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PHYTOCHEMICAL SCREENING, ANTIOXIDANT ASSAY OF *JUNIPERUS RECURVA* AND STUDY OF IT'S IN VITRO CYTOTOXICITY AGAINST BREAST CANCER CELL LINES

JAYA BHANDARI[1], ASMA NAQVI[2], PRASODHAN NIRAULA[1], PRATIKSHYA THAPA[1], NITA THAPA[1], NIKITA SHRESTHA[1] AND BHUPAL GOVINDA SHRESTHA*[1]

[1]Department of Biotechnology, Kathmandu University, Nepal
[2]Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences, University of Karachi, Pakistan

ABSTRACT

Over 70% of anticancer drug has their origin as natural products and only a small part of the massive reservoir of the bioactive components on Earth has been studied. This study comprises the phytochemical screening, antimicrobial tests, antioxidant (DPPH) assay, and in-vitro cytotoxicity against MCF-7 (Breast cancer) cell lines of different plant-parts of Nepalese medicinal plant *Juniperus recurva*.This is the first study of the effects of *Juniperus recurva* against the breast cancer (MCF-7) cell lines. Two different extracts of leaves of two different parts of the tree of *J. recurva* (JRL1 and JRL2) and two extracts of seeds of *J. recurve* JRSO(Outer coat of seed) and JRSib (Inner black seed without the coat) were taken for studies. The zones of inhibitionin the antimicrobial tests of the extracts of a leaf extract (200mg/mL) against *B. cereus, B. thuringiensis, E. coli, P. aeruginosa, B. subtilis* and *P. mirabilis* were17mm, 15mm, 10mm, 10mm, 15mm and 12 mm respectively and of seed extract (200mg/mL) were 11mm, 10mm, 12 mm, 16mm, 15mm 13mm for seeds respectively and in the same sequence against different microorganisms. The percentage of DPPH scavenging activity is found to increase with the increase in concentration. The highest percent DPPH scavenging activity is found at the maximum concentration used i.e. 10 mg/mL corresponding to the value of 89.25%, 45.01%, 25.74%, 75.09% and 32.81% of ascorbic acid for JRL1(Leaf extract), JRL2 (Leaf extract), JRSib (Seed extract) and JRSO (Seed extract) respectively. MTT assay was carried to determine the IC50 values of different plant-parts extracts. All the plant parts are found to be weakly cytotoxic against MCF-7 with IC50 values of 40.38µg/mL for JRL1 and 50 µg/ml for all other extracts named JRL2, JRSO and JRSib as compared to Standard Doxorubicin i.e. IC50=1.56±0.05µM. Further work on isolation of pure compounds by bioassay guided extraction from the plant *J. recurva* is suggested so that the higher activity of the pure compounds towards MCF7 and other cancer cell lines can be investigated.

KEYWORDS*: Juniperus recurva*, MTT, Cytotoxicity, MCF7, antimicrobial tests, antioxidant assay

