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STUDIES ON PHYTOCHEMISTRY, ANTI-CANCER PROPERTIES OF BETULA UTILIS

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Abstract: **Betula is a versatile plant with several applications. D. Don (family Betulaceae) is a long-lived tree that has been known in Sanskrit for about 400 years as Himalayan birch, bhojpatra, or Bhurja. Its usefulness as a therapeutic plant is well acknowledged. Birch bark has recently been the topic of scientific study and industry given the presence contains triterpenoids including such betulinic acid as well as oleanolic acid, that are well for their anticancer actions. A new anticancer medication, betulinic acid promotes apoptosis in the cancer cells. Therefore, It was unique from other cancer treatments. A number of B. utilis species have been reported to contain phenolics as well as flavonoids. Suitable investigations were carried out to assess phytochemicals qualitatively using various solvents, as well as anticancer activities utilising the SRB test.**

Keywords: **phytochemicals, anticancer medication, cancer cells.**

1. INTRODUCTION

There are several uses for Betula, a plant. Betulaceae tree D. Don (also called as Himalayan birch, bhojpatra, as well as Bhurja in Sanskrit) is a long-lived tree which has been recognised for about 400 years. It's well accepted that this plant has medical properties. Because of its high altitude and sensitivity to soil instability and/or severe snowfall, the Betula utilis tree can only be found in the Himalayas. Various ailments and diseases, such skin disinfection, wound healing, bronchitis, leprosy, convulsions, as well as ear and blood issues may be treated using the stem bark of B. utilis. It has been shown to possess antibacterial, antihyperglycemic, antitumor, antioxidant, as well as anti-HIV activities pharmacologically.

The anticancer qualities of triterpenoids like betulinic acid as well as oleanolic acid, found in birch bark, have spurred recent industrial and scientific interest in the bark. Betulinic acid, a new anticancer medication, causes cancer cells to die by triggering apoptosis. As a result, it varies from traditional anticancer drugs. Phenolics and flavonoids are two more types of chemicals found in B. utilis species.

The measurement of components in B. utilis was previously reported using high-performance thin-layer chromatography16, which had limited sensitivity and resolution. As a result, for progressive output in this work, ultra-high-performance liquid chromatography (UHPLC) is utilised. Because of its unique and better grade sensitivity, the UHPLC- triple quadrupole/linear ion trap (QqQLIT) mass spectrometer makes it simple to identify analytes in ultra-low concentrations. Because of its precision, sensitivity, as well as ability to measure several analytes in complex matrices at the same time, the multiple reaction monitoring (MRM) approach is chosen in this investigation.

2. LITERATURE REVIEW

(Selzer et al., 2000) The effects of betulinic acid on malignant melanoma cells were investigated both on its own and in conjunction with irradiation. Betulinic acid was strong and continuously suppressed the proliferation and colony formation of all the human tumor cell lines. When betulinic acid and ionising radiation were used in colony-forming tests, the effect on development suppression was greater. Human melanoma cells were also caused to die by betulinic acid, according to this study.

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(Zuco et al., 2002) evaluation of betulinic acid as well as doxorubicin's cytotoxicity on melanoma and other tumour cell lines (also an anticancer drug). Various cell lines with different p53 statuses were investigated as well. Cell lines from melanoma, melanoma, small as well as non-small cell lung cancer as well as cervical and ovarian carcinomas were all repressed by betulinic acid were tested in vitro. An antiproliferative impact was detected on all tested lines in a limited dosage range (1.5-4.5 mg/ml).

(Fulda and Debatin 2005) A new cytotoxic medication, betulinic acid, has been shown to be effective in the treatment of neuroectodermal tumours including such neuroblastoma, medulloblastamas, glioblastomas as well as Ewing's sarcoma. Betulinic acid was shown to have substantial anticancer action in primary tumour cell cultures from across all neuroblastoma, medulloblastoma, and the most glioblastoma patients, with just an ex vivo ED50 in neuroblastoma cells of having 3-15 mg/ml that have been resistant to CD95 or doxorubicin-induced apoptosis. According to these findings, in vivo treatment of neuroectodermal malignancies might benefit from betulinic acid.

