When the researchers used BUE at a concentration of 50 g/ml, they discovered that strain C had an increased chance of surviving in average. *elegans* by 35.99 percent, in addition to higher survival in stressful circumstances. When they did this, they found that strain C also had a higher survival rate. In addition to this, researchers found that the quantity of reactive oxygen species (ROS) that were present in the cells had decreased by 22.47 percent. When the CL4176 gene was expressed in transgenic worms, there was a noticeable delay in the start of the amyloid-induced paralysis. When it was found out that supplementing with BUE would result in a reduction in the number of occasions in which the NL5901 transgenic strain demonstrated -synuclein aggregation, it came as a big surprise to everyone. Gene-specific mutant studies demonstrated that the *sir-2.1* gene is not required for the BUE-mediated lifespan extension mechanism. However, the *daf-16*, *hsf-1*, and *skn-1* genes are required for this process. According to the results of an experiment that evaluated the expression of transgenic sensor genes, stress-protective genes like *Sod-3* and *Gst-4* revealed an increase in inactivity after being treated with BUE treatment. This was shown by the outcomes of the investigation. The findings of this study lead the researchers to the conclusion that the BUE-induced extension of lifespan is regulated by ROS scavenging activity, in combination with a broad range of other processes that are associated with ageing. Because of this, there is a possibility that BUE may be able to extend people's lives and arrest the course of neurodegenerative illnesses.

(Mohapatra et al., 2019) Mountains are home to some of the most remarkable ecosystems in the world,

which may be identified by their distinct acute vertical biological gradients and simultaneous altitudinal vegetation zonation. Mountains are home to some of the world's tallest mountains. Alpine treelines, which are the upper boundaries of relatively continuous tree stands, are the most obvious vegetation limits; as a result, they have attracted a significant amount of scientific study for a very long time. This is due to the fact that treelines are the upper boundaries of relatively continuous tree stands. This is because treelines are the most noticeable indicators of the boundaries between different types of vegetation. The lowering of the average yearly temperature may be responsible, on a global scale, for the movement of treelines in alpine mountain ranges. This phenomenon has been seen in many parts of the globe. The placement of the treeline, its spatial pattern, as well as the dynamics both at the landscape and the local scale are all influenced by the complex interactions between the factors and processes that led to its development. These interactions may be broken down into three categories: As a consequence of future changes to the environment's temperature and precipitation patterns, it has been hypothesised that treelines would move to more elevated latitudes. In order to determine whether or not there is the possibility for changes in the ecosystem, it is essential to first investigate the components that serve as a foundation and then develop an appropriate model of the treeline ecotone by placing it within the framework of the current climatic circumstances. After then, proposed shifts will be able to be reviewed. On the basis of the most common weather patterns, statistical models are used in order to arrive at an estimation of the ecological niche that is inhabited by a particular species. This is accomplished by taking into consideration the circumstances that are now present. These models serve as the foundation for establishing how accurate future distribution estimates may be based on the conditions of the upcoming climate by providing the data necessary. Even though it is the world's highest mountain range, the Himalayas are often underrepresented in papers that deal to scientific study. This is despite the fact that they are the most extensive mountain range on the planet. This is particularly true in reference to the research that primarily focuses on modelling. There seems to be a number of challenges that must be conquered in order to mimic treeline species in high-altitude habitats that are geographically separated from one another. The ease with which occurrence data may be accessed and the availability of environmental parameters of a high enough quality are two of the challenges that are involved with this endeavour. In this chapter, we examine the most recent findings that were achieved by modelling the ecological niche that the Himalayan birch, also known as *Betula utilis*, inhabits in the Himalayan mountain range while taking into account the conditions of the climate as it now stands. Because of its widespread distribution as a species that forms treelines over the whole of the Himalayan arch, *B. utilis* is an attractive species to explore for the purpose of modelling because of the data it can supply. This is due to the fact that *B. utilis* has a huge range. As a result of the fact that it has fewer specific ecological requirements and a high capacity for adaptability than other tree species, it is becoming more significant as a pioneer tree species for prospective succession developments along treelines that will take place under prospective climatic conditions. This is because these developments will take place under prospective climatic conditions. This is because projects of this nature will take place along treelines in the future. A comparative investigation of the underlying climatic, topographical, and phenological plant features was carried out as part of a synergistic strategy in order to determine the prospective and actual distribution of the target species. This was done in order to determine the efficacy of the strategy. This was