BHUPAL GOVINDA SHRESTHA
Department of Biotechnology, Kathmandu University, Nepal

***Corresponding author**

INTRODUCTION

The development of preventive and newer strategies of therapy may come from the botanical explorations used in ethno-medicines [1]. Medicinal plants are considered the only potential source for drug development and many novel products has gone into clinical trials[2]. Scientists are investigating properties of medicinal plants in order to develop novel drugs from natural products [3]. Medicinal herbs and the herbal medicines have a profound scope and have been used to find the anticancer effects in them [4]. Research on anticancer drugs are focusing to the plant products such as resveratrol, ricin, epigallocatechin-3-gallate, oridonin, juglone, quercetin, matrine, nordihydroguaiaretic acid and the investigations have also shown that the antitumor mechanisms of plant extracts include induction of apoptosis, cytotoxicity, redifferentiation, anti-angiogenesis, anti-invasion and anti-metastasis [5]. Various research papers discuss the genetic and biochemical mechanisms of carcinogenesis and the cancer preventing role of phytochemicals such as polyphenols by signal transduction and genetic changes [6]. Only a small part of the massive reservoir of the bioactive components on Earth has been studied and used as significant anticancer agents [7]. Cancer these days is regarded as the second leading cause of mortality over the world and many plant products have shown very promising anti-cancer properties in vitro still to be evaluated in humans [8]. For example, the anticancer potential of ginger is linked to the pungent vallinoids, viz. [6]-gingerol and [6]-paradol, as well as some other components like shogaols, zingeroneetc present in ginger [9]. The natural products of *Scurrulaatro purpurea* (Loranthaceae), the alkynic fatty acid octadeca-8, 10, 12-triynoic was reported to reveal a potent inhibitory effect on cancer cell invasion in vitro [10]. The researches in the world speculated that *Limoniastrum guyonianum* aq. gall extract showed important inhibitory activity against calpain and caused an indicative and concentration dependent upregulation of p16INK4A [11]. Research has been focused on the use of plant products for cancer therapy [12]. Anti-cancer evaluation of carboxamides of furano-sesquiterpene carboxylic acids from the soft coral *Sinularia kavarattiensis* indicated that the extracts were active against five human cancer cell lines [13]. The aqueous *Peltophorum pterocarpum* wood extracts are reported to show potent inhibitory effect against Epstein-Barr virus early antigen (EBV-EA) [14]. Tocopherols (vitamin E) and tea polyphenols have been reported to exhibit anticancer properties [15]. Anticancer chemothreupatic properties of plants of the Cactaceae family have been studied recently [16]. In-depth study of tumor metastasis and drug resistance and use of herbal medicines for their inhibition are increasing [17]. Similarly, bioactive compounds can revert epigenetic alterations in a variety of cancers in vitro and in vivo and they exhibit anticancer effects by targeting signaling pathways inside the cells [18]. It has been studied that limonoids can inhibit cell metastasis in cell culture and animal studies [19]. Crocetin, an important plant product, has shown significant potential as an anti-tumor agent in animal models [20]. Researchers have been speculated the efficiency of plant products to modulate the signaling pathways related to cancer [21]. The terpenoids appears to be promising anticancer component and will potentially increase the scope of cancer chemoprevention by using natural products [22]. A review discusses the genetic and biochemical mechanisms of carcinogenesis and the role of natural bioactive compounds like polyphenols in cancer treatment by signal transduction and change in gene expression [23]. Similarly the chemopreventive efficiency of some of the phytochemicals has been demonstrated, as observed in rodent carcinogenesis models in some studies [24]. Finding novel drugs is still a priority goal for cancer treatment, providing the increasing resistance to chemopreventive drugs [25]. Cephalostatin 1, OSW-1, ritterazine B and schweinfurthin are natural products that potently and in some cases selectively, inhibit the growth of cultured human cancer cell lines [26]. Natural products targeting the ubiquitin-