(Mishra et al., 2016) Scientists looked in the Betula utilis peel for an anti-cancer bioactive chemical. As a means of fractionating the methanol-extracted Betula utilis Bark (BUB), they researchers used many solvents, including hexane, ethyl acetate, chloroform, and water. Tested in vitro against nine distinct cancer cell lines and ethyl acetate was revealed to be the most powerful anticancer fractions. These six triterpenes, comprising betulin, -amyrin, lupeol, and -oleanolic acid (UA), were identified by spectroscopy after they had been extracted from the BUB extract using the ethyl acetate method and their structures were determined. It was the first time that -amyrin as well as UA were found in Betula utilis. The cytotoxic impact of isolated triterpenes on breast cancer cells was found to be more pronounced versus non-tumorigenic breast epithelial cell lines when tested in vitro (MCF 10A). UA's tumour cell-specific apoptotic impact was mostly responsible for the increase of DR4, DR5, as well as PARP cleavage in MCF-7 cells compared to non-tumorigenic MCF-10A cells in the extrinsic apoptosis pathway. The anti-cancer effects of UA are also influenced by its ROS generation as well as mitochondrial membrane potential disruption. UA also halts the spread of breast cancer. Overall, we found a unique source of UA with significant tumour cell specific cytotoxic properties, suggesting that it might be used to treat breast cancer.

(Pandey et al., 2020) B. utilis ethanolic extract (BUE) was shown to extend longevity and reduce amyloid toxicity in Caenorhabditis elegans. The worms' life spans were assessed in a variety of laboratory and high-stress conditions (including oxidative and high heat). BUE was also shown to slow the ageing process as measured by age-related biomarkers. GFP-tagged mutants and strains were also employed to examine the molecular mechanism under the beneficial benefits of BUE supplementation. Using BUE (50 g/ml), they found that C had a greater chance of surviving on average. elegans by 35.99 % as well as improved survival in stressful situations. There was a 22.47 percent reduction in reactive oxygen species (ROS) in cells as well. The amyloid-induced paralysis in CL4176 transgenic worms was delayed. Supplementation with BUE reduced the number of -synuclein aggregation with the NL5901 transgenic strain, which was unexpected. BUE-mediated lifetime extension is reliant on the daf-16, hsf-1, as well as skn-1 genes, but not the sir-2.1 gene, as according gene-specific mutant studies. Stress-protective genes which are Sod-3 and Gst-4 saw an uptick in activity after BUE therapy, a transgenic sensor gene expression experiment found. The current data revealed that BUE-induced lifetime extension was mediated by ROS scavenging activity in conjunction with various aging pathways. As a result, BUE may have the ability to extend life and slow the course of neurodegenerative diseases.

Aims and objectives

The goal of this study is to learn more about Betula utilis' phytochemistry, anti-diabetic, and anti-cancer activities.

The present study has been categorized into the objectives given below to fulfill the aim of the study:

- Selection of medicinal plants from different geographical regions of India will be collected.
- Phytochemical studies of different solvent extracts of selected medicinal plants will be studied by standard spectroscopic methods.
- Different solvent extracts for various biological activities i.e. Anticancer activities will be screened.

3. MATERIALS AND METHODS

Reagents, chemicals and materials

It was collected from Jammu & Kashmir, Himachal Pradesh, Sikkim, as well as Uttarakhand, where there were 27 accessions of the Himalayan Silver Birch. CSIR-National Botanical Research Group plant taxonomists in Lucknow, India verified the samples.

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Sigma Aldrich Ltd provided 96 percent purity standards for the analysis of betulinic acid, apigenin, caffeic acid, ferulic acid, caffeic, catechins, and oleanolic acids. Quercetin and luteolin analytical reference standards were given by Extrasyntheses (purity percentage 95 percent). For example: (Genay, France).

Fluka and Sigma-Aldrich provided the mobile phase solvents, which included LCMS-quality methanol, acetonitrile, and formic acid. A Milli-Q water purification system is employed to create ultra-pure water for the mobile phase (Millipore Corporation, Bed- ford, MA). They purchased Millipore 0.22-m syringe filters (Billerica, MA, USA).

