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Research Article

Nutritional and Anti-Nutritional Study of Juniperus Excelsa (M.Bieb) of Ziarat, Balochistan, Pakistan

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Abstract

The ecological entity and juristic personality of River Ganga traced from the standpoint of the legal status vis-à-vis the reverJuniper forest of Ziarat, Balochistan, Pakistan is the second largest forest in the world. Ziarat has world oldest Juniper trees. The Juniper forest of Ziarat is in a very bad shape by over- grazing, excessive cutting and overexploitation by the local people. The berries were powdered and used to analyze the nutritional and antinutritional compositions; like (Alkaloids, Flavonoids, Terpenoids, Saponins, Pectin, Total Phenols and Steroid contents) were reported in different concentration. Also reported the composition of proteins and carbohydrates, some vitamins (A, B1, B3, and C) were found in varying concentration. These all chemical compounds obtained the responsible for nutritional and antinutritional values. The all chemical compositions suggested that the berries of Juniperus excelsa has the cheap source of nutritional compounds and also have a strong source of anti-oxidant compounds. The presences of these compounds justify the medicinal uses of these berries.

Introduction

Commonly known as the coniferous plants, Junipers belong to genus Juniperus of the family Cupressaceae The Genus Juniperus is monophyletic [1,2] that consists of almost seventy species [3] spread all over Africa and the Northern Hemisphere [4]. Juniperus is the third largest genus among the conifers found in the world [3] Plants belonging to this genus grow slowly [5] and can live as long as up to 2000 years [6]. These coniferous plants can be found in all sizes ranging from small flat shrubs to giant forest trees. One thing that stands Junipers out in other plants is that they can survive in different sites enduring extreme and rapid fluctuations in temperature. They can grow in the arid places where other plants cannot survive [3]. There is a wide range of wood and non-wood products created with Junipers, which are important for societies of people [7,8] . The wood produced by Junipers is scented and anti-decay. It has a large usage for producing furniture, paneling as well as barriers [9,10]. Furthermore, the coniferous plants are a great source to get fuel wood that produces little ash and smoke when is on fire [11]. A large amount of essential oil exemplifies Junipers' wood, berries, needles, and seeds, and is used in making of some pharmaceutical products [12] Additionally, Junipers contain a large quantity of triperthen, resin, tannin, lignin, flavanoid and tannin [13]. An assortment of Juniper species is used in the preparation of different herbal medicines for the treatment of different ailments. For example, Junipers' roots make the best cure for cough, rheumatism, and tuberculosis whereas the leaves and cones are important for several antiseptic usages. In general, Juniper berries are known as safe medication, which also serves as the best urinary antiseptic, and can treat arthritis and heal wounds [14]. Unfortunately, the Ziarat juniper forest is at a huge risk of devastation because of over-grazing, excessive cutting and over-exploitation by the rural population for household and medicinal purposes [15]. Reported that Micro-propagation is an effective technique and also optimized different protocols of Junipers. This paper optimized the protocols for in-vitro micro-propagation of J. excelsa for its conservation, afforestation and restoration of Juniper forest of Ziarat.

Materials and Methods

Nutritional analysis of juniper berries

Estimation of Protein

Ten grams of the sample was weighed and transferred into a Kjeldahl flask. Four tablets of Kjeldahl catalysts (tablet contain 1 g of Na₂SO₄ and 0.5 g of selenium) were added. Concentrated H₂SO₄ (20 ml) and glass beads were introduced to avoid bumping on heating. The flask was set in the fume cupboard; heated gently immediately and then continue heating until a slight charring begin to clear and the mixture become colourless. The heating process was approximately one hour. The flask was allowed to cool to room temperature and slowly washed the long neck flask with 20 ml of distilled water into 500 ml distillation flask. Distillation: Pieces of hot cleaps were added into the flask and connected up to the splash head and water cooled condenser. NaOH solution (5%, 4 ml) was added in the dropping funnel and 50 ml of 2% boric acid into the 250 ml receiving flask with methyl red indicator. The dropping funnel tap was opened slowing to allow the 5% NaOH to enter the boiling flask. The distillation flask was heated to boiling with water passing through the condenser. Distillation continued until about 150 ml was collected in the receiving flask. The content of the flask was titrated with 0.1 M HCl until pink end point. The reading was recorded and blank was ran along the same treatment [16].

$$\text{Nitrogen \%} = \frac{((VS-VB) \times \text{normality of HCl} \times 0.014 \times 100)}{(\text{Weight of sample in gram})}$$

VS = Volume of acid used to titrate sample.

VB = Volume of acid used to titrate blank.

N = 0.1 M of acid.

% crude protein = N% × conversion factor (6.25)s



Estimation of Carbohydrate

In a 50ml of conical flask 1g of ground sample was taken and 10ml of distilled water was added with a continued shaking for 2min. After that 13ml of 52% of per-chloric acid (HOCLO_3) were added the solution was kept on a shaker for 20min. It was then filtered through Whatman 44. The contents were diluted to make it 250ml. The absorbance was measured in a visible spectrophotometer at 630nm [17].