proteasome system as well as synthetic compounds with potent inhibitory potential is recently being studied [27]. A review article suggests microtubule-binding natural products can contribute in developing cancer drugs [28]. The modulation of apoptosis signaling pathways by natural compounds is studied to have constituted a key event in their antitumor efficacy [29]. A natural product named [6]-Gingerol (1-[4'-hydroxy-3'-methoxyphenyl]-5-hydroxy-3-decanone) suppress tumor necrosis factor alpha (TNF-α) expression [30]. In the same way, some recent researchers shed light on the finding of plant products with an efficacy to be developed as a possible alternative therapy for cancer or for its cure [31]. Few researches focus on the functions of NF-β, its role in human cancer and the therapeutic potential and benefit of targeting NF-β by natural products in cancer chemoprevention [32]. One of the potential strategies for preventing cancers is using food-based natural products [33]. Researchers addressed the inhibition of the nuclear factor-β (NF-β) pathway by thioalkaloids promising adjuncts to classical cancer chemotherapy [34]. The antiproliferation activity of a black carrot anthocyanin-rich extract on human cancer cells (HT-29 colorectal adenocarcinoma and HL-60 promyelocytic leukemia) and metabolism of anthocyanin is studied [35]. From all of the above information, we can infer that there is growing interest in the use of natural products and plants for the treatment and prevention of cancer and many plant products are currently evaluated in preclinical phase as promising anticancer agents [36]. The normalization of inappropriately over-amplified signaling cascades implicated in chronic inflammation-associated carcinogenesis by use of COX-2 specific inhibitors has been recognized as a practical strategy in molecular target-based cancer prevention using anti-inflammatory phytochemicals [37]. New effective mantra for cancer prevention "building customized mechanism-based chemoprevention cocktail of naturally occurring substances" is advocated which can be attained by utilizing the multidisciplinary approach towards natural products and cancer [38]. *Juniperus recurva* known as Himalayan Juniper is a large shrub commonly found in hilly region of Nepal. It can also be tree having height of 6-20 m and a trunk up to 2 m diameter and a broadly conical to rounded or irregular crown. In Nepal it is widely spread in east side of Himalaya region. It mainly grows at altitude of 3000-4000 m.

MATERIALS AND METHODS

Collection of Plant Materials

Juniperus recurva leaves and seed were collected from Panauti area of Kavre district and surrounding areas of Nepal. The leaves and the seeds were then dried in the shade, left over for 15 days and macerated and powdered with the help of kitchen grinder. The powder of leaves (JRL1 and JRL2), the powder of seeds (JRSOand JRSib) were separately labeled and stored in airtight bottles.

Preparation of Plant extracts

The dry powder of JRL1, JRL2, JRSO and JRSib, 100 gm each were dipped in 80% aq. Ethanol for three days stirring twice a day. The solution at the end of third day is filtered and then concentrated in high vacuum-rotator evaporator and finally all the solvent is evaporated using freeze dryer to get the semi-solid plant extract. The same procedure is repeated three times and the freeze-dried extract is collected together. The yield of the plant powder JRL1, JRL2, JRSO and JRSib from 100gm each, in aq. Ethanol weighed after the freeze drying was 15.063gm, 14.016gm, 12.467gm and 12.885 gm respectively. The extracts were then kept in glass vials with airtight caps and stored at 4 degree centigrade.

Phytochemical Screening

Test for Alkaloids

5ml of extract was concentrated to yield a residue. Residue was dissolved in 3ml of 2% (v/v) HCL. Few drops of Mayer's reagent was added. Appearance of the dull white precipitate indicated the presence of basic alkaloids.

Test for Coumarin

4ml extract solution was taken; 1-2 drops of water (hot) was added. Volume was made half

(UV fluorescence). 10% NH_4OH was added to another half volume (strong fluorescence).Presence of green fluorescence indicated the presence of Coumarin.

Test for Saponins
2ml extract was shaken vigorously for 30 seconds in a test tube. Persistence of thick forth even after 30 minutes indicated the presence of saponins.

Test for Glycosides
2ml of the extract was dried till 1ml.1-2ml NH_4OH was added and shaken. Appearance of cherish red color indicated the presence of glycosides.

Test for Reducing Sugars
0.5 ml of extract was taken and1ml distill water was added. 5-8 drops of Fehling's solution (hot) was added. Presence of brick red precipitation indicated the presence of reducing sugar.

Test for steroids
1ml extract was dissolved in 10 ml chloroform. Equal volume of conc. H_2SO_4 was added by the side of test tube. Upper layer turned red and sulphuric acid layer turned yellow with green fluorescence. This indicated the presence of steroids.

Test for Quinone
1 ml of extract was taken.1ml of conc. H_2SO_4 was added. Formation of red color indicated the presence of quinone.

Test for Terpenoids
5 ml of extract was taken and mixed with 2 ml of chloroform.3ml of conc. H_2SO_4 was added to form a layer. Reddish brown precipitate formation at the interface formed indicated the presence of terpenoid.