Extraction and sample preparation

The soluble components were extracted from the powdered bark (10.0 g) by crushing it and sieving it through a 40-mesh sieve inside an extractor at room temperature for 36 hours. The extractor was kept at room temperature. Rotatory evaporator was used to evaporate the solvent at 45°C in order to get a totally dry extract. Table S1 shows the extractive yields from samples treated in triplicates. Each sample's dried residue (1 mg) were accurately measured and diluted in the 1 mL of methanol for analysis. Ultrasonics (Berlin) were employed throughout the whole mixing procedure using a Bandelin Sonorex ultrasonicator. By using a syringe filter the combined solutions were filtered with a 0.22 m diameter. Methanol was used to dilute the filtrates in order to get the final working concentrations. Afterwards, the desired finished solution were vortexed for 30 seconds to remove the spikes. For the UHPLC-MS/MS study, a 5 litre aliquot was all that was needed to be injected into the apparatus during the extraction process.

For qualitative analysis

Chemicals:

Extraction procedure:

After 72 hours of maceration with freshwater, methyl alcohol, hexane, petroleum ether, alcohol, dichloromethane, and carbon tetrachloride, ten grammes of powdered plant components were extracted. Using a rotary evaporator, the macerates were filtered and concentrated at 40-50 °C. Until they were needed, the extracts were kept at a temperature of 4 degrees Celsius.

Phytochemical analysis

DCM, hexane, chloroform, methanol, as well as water extracts were used to identify phytochemical components such as anthraquinone, phytosterols, tannins, as well as steroids (ethyl acetate). Other metabolites such as flavonoids as well as alkaloids were also detected.

Evalution of TPC

The phenolic content is determined and calculated using Folin-Ciocalteu with minor adjustments (TPC). First, % sodium carbonate solution was mixed with 1N Folin-Ciocalteu, and then 1N Folin-Ciocalteu reagent was added (1N). Samples are swirled about in a vortex. After mixing, it was allowed to remain in the reaction mixture about 30 more minutes. The Water is used to keep the solution at a maximum volume of 12.5 mL. At a wavelength of 765 nm, the absorption level was observed.. Extracts were quantified in mg GA/g extract in order to determine their phenolic content.

Determination of total flavanoid content

30 minutes at room temperature were used to mix one millilitre of the extract (containing 100 micrograms of extract per millilitre) with three millilitres of methanol, two millilitres of potassium acetate solution (1 M), two millilitres of aluminium chloride (10 percent), and five millilitres of denatured alcohol (1 M). Measurement of the mixture's absorbance at 420 nm was carried out by using a UV spectrophotometer. Milligrams of rutin equivalent per grammes of extract (mg RU/g of extract) was used to assess flavonoids in extracts.

Preparation of standard solution

The liquid components were extracted from the powdered bark (10.0 g) by sieving it through a 40-mesh sieve inside an extractor (a sealed container) for 36 hours at room temperature. When the extractor was not in use, it remained at room temp Rotatory evaporator calibrated to 45°C was used to evaporate the solvent under decreased pressure in order to get a totally dry extraction. Three different experiments were performed to determine the extraction yield for each sample. One millilitre of methanol was used to dissolve one milligramme of dried residue from each sample. Throughout the mixing

process, a Bandelin SONOREX ultrasonicator (Berlin) was used. By using 0.22 micron- diameter syringe filters the combined solutions are filtered. Methanol was used to dilute the filtrates in order to get the final working concentrations. Once the internal standards (50 L) were added, the functional solutions were vortexed for 30 seconds. It was revealed that the UHPLC-MS/MS apparatus could handle merely a 5 L aliquot for injection.

Cytotoxic action

Each of the six Betula utilis extracts was tested against nine cancer cell lines for in vitro cytotoxic activity. Ethyl acetate as well as chloroform were found to be the most potent extracts. Extracts of Betula utilis bark were chromatographed over silica gel in order to identify the cytotoxic component.