done in order to take into account the fact that different types of plant life are impacted by a wide range of climatic conditions, topographies, & phenologies. The reason this was done is because of the previous sentence. The data that are presented here provide a fresh beginning point for additional research that aims to replicate the distribution of the species as a reaction to a variety of climatic scenarios, either from the past or from the future. This research could be conducted either in the present or in the future. In addition, the tactics that have been detailed in this article may also be beneficial to other species that dwell above treeline and on high mountains.

Aims and Objectives

- The purpose of this research is to get further knowledge on the phytochemistry, anti-cancer, and anti-diabetic properties of *Betula utilis*. In order to accomplish the purpose of the study, the current research has been broken down into the following categories and objectives:
- A variety of medicinal plants from India's various geographical locations will be gathered for collection.
- Phytochemical studies of different solvent extracts of selected medicinal plants will be studied by standard spectroscopic methods.
- Screening will be performed on a variety of solvent extracts to investigate their potential for a broad range of biological activities, including anticancer properties.

Materials And Methods

Reagents, Chemicals and Materials

A total of 27 specimens of the Himalayan Silver Birch were discovered in the states of Jammu and Kashmir, Himachal Pradesh, Sikkim, and Uttarakhand. This information was obtained from the respective states. The samples were reviewed by plant taxonomists from the CSIR-National Botanical Research Group in Lucknow, India, to validate that they were true representations of the species in question.

For the purpose of easing the process of doing research on betulinic acid, apigenin, caffeic acid, ferulic acid, caffeic, and catechins, Sigma Aldrich Ltd. offered purity standards that were 96 percent effective.

Extrasyntheses was the company that offered their services in order to provide the analytical reference standards for quercetin & luteolin (puritypercentage 95 percent).

Take for instance the following: (Genay, France).

Both Fluka and Sigma-Aldrich were responsible for supplying the mobile phase solvents. These solvents were of an LCMS quality, and they included methanol, acetonitrile, & formic acid respectively. The manufacturing of ultra-pure water that can be used for the mobile phase is made possible via the utilization of a Milli-Q water purification system (Millipore Corporation, Bedford, MA). Through their acquisition, they were able to get Millipore 0.22-μm syringe filters. (Billerica, MA, USA).

Extraction and Sample Preparation

Powdered bark weighing 10.0 grams was subjected to crushing and sifting through a 40-mesh sieve while being held within an extractor @ room temperature for a duration of 36 hours in order to extract the soluble components. The temperature of the extractor was maintained at room temperature. In order to get a completely dry extract, a rotary evaporator was used in the process of evaporating the solvent at 45 degrees Celsius. The extraction yields of samples that were processed in triplicate are shown in Table S1. In order to conduct the analysis, the dried residue of each sample was precisely measured to be 1 mg and then diluted in 1 mL of methanol. Throughout the whole of the mixing process, an ultrasonicator made by Bandelin Sonorex was used, and ultrasonics, also known as Berlin waves, were used. Filtration of the mixed solutions was accomplished by utilising a syringe filter with a diameter of 0.22 metres. To obtain the final working concentrations, methanol has been used to reduce the filtrates in order to achieve this goal. After that, the completed solution that was wanted was vortexed for a period of thirty seconds in order to eliminate the spikes. During the extraction procedure, the only thing that needed to be introduced into the apparatus was an aliquot that was 5 litres in size for the UHPLC-MS/MS investigation.