DPPH Assay
The reaction mixture contained 10 ml of (0.5mmol) test samples (in DMSO), 90 ml of 0.1 mol phosphate buffer (pH 7.4), 40 ml of 0.2mmol β-nicotanamide adenine dinucleotide (NADH) and 40 ml of (0.081mmol) nitro blue tetrazolium (NBT). The reaction was initiated by the addition of 20 ml of (0.008mmol) phenazinemethosulphate (PMS). The solutions of NADH, NBT and PMS were prepared in phosphate buffer. The formation of superoxide was monitored by measuring the absorbance of the blue formazan dye after 5 min at 560 nm against the DMSO treated blank solution. Ascorbic acid was used as positive control.

Agar plate diffusion method for antimicrobial activity
Medium was dissolved and autoclaved at 121° C for 15 min, cooled up to 45° C and then 40-50 ml media was poured in sterile 14 cm diameter Petri plate, and then allowed to solidify and kept at room temperature to check the sterility of the prepared media.

Preparation of plant extract
Stock solution of 400mg/ml was prepared by weighing 200mg of plant extract in 1.5 ml eppendorf tube and the final volume of 0.5ml of DMSO was added by micropipette. Extract was completely dissolved by vortexing for 5-10 minutes. Test solution of 200mg/ml, 100mg/ml, 50mg/ml, and 25mg/ml concentration was prepared.

a. Preparation of inoculums
Each culture to be tested was streaked onto nutrient agar to obtain isolated colonies. Overnight incubation was done at 37°C.Then isolated colonies were transferred with the help of a sterile loop onto Muller Hinton Broth. Overnight incubation was done at rotary shaker at 37°C.

b. Inoculation Procedure
For inoculation, a sterile cotton swab was dipped into the suspension and was pressed firmly against the inside wall of the tube just above the fluid level and the swab was rotated to remove excess liquid. The cotton swab was streaked over the entire surface of the medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculums. Finally, swabbing was done all around the edge of the agar surface. For plant extract, the disc was made from No.1 Whatman filter paper by the

help of punching machine. Discs were sterilized by autoclaving. After sterilization, the discs were dipped into the desirable concentration of the plant extracts prepared initially in a sterile condition. The discs were applied to the plates as soon as possible, but no longer than 15 minutes after inoculation. The discs were placed individually with sterile forceps, and then gently pressed down onto the agar (In general, place no more than 12 disks on a 150-mm plate and no more than 4 disks on a 100-mm plate after the discs were placed on the plate) the plate was inverted and incubated at 37°C for bacteria and fungi was placed without inverting for 24 hours. After incubation, the diameter of the zones of complete inhibition was measured (including the diameter of the disc) and recorded it in millimeters. The measurements were done with a ruler on the undersurface of the plate without opening the lid.

MTT/Cytotoxicity Assay

Cytotoxic activity of plant extract was evaluated in 96-well flat-bottomed micro plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. For this purpose, MCF7 cells (Breast Cancer) were cultured in Dulbecco's Modified Eagle Medium, supplemented with 5% of fetal bovine serum (FBS), 100 IU/ml of penicillin and 100 µg/ml of streptomycin in 75 cm^2 flasks, and kept in 5% CO_2 incubator at 37^oC. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture with the concentration of $1x10^5$ cells/ml was prepared and introduced (100 µL/well) into 96-well plates. After overnight incubation, medium was removed and 200 µL of fresh medium was added with different concentrations of plant extracts. The stock solution of the extract was first prepared (50µg/ml) in DMSO and is serially diluted up to the concentration of 0.78125µg/ml. After 48 hrs, 200 µL MTT (0.5 mg/ml) was added to each well and incubated further for 4 hrs. Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction to formazan within cells was calculated by measuring the absorbance at 570 nm, using a micro plate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC_{50}) for MCF7 cells. The percent inhibition was calculated by using the following formula

% inhibition = 100-[(mean of O.D of test compound – mean of O.D of negative control)/ (mean of O.D of positive control – mean of O.D of negative control)*100].
The results (% inhibition) were processed by using Soft-Max Pro software (Molecular Device, USA). IC_{50} was calculated using EZ-Fit Software.