Sample preparation and cell culture

On human cancer cell lines such A549, Colon, Breast, Head/Neck/Cerebral (FaDu), Prostate Ovary, and Brain, RPMI-1640 medium was tested (A-172) Stock solutions with a concentration of 100 mg/mL is produced by dissolving the test samples/molecules in DMSO. The stock solutions are kept at a temperature of -20°C. The stock solution was diluted in culture medium to obtain a working solution of 200 g/mL prior to testing.

Assay for cytotoxicity (SRB assay)

To determine cell viability, colorimetric SRB (sulforhodamine B) assays were utilised. Before being examined, cells were grown for 24 hours at 37°C in a CO2 incubator after being seeded into each well of a 96-well plate in a growth medium containing 100 percent serum. A suitable dose of vehicle or fractions was then applied to the adherent cells. An overnight fix with ice-cold TCA 50 percent was followed by 48 hours of staining with SRB in 0.1 acetic acid and finally air drying of the cells. Plate readers were used to measure the absorbance at 510 nm of the dye that had been released from its binding solution in 10mM Tris acid (Epoch Microplate Reader, Biotek, USA). We utilised the formula [100-(Absorbance for untreated cells/Absorbance of vehicle-treated cells) X 100]is used to determine the fractions' cytotoxic potential.

Screening samples that inhibited cancer cell growth by 75% or more at 10specific charge were referred to as "Hits" during the first screening, and they were then tested on cancer cell lines to figure out their half-maximum inhibitory concentration (IC50) with Graph Prism software.

4. RESULTS AND DISCUSSION

Biochemical screening of B. utilis's stem bark as well as roots is shown in Table 1. All twelve phytochemicals tested were discovered in various solvent extracts. The stem bark had the most phytochemicals, whereas the leaves and seeds contained the fewest. As it turned out, the stem bark contained a startling array of compounds, from anthraquinones to steroid hormones to anthraquinone glycosides to phytosterols to saponins and proteins. According to these studies, the stem bark contains more phytochemicals than either the roots or the leaves. The methanol extracts of stem bark was discovered to have more components than the extracts of leaves and roots.

Table 1: Qualitative analysis of the phytochemicals in bark, leaves and roots

	Bark							Leaves							Roots						
Phytoconstituents	A	M	E	C	PE	H	DCM	A	M	E	C	PE	H	DCM	A	M	E	C	PE	H	DCM
Flavonoids	+	+	+	+	+	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	-
Alkaloids	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	-
Terpenoids	+	+	+	+	+	+	-	-	+	+	-	-	-	+	-	+	+	-	-	-	-
Cardiac Glycosides	+	+	-	+	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-
Saponins	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenolics	+	+	-	-	-	+	+	+	+	+	+	+	+	+	-	+	-	-	-	-	-
Proteins	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Amino acids	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Anthraquinone glycosides	_	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
Phytosterols	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tannins	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-
Total no. of phytoconstituents	10	11	3	6	6	2	3	1	7	7	3	2	1	2	nil	4	2	1	1	nil	nil

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Free radical scavenging activity and quantities of total flavonoid and phenolic compounds in bark, leaves, and roots were studied by using water and six different organic solvents like methanol, ethanol, dichloromethane, chloroform, petroleum ether, and hexane. Folin-Ciocalteu reagent has been used to measure total phenolic content in the sample. It was measured in milligrammes of gallic acid equivalency per millilitre of solvent for each B. utilis extract (GAE). There were total phenolics in extract ranging from 0.070.002 mg GA/g to 33.6 2.1 mg GA/g, according to Table 2. Total phenolic content of 33.6 2.1 mg GA/g is found in the root extracts. While looking at the total chemical content of each extract, researchers discovered the greatest concentration of phenolic compounds in peel extract by using solvents with moderate and high polarity. An average dose of 0.08 mg GA/g is found in the bark extract, while the maximum dose is found to be 16.9 mg GA/g.