Regarding Qualitative Research: Procedure For The Extraction Of Chemicals

In order to effectively extract 10 grams of powdered plant components, it was necessary to macerate the plant material for a total of 72 hours using freshwater, methyl alcohol, hexane, petroleum ether, alcohol, dichloromethane, and carbon tetrachloride. The macerates were heated to temperatures between 40 and 50 degrees Celsius, filtered, and then concentrated using a rotary evaporator. The extracts were stored in a refrigerator at a temperature of 4 degrees Celsius until it was time to utilise them.

Phytochemical Analysis

In order to detect phytochemical components of the plant, such as anthraquinone, phytosterols, tannins, and steroids, extracts of the plant were subjected to processing with DCM, hexane, chloroform, and methanol in addition to water (ethyl acetate). Additionally, it was found that the sample included other metabolites, such as flavonoids and alkaloids.

Evaluation Of TPC

The Folin-Ciocalteu method, with a few minor modifications, is used to ascertain and compute the phenolic content (TPC). Following the mixing of the percent sodium carbonate solution with the 1N Folin-Ciocalteu reagent, the next step was to add the 1N Folin-Ciocalteu reagent (1N). A vortex is used to rotate and agitate the samples. After it had been mixed, it was left to sit in the reaction mixture for about another half an hour. Water is used in the process of keeping the volume of the solution at a maximum of 12.5 millilitres at all times. At a wavelength of 765 nanometers, the degree of absorption that was recorded was at its maximum. In order to determine the amount of phenolics included in the extracts, we measured the quantity of each extract in milligrammes of GA per gramme of extract.

Determination Of Total Flavonoid Content

One millilitre of the extract, which contained one hundred micrograms of extract per millilitre, was combined

with three millilitres of methanol, two millilitres of potassium acetate solution (1M), two millilitres of aluminium chloride (10 percent), and five millilitres of denatured alcohol over the course of thirty minutes. The extract contained one hundred micrograms of extract per millilitre. After then, it was left to rest at room temperature until it was ready to use (1M). We were able to determine the absorbance of the mixture by using an ultraviolet spectrophotometer, and we found that it peaked at 420 nanometers. The quantity of flavonoids found in extracts was measured in milligrammes of rutin equivalent for every gramme of extract. This was done so that comparisons could be made (mgRU/gofextract).

Preparation Of Standard Solution

After putting the powdered bark (10.0 g) in an extractor (a container that was sealed) for 36 hours at room temperature, the liquid components are extracted from powdered bark by passing it through a sieve with a mesh size of 40. The liquid components are then collected in a separate container. After that, the liquid components are placed in a separate container to be stored. The temperature in the room was continually kept at the same level regardless of whether or not the extractor was running. In order to achieve a totally dry extraction, a rotatory evaporator had been used to evaporate the solvent at a temperature of 45 degrees Celsius while the pressure was lowered. This was done while the pressure was being lowered. This was done while maintaining a constant temperature. Three separate tests were carried out so that the amounts of extractive material that could be recovered from each sample could be determined. One millilitre of methanol was used to dissolve one milligrams of dried residue from each sample. An ultrasonicator modelled after the Berlin Bandelin SONOREX was used for each and every stage of the mixing process. Syringe filters having a diameter of 0.22 microns are used in order to filter the combined solutions after they have been mixed. In order to accomplish this objective, the filtrates have been diluted with methanol so that the desired final working concentrations may be obtained. After the internal standards of the functional solutions' total volume of 50 L were added, the mixture was vortexed for a total of thirty seconds before being put into the next step of the process. It became out that the UHPLC-MS/MS equipment could only handle an aliquot of injection material of 50 L.

Cytotoxication

Each of the six Betula utilis extracts was tested against nine cancer cell lines for in vitro cytotoxic activity. It was discovered that ethyl acetate and chloroform produced the best results when used as extracts. In order to determine the cytotoxic component, chromatographic separations were performed using silica gel on extract of Betula utilis bark.