RESULTS AND DISCUSSIONS

Table1
Phytochemical screening of different plant extracts of Juniperus recurva

Phytochemical	JRL1	JRL2	JRSib	JRSO
Alkaloids	-	-	-	+
Tannins	-	-	+	-
Reducing sugar	-	-	-	-
Coumarin	+	+	+	+
Glycosides	-	-	+	-
Quinone	+	+	-	-
Steroids	-	-	-	+
Terpenoids	-	-	-	+
Saponin	-	-	+	-

In the phytochemical screening for the presence of different phytochemicals like Alkaloids, Tannins, Reducing Sugar, Coumarin, Glycosides, Quinones, Steroids, Terpenoids and Saponin of the different plant parts of *Juniperus recurva*, JRL1, JRL2, JRSib and JRSO, the extracts JRL1 and JRL2 showed the presence of similar phytochemicals as they are both the extracts of leaves of *Juniperus recurva* from different position in tree. JRSib and JRSO showed a bit difference in the presence of different phytochemicals. JRL1 and JRL2 showed only the presence of Coumarins and Quinones with the absence of other mentioned phytochemicals. JRSib showed the presence of Tannins, Coumarin, Glycosides and Saponin and JRSO with the presence of Alkaloids, Coumarins, Steroids and Terpenoids with the absence of other phytochemicals mentioned above. The presence of alkaloids and Coumarin in the JRSO is a good indication of being anticancer activity. In the same way the presence of coumarin all the extracts may attribute to the anticancer activity that the plant may have. The presence of terpenoids only in the JRSO may attribute to the smell of the seeds of *Juniperus recurva* and may contribute to some anticancer properties.

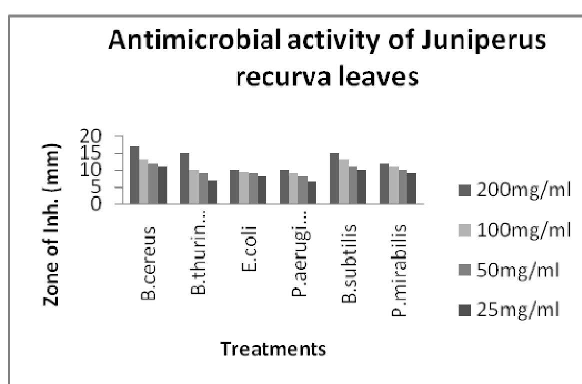

Figure 1
Antimicrobial Activity of (JRL1+JRL2) mixed

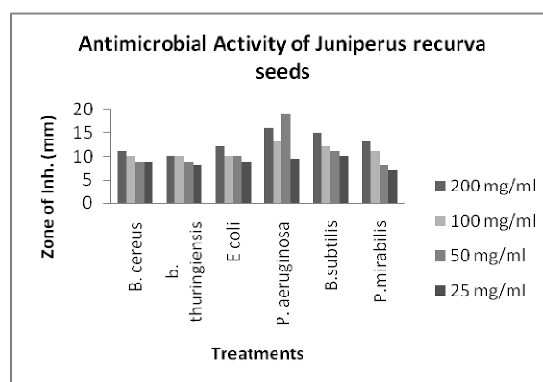

Figure 2
Antimicrobial Activity of (JRSO +JRSib) mixed

Antimicrobial assay was performed with all the plant extracts by agar plate diffusion method. Five antibiotics were used as standard drugs. Six different bacteria *B. cereus, B. thuringiensis, E. coli, P. aeruginosa, B. subtilis* and *P. mirabilis* are used for the assay. The antimicrobial activity was assayed by measuring the diameter (Zone of Inhibition in millimeters) ZOI of different extracts on the agar disc plate. The plant extracts were divided into two parts viz. leaves(JRL1 and JRL2 mixed) and seeds (JRSO and JRSib)