It containes the highest amount of phenols (23.6 0.67 mg GA/g) among the seven leaf extracts studied. From 0.34 mgGA/g to 23.6 mgGA/g, phenolic chemicals may be found in different leaf extracts. The preponderance of phenolic compounds are flavonoids. Plant preparations from B. utilis leaves, bark, as well as roots were tested for flavonoids using a spectrophotometric approach including AlCl3. Flavonoids were found in the analysed extracts as shown in Table 3. Flavonoids may be found in varying concentrations in plant extracts from the aerial and subterranean parts of the plant. There is a wide range of flavonoid concentrations in plant extracts, from 0.15 RU/g to 7.23 RU/g. Methanol, ethanol, and water extracts have the largest quantities of flavonoids in leaves, but DCM, chloroform, petroleum ether, and hexane extracts have modest levels of flavonoids. Root extracts contain less flavonoids than bark extracts, although the differences are minor. Methanolic and ethanolic extracts from bark had the greatest concentration of flavonoids.

Table 2: different solvents' effect on the extraction of total phenols from different parts of b. utilis

	Total phenols mg GA/g DW						
Parts	**Water**	**Methanol**	**Ethanol**	**Dichloromethane**	**Chloroform**	**Petroleum ether**	**Hexane**
Bark	3.5 ± 0.4	16.9 ± 1.9	6.5 ± 0.2	1.2 ± 0.02	0.5 ± 0.02	0.08 ± 0.005	0.2 ± 0.003
Root	11.1 ± 0.27	33.6 ± 2.1	29.1 ± 0.67	0.3 ± 0.02	0.4 ± 0.03	0.03 ± 0.003	0.07 ± 0.002
Leaf	23.6 ± 0.67	21.8 ± 0.5	12.6 ± 0.38	0.7 ± 0.02	0.9 ± 0.19	0.34 ± 0.02	0.5 ± 0.02

The standard error of the mean of three replicates is used in all analyses. Gallic acid concentrations in mg/g dry plant material were given.

Table 3: effect of different solvents on flavonoid extraction from different parts of b.utilis

	Total flavonoids mg Ru/g DW						
Parts	**Water**	**Methanol**	**Ethanol**	**Dichloromethane**	**Chloroform**	**Petroleum ether**	**Hexane**
Bark	0.4 ± 0.038	4.3 ± 0.08	3.2 ± 0.012	0.8 ± 0.05	0.5 ± 0.05	0.3 ± 0.08	0.3 ± 0.11
Root	0.34 ± 0.02	1.3 ± 0.03	1.1 ± 0.02	0.8 ± 0.07	0.5 ± 0.009	0.3 ± 0.008	0.6 ± 0.04
Leaf	1.3 ± 0.018	7.3 ± 0.31	5.3 ± 0.03	0.2 ± 0.003	0.8 ± 0.6	0.2 ± 0.007	0.3 ± 0.018

The standard error of the mean of three replicates is used in all analyses. It's given in mg rutin per gramme of dried plants.

Compound characterisation using UHPLC-ESI-MS/MS

Triterpenoids

OLEA and BETULINIC acids, both 455.800 (M-H), generated product ions at 392 and 438 (m/z) for the loss of CO2H and OH when OH and CO2H were present in the reaction (after successive loss of OH, CO, and H2O).

Phenolics

Carbamazepine and caffeic acid were recognised by their respective m/z values of 353.100 (M-H) for chlorogenic acid, and 178.800 and 134.800 for caffeic acid (M-44; loss of CO2). ferulic acid had a parent ion m/z of 193.00, whereas the final product had 134.000 ion m/z. Loss of CH3 as well as CO2 in M-59

Flavonoids

The molecular ion at m/z 301.100 and the product ion at m/z 151.000 were used to identify quercetin. (M-150; C7H204 is missing). The precursor ion of kaempferol was detected at m/z 239.000 (M-46; loss of CH2O2), while the parent ion of apigenin was discovered at m/z 269.000, and the product ion was discovered at m/z 117.100, while the catechin parent ion was discovered at m/z 289.100.

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Quantitative analysis in B. utilis

In B. utilis stem barks collected from different parts of the world, Bioactive substances were determined using the UHPLC-ESI-MS/MS technique. During a quantitative analysis of 27 accessions, significant differences in the quantities of terpenoids, phenolics, and flavonoids were discovered. Among all the measured analytes, All B. utilis stem bark samples, as indicated in the table below, had the highest concentrations of betulinic acid as well as oleanolic acid.