Sample Preparation and Cell Culture

After putting the powdered bark (10.0 g) in an extractor (a container that was sealed) for 36 hours at room temperature, the liquid components are extracted from the powdered bark by passing it through a sieve with a mesh size of 40. The liquid components are then collected in a separate container. After that, the liquid components are placed in a separate container to be stored. The temperature in the room was continually kept at the same level regardless of whether or not the extractor was running. In order to achieve a totally dry

extraction, a rotatory evaporator was being used to evaporate the solvent at a temperature of 45 degrees Celsius while the pressure was lowered. This was done while the pressure was being lowered. This was accomplished while the temperature was held steady throughout. The quantities of extractive material which could be extracted from each sample were determined during the course of three distinct experiments that were carried out in isolation from one another. One milliliter of methanol was used to dissolve one milligrams of dried residue. The RPMI-1640 medium was evaluated using a wide range of human cancer cell lines, such as A549, Colon, Breast, Head/Neck/Cerebral (FaDu), Prostate Ovary, & Brain, amongst others. The results of these evaluations were positive. The outcomes of these examinations came back favourable (A-172). In order to produce stock solutions with a concentration of one hundred milligrams per millilitre, one must first soak the test samples or molecules in DMSO. This allows one to construct the stock solution. This will bring about the outcome that is wanted. At all times, the stock solutions are maintained at a temperature of -20 degrees Celsius, since this is the standard that is enforced. After initially diluting the material in culture medium, a working solution was produced from it that had a concentration of 200 grams per millilitre. Before any tests were performed, this preparation was made.

Assay For Cytotoxicity (SRB assay)

For the purpose of determining whether or not cells are still alive, colorimetric SRB tests, also known as sulforhodamine B tests, were used. After the cells were seeded into each well of a 96-well plate in a growing medium consisting entirely of serum, they have been allowed to develop for 24 hours at 37 degrees Celsius in an incubator containing carbon dioxide before being examined. Prior to this, the cells were allowed to grow in an incubator at a temperature of 37 degrees Celsius. After that, an adequate amount of vehicle or parts were given to the cells that had adhered to the dish. The cells were initially fixed with ice-cold TCA at a concentration of 50 percent for a whole night, then stained with SRB that had been dissolved in 0.1 acetic acid for the following 48 hours, and then dried with air. The process began with fixing the cells with TCA. After the dye was separated from its binding solution, plate readers were used to analyse its absorbance in 10 mM Tris acid at 510 nm. This was done so that the results could be interpreted (Epoch Microplate Reader, Biotek, USA). In order to determine the cytotoxic potential of the fractions, we used the formula $[100 - (\text{Absorbance for untreated cells} / \text{Absorbance of vehicle-treated cells}) \times 100]$. This allowed us to compute the cytotoxic potential of each fraction. With the use of this formula, an assessment of the fractions' potential toxicity may be made.

During the preliminary testing, compounds that suppressed the growth of cancer cells by a particular charge and more than 75 percent were referred to be "Hits." Using the Graph Prism programme, the half-maximal inhibitory concentration (IC₅₀) of these samples was then determined by testing them on cancer cell lines.

CONCLUSION

The Himachal Pradesh access regions were the ones that turned up the greatest quantities of foroleanolide and betulinic acid (24.2 mg per gram and 22.2 percent, respectively). According to this research, the harvest of *B. utilis* from Himachal Pradesh is the most reliable source for obtaining phyto constituents with the highest level

of activity. The findings of this research will lead to the establishment of standards of quality for *B. utilis*. In the current study, researchers investigated if the peel of *Betula utilis* included any anticancer characteristics. Because betulinic acid is present, the bark of *Betula utilis* is thought to have cytotoxic properties. On either hand, the findings of the most recent study suggested that UA was much more active than betulinic acid.

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