since they are the respective plant parts. The different concentrations of the *Juniperus recurva* leaves and *Juniperus recurva* seeds used were 200mg/ml, 100mg/ml, 50mg/ml and 25mg/ml for each of leaves and seeds. The leaves and seeds are taken with no further division for the ease of comparison of different plant parts to the standard antibiotics. The standard antibiotics used are Gentamicin (GEN^{10}), Ciprofloxacin (CF^{30}), Chloramphenicol (C^{30}), Cephotaxime (CTX^{30}) and Tetracycline (TE^{30}).

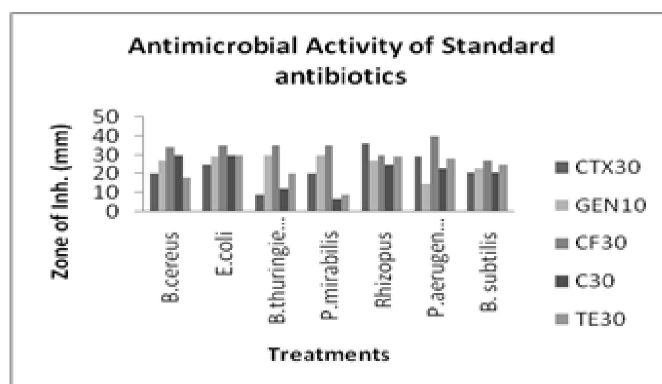

Figure 3
Antimicrobial Activity of Standard antibiotics against different bacteria

Both the *Juniperus recurva* leaves and seeds showed the concentration dependence antimicrobial activity towards the bacteria used with the highest ZOI being 17 mm for the *Juniperus recurva* leaves at 200 mg/ml. The ZOI at the highest concentration i.e. 200mg/ml against *B. cereus, B. thuringiensis, E. coli, P. aeruginosa, B. subtilis* and *P. mirabilis* are 17mm, 15mm, 10mm, 10mm, 15mm and 12 mm for leaves respectively and 11mm, 10mm, 12 mm, 16mm, 15mm 13mm respectively for *Juniperus recurva* seeds (Fig: 1 and Fig: 2) The gradual decrease in concentration shows the similar decrease in ZOI from 200mg/ml to 25mg/ml , the observation of which is represented in Fig: 1 and Fig: 2. The highest ZOI among the five standard antibiotics used is of CF30 which is 34mm, 35mm, 35mm, 35mm, 40mm, and 27mm against *B.cereus, E.coli, B.thuringiensis, P.milabilis, P. aerugenosa* and *B. subtilis* respectively (Fig:3). The other observations are represented in Fig: 3. *The P. mirabilis* and *B. thuringiensis* are found to be less susceptible towards the standard drugs in comparison to other bacteria mentioned and in the same way *P. aeruginosa* and *E.coli* are found to be less susceptible towards the antibacterial effect of *Juniperus* leaves and *B.cereus*and *B. thuringiensis* is found to be less susceptible towards the extracts from seed of *Juniperus recurva.*