Golaba, Kullu, Himachal Pradesh, stem barks had the highest quantities of the triterpenoids betulinic acid (24.2 mg g1) as well as oleanolic acid (22.2 mg g1). This is consistent with the results from stem bark obtained from the same location, which showed higher levels of all flavonoids. studied, including quercetin (0.116 mg g1), luteolin (1.320 mg 1), kaempferol (0.439 mg 1), and catechin (1.17 mg 1). (0.720 mg g1). For phenolic acids, samples from Himachal Pradesh showed higher concentrations of the caffeic acid (0.063 mg g1) as well as chlorogenic acid (0.417 mg g1), whereas samples from Kashmir had lower concentrations of the caffeic acid (0.063 mg g1), ferulic acid (0.417 mg mg1) (0.217 mg g1).

Figure 1 depicts a graphical depiction of these findings, which illustrates the chemical variances among bioactive substances as a result of varied geographical locations, altitudes, and collecting times. If a plant's therapeutic medicinal properties and effectiveness are affected by the quantity of active ingredients in its bioactive analytes, then the effects will be felt immediately. This means that choosing and quantifying active chemical markers is critical for achieving the desired pharmacological effect.Using this method, researchers found that eleven compounds that may be used as major indicators of quality control of B. utilis in the hereafter could be thoroughly evaluated. This comparative variation analysis is crucial again for the quality control of B. utilis.

Samples	Betulinic acid	Oleanolic acid	Caffeic acid	Catechin	Chlorogenic acid	Ferulic acid	Apigenin	Kaempferol	Luteolin	Quercetin
NBMP-1	7.64	9.42	0.063	0.44	0.30	0.046	0.017	0.014	0.013	0.046
NBMP-2	16.8	18.4	0.017	0.58	0.25	0.037	0.028	0.028	-	0.051
NBMP-3	23.3	25.8	0.045	0.32	0.06	0.042	0.018	0.057	-	0.022
NBMP-4	14.2	14.7	0.023	0.26	0.10	0.039	-	-	-	0.042
NBMP-5	9.91	10.8	0.045	0.51	0.16	0.041	-	-	-	-
NBMP-6	17.9	17.7	0.033	0.29	0.26	0.065	-	-	-	-
NBMP-7	12.4	12.3	0.051	0.57	0.21	0.046	-	-	-	0.038
NBMP-8	6.29	6.84	0.020	0.56	0.42	0.043	0.0198	-	-	0.027
NBMP-9	18.9	18.2	0.030	0.19	0.05	0.040	-	-	-	-
NBMP-10	6.26	6.3	-	0.60	0.32	0.042	-	-	0.014	0.036
NBMP-11	5.94	6.26	0.029	0.51	0.15	0.038	-	0.019	-	0.058
NBMP-12	3.33	3.87	-	0.41	0.10	0.047	-	0.044	-	0.042
NBMP-13	8.01	7.72	0.015	0.28	0.12	0.079	-	0.054	-	0.037
NBMP-14	14.9	15.5	0.013	0.31	0.09	0.043	0.049	-	-	-
NBMP-15	13.9	14.2	0.018	0.33	0.25	0.063	-	-	-	-
NBMP-16	8.07	7.9	0.029	0.49	0.20	0.046	-	-	-	0.040
NBMP-17	6.63	6.63	0.012	0.35	0.14	0.046	-	-	-	0.043
NBMP-18	13.2	12.9	0.015	0.43	0.18	0.111	1.17	0.439	1.320	0.116
NBMP-19	14.4	13.3	-	0.43	0.14	0.047	0.017	0.048	-	0.048
NBMP-20	6.05	5.18	0.010	0.28	0.16	0.045	-	0.009	-	0.040
NBMP-21	4.22	3.83	-	0.47	0.34	0.054	0.024	0.046	-	0.042
NBMP-22	24.2	22.2	0.031	0.11	0.04	0.217	-	0.046	-	-
NBMP-23	1.32	1.21	-	0.72	0.16	0.036	0.020	0.066	-	0.041
NBMP-24	0.09	-	0.046	0.50	0.04	0.154	0.019	0	0.133	0.071
NBMP-25	19.0	16.5	0.055	0.25	0.15	0.124	0.021	0.0172	0.094	0.115
NBMP-26	11.5	12.1	-	0.35	0.13	0.044	-	-	-	-
NBMP-27	18.3	17.5	0.033	0.37	0.04	0.110	-	-	-	0.068