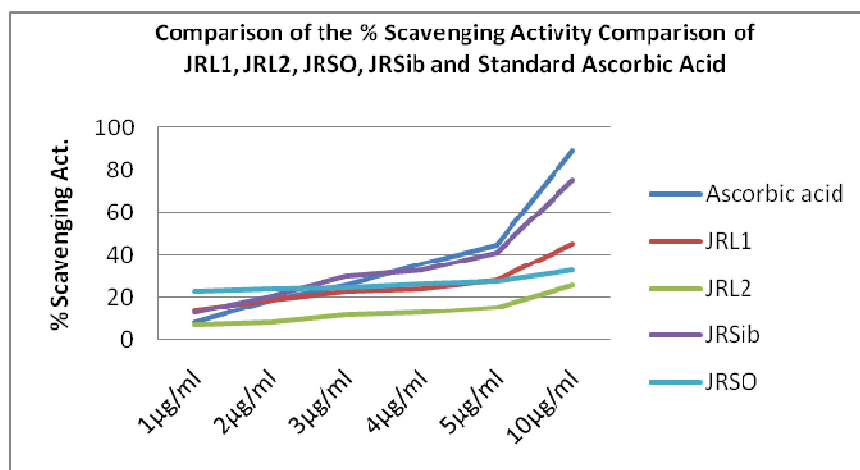

Figure 4
Comparison of the % Scavenging Activity Comparison of JRL1, JRL2, JRSO, JRSib and Standard Ascorbic Acid

The percentage DPPH scavenging activity of different extracts of *Juniperus recurva* ; JRL1, JRL2, JRSO and JRSib are compared with the standard Ascorbic acid and studied (Fig: 4). The % scavenging activity is measured at different concentrations of the extracts ; 1mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml and finally 10 mg/ml. The comparison in the Fig: 4 showed that the percentage scavenging activity is increased with the increase in the concentration being highest at the maximum concentration used i.e. 10 mg/ml corresponding to the value of 89.25%, 45.01%, 25.74%, 75.09% and 32.81% of Ascorbic acid, JRL1, JRL2, JRSib and JRSO respectively. Similarly the IC-50 value was calculated from the graphs of the Regression analysis and found to be 5.62µg/ml of Ascorbic acid 21.42 µg/ml of JRL2, 6.34 µg/ml for JRSib, 25.07 µg/ml for JRSO and 11.48 µg/ml for JRL1. In turn, these free radicals can start chain reactions and can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. Antioxidants compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. These compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Screening of plants for this is done by measuring the antioxidant activity by various in vitro activities such as DPPH method, Nitric Oxide method, etc and in vivo models using rats and mice. Free radicals that are commonly used to assess antioxidant activity in vitro is 2, 2-diphenyl-1-picrylhydrazyl (DPPH). So higher the % scavenging activity, higher the antioxidant activity and higher the anticancer property it may possess. Screening of the different plant-parts of *Juniperus recurva* resulted to be weakly active against MCF-7(Breast Cancer) cell lines. The cytotoxic effect or the inhibitory effect of these extracts is compared with standard Standard (Dexorubicin). The percentage Inhibition is found to be concentration dependent. The IC50 values calculated were 40±0.06 µg/ml for JRL1 and 50±0.06 µg/ml for JRL2, JRSO and JRSib as compared to standard Dexorubicin i.e. 1.56±0.05µM. From this finding only weak activity can be attributed to all different plant extracts used. The following results were observed and the cytotoxicity against MCF7 cell lines were studied.