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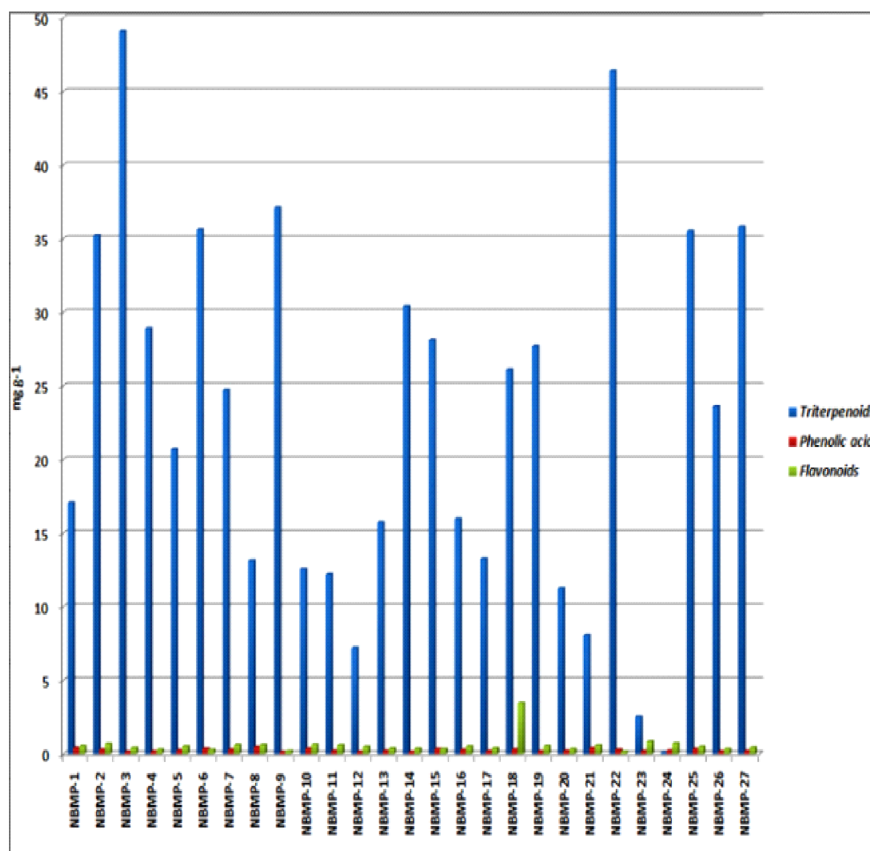

Figure 1: geographical variation of the reference analytes

The bark was fractionated using n-hexane and chloroform, as well as ethyl acetate and butanol, after it was extracted from the Betula utilis tree with methanol, . Using SRB assay, extracts were tested against nine distinct human cancer cell lines for the in vitro cytotoxic activities, including A172, MCF-7, DLD1, PLC/PRF5, A549, SK-OV3, DU145, and Caki-1 (all of which are human cancer cell lines) for glioblastoma and colorectal adenocarcinoma. Chloroform, chloroform, and methanol extracts are shown to be the most active in comparison to the other extracts (Fig 1). We used the extract of ethyl acetate to isolate pure molecules since it was shown to be the most suited in terms of cytotoxic activity and extractive yield.

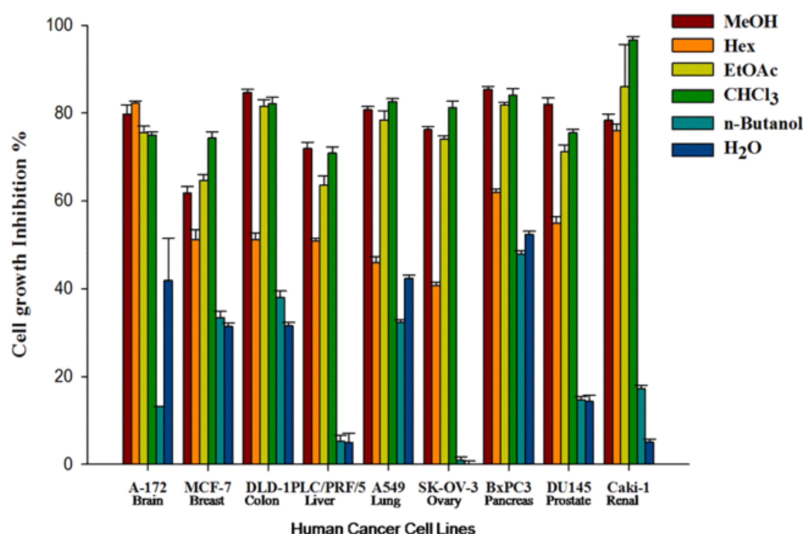

Figure 2: Inhibition of cell proliferation by solvent extracts of Betula utilis bark in nine distinct cancer cell lines as a percentage.

Cytotoxic activity of isolated triterpenes

Unguanositol and betulinic acid were found to be solubilized in DMSO in the presence of four identified triterpenes: betulinic acid, oleanoic acid, beta amyrin, and unguanositol (UA). Four soluble triterpenes were tested in vitro against seven human cancer cell lines: MCF-7 breast, MCF-7 colon and MCF-7 lung cancer cell lines, as well as HeLa head and neck cancer cell lines (HeLa). Figure 3 displays the inhibition percent . UA, one of four triterpenes identified, was shown to be cytotoxic to various cancer cell lines, having the maximum cytotoxicity in breast cancer.

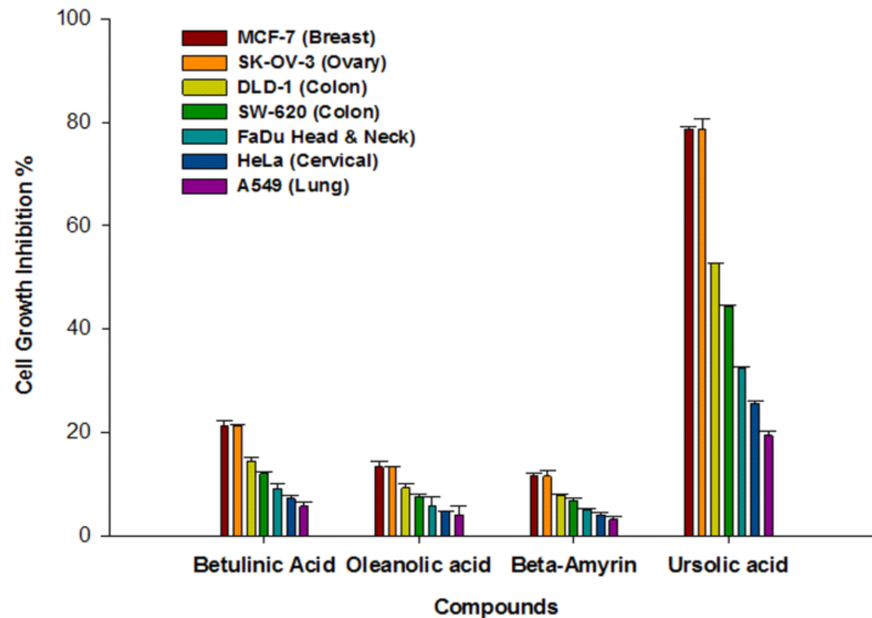

Figure 3: Cytotoxic activity of Isolated Triterpenes (10µM) against seven human cancer cell lines.

5. CONCLUSION

For oleanolic acid and betulinic acid, the highest concentrations were discovered in the Himachal Pradesh accessions (24.2 mg per gramme and 22.2%, respectively). The Himachal Pradesh collection of B. utilis was shown to be the best place to get the most active phytoconstituents from this study. Quality standards for B. utilis will be developed as a result of this study. Anticancer properties were explored from the peel of Betula utilis in the present study. Betula utilis bark is hypothesised to have cytotoxic characteristics because of the existence of betulinic acid. However, the current investigation demonstrated that UA was more active than betulinic acid.

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