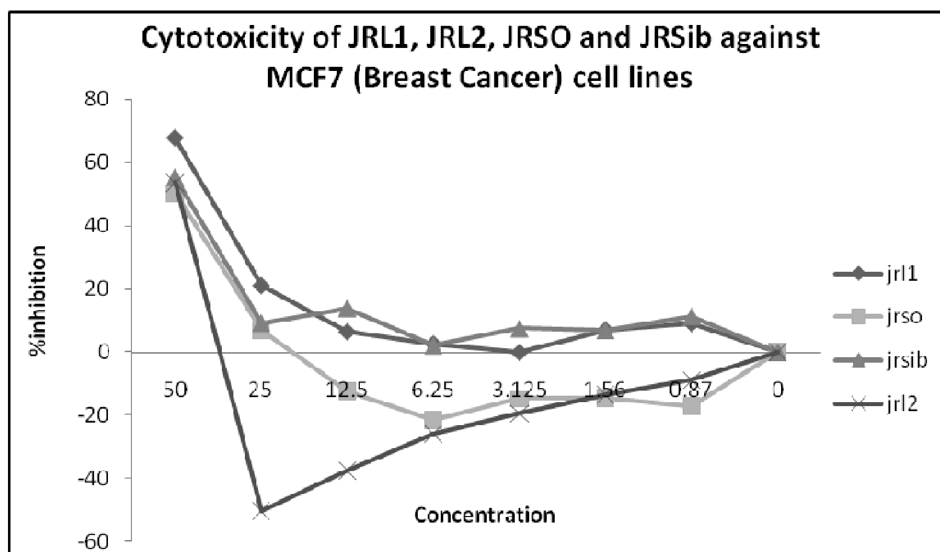

Figure 5
Cytotoxicity of JRL1, JRL2, JRSO and JRSib against MCF-7 (Breast Cancer) cell Lines

JRL1 showed 67.82% inhibition at 50µg/ml which was the highest dose of the extract used and then it gradually decreased and again slightly increased at 1.56 µg/ml and 0.78 µg/ml (Fig:5) for second-least and the least concentration utilized. This might be because of synergic effects of the different compounds present in the extracts, but the increase in %inhibition in the lower dose might be significant and could be attributed to the fact that the synergic effect of the interfering compounds or molecules is also decreased with the decrease in concentration which might have accounted to slight rise in the %inhibition at the lower dose of JRL1. In case of JRL2, the inhibitory effect is decreased significantly from 50µg/ml to 25µg/ml. The concentration dependence of the inhibitory effect was found to be prevailed in case of JRSO and JRSib somehow. The extract JRSO show a similar significant decrease from the highest dose to second highest dose accounting a decrease of %inhibition from 50.40% to 6.77%, but on contrary to this figure JRSib has again showed a gradual decrease in %inhibition up to 6.25 µg/ml concentration then a slight gradual rise the lower concentrations. The slight rise in the %inhibition at the lower concentration used might again, as in case of JRL1 can be described as because the apprehending compounds in the extracts might as well decrease. The similar pattern of inhibition in the two different extracts suggest that the synergic effect that is actually interfering with the inhibitory effect might be higher in the higher dose, from which we can infer that if we can isolate pure compounds with bioassay guided extraction, there is a possibility to get the higher inhibitory effect against MCF7 cells from the plant *Juniperus recurva*. Separation of chemical constituents by column chromatography followed by spectrometric analysis would reveal the active components present in this plants. Our works are currently under progress towards that direction too. The study of anticancer properties of natural products of *Juniperus recurva* provide a high diversity of chemical structures with specific biological and medicinal activity which are potent inducers of caspase-dependent programmed cell death (apoptosis) in malignant MB231 breast cancer cells and further elucidation revealed that Deoxypodophyllotoxin (DPT) isolated from *Juniperus* concomitantly inhibited cell survival pathways mediated by the MAPK/ERK and NF B signaling pathways within hours of treatment [39]. The other medicinal plants found in Nepal

are also found to have cytotoxic effect against different cancer cell lines. For example, Cell viability assay of plant extracts from *Berberis aristata* showed a significant cytotoxicity to MDA-MB-231 and U-87 MG human cancer cell line compared to NIH/3T3 standard embryonic fibroblast cell lines of mouse[40]. Similarly, Withanone from *Withania somnifera* (Ashwagandha) has been identified to have p53-activating tumor-inhibiting property[41]. Ashwagandha leaf powder was non-toxic and anti-tumorigenic in mice assays and caused an abrogation of mortalin-p53 interactions and reactivation of p53 function [42].

CONCLUSION

The result of phytochemical analysis revealed that the active principles responsible for the antibacterial activities are the presence of alkaloid, coumarin and glycosides. The results supports that the selected plant extracts possessed significant antioxidant activities. From the analysis discussed above, it can be concluded that the different extracts of plant *Juniperus* possess anticancer properties against MCF7 (Breast Cancer) cells. Further experiments and extraction of the pure compounds might possibly open the door to finding a more potent constituents from the plant *Juniperus recurva* as the activity of the pure compounds would be higher than the extracts because a mixture of compounds are present in extracts and their collective interfering or synergic effects.

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