Carbohydrate % = 25 x absorbance of dilute sample wt. of sample x absorbance of dilute standard.

Estimation of Vitamin A (Retinoid)

The standard methods of AOAC were followed. Ground sample 0.5g was taken and 2.5ml of 12% alcoholic potassium hydroxide was added in a flask and kept in water both at 60 °C for 30min. It was filtered and transferred to a separating funnel containing 10-15ml of petroleum ether and were shaken gently. After that the lower aqueous layer was taken out in another separating funnel and discarded. The upper layer petroleum ether which contained carotenoids was collected. It was repeated until the aqueous layer became colourless. For removing the excessive moisture in the petroleum ether few drops of anhydrous sodium sulphate was added. The final volume was noted. The absorbance was measured through visible spectrophotometer at 450nm while petroleum ether was used as blank.

Estimation of Vitamin B1 (Thiamin)

To 5g of ground sample 50ml of ethanol sodium hydroxide was added and mixed well. It was filtered, 10ml of filtrate was taken and homogenized with 10ml of potassium dichromate and a coloured solution was developed. The absorbance was read in through visible spectrophotometer at 360 nm [18].

Estimation of Vitamin B3 (Niacin)

5g of ground sample was homogenized with 50ml of 1N sulphuric acid, it was shaken well for 30min. Before the filtration 03 drops of ammonia solution were added, 10ml of filtrate were added 5ml of 0.02N, H_2SO_4 also added. The absorbance was read through visible spectrophotometer at 470nm [22].

Estimation of Vitamin C (Ascorbic Acid)

In a 25ml of conical flask 1g of ground sample was taken with 10ml of oxalic acid (0.05M)-EDTA (0.02M) solution and allowed standing for 24h. After that the solution was filtered through Whatman filter paper No.1 (054um). Then 2.5ml of each sample was transferred to a volumetric brown flask, after which 25ml of oxalic acid (0.05M)-EDTA (0.02M) were added. Meta phosphoric acid was added with acetic acid (0.05M), sulphuric acid (5% v/v) solution (1ml) and ammonium molybdate solution (2ml) and distilled water to achieved 25ml of volume. The absorbance was measured by a visible spectrophotometer at 760nm [19].

Anti-Nutritional Analysis of Juniper Berries

Quantitative Phytochemical Analysis

The quantitative phytochemical analyses to estimate the concentrations of alkaloids, flavonoids, terpenoids, saponins, pectin and tannins in the berries of J excelsa.

Estimation of Alkaloids

Than 5g of ground sample was taken a 250ml beaker. To acetic acid 20%, ethanol was added it was covered and allowed to stand for 4 hrs. Then this was filtered and extract was concentrated and was used a water bath to evaporate one-quarter of the original volume. In extract the ammonium solution was added drop-wise until the precipitation was completed. The solution was allowed to settle then the precipitate was collected by filtration and weighted the solution [20].

$$\% \text{ Alkaloids} = \frac{\text{Final weight of sample}}{\text{Initial weight}} \times 100$$

Estimation of Flavonoids

Ground sample 5g was taken into a 250ml titration flask to it 100ml of 80% methanol was added at room temperature and were shaken in an electric shaker for 4 hrs. Then

filtered the solution through Whatman filter paper No.1, this process was repeated twice. The filtrate was later transferred into a crucible and evaporated to dryness over water bath and weighted [21].

Estimation of Terpenoids

100g of ground sample were taken separately and were soaked in alcohol for 24 hrs. Then filtered the solution and the filtrate was extracted with petroleum ether, the ether extract was treated as total terpenoids.

Estimation of Saponins

The ground 20g sample was taken, 200ml of ethanol was added it was heated over a hot water both for 4h with continued stirring at 55 °C, 200ml of 20% ethanol was added in residue for re-extract. The extracts were reduced to 40ml over water bath. The reduced extract was transferred into a 250ml separating funnel. 20ml of diethyl ether was added and was shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. This purification process was repeated thrice. With 10ml of 5% aqueous sodium chloride the 60ml of normal butanol extracts were washed twice the remaining solution was heated in water bath. After the evaporation the sample was dried in the oven. The saponin content were calculated in percentage. (Obadoni & Ochuko, 2001)

$$\text{Saponins \% in sample} = \frac{\text{Weight of final filtrate}}{\text{Weight of Sample}} \times 100$$

Estimation of Pectin

In a beaker 40ml of distilled water was added along with 5g of ground sample and was boiled gently for 1h. The solution was transferred to a 50ml of volumetric flask and was diluted up to the mark by distilled water and was shaken well. Then filtered into a 50ml of volumetric flask. After which 10ml of filtrate was taken into an 80ml of beaker. 30ml de-ionized water and 1ml of sodium hydroxide was added with continue stirring then was allowed to stand overnight. After that 5ml of 1N acetic acid was added with a constant stirring and was left to stand for 5min. Then 1N and 2.5ml of calcium chloride solution was added and was allowed to settle for 1h and then the solution was boiled for 1min. The solution was filtered through Whatman filter paper No.41 and was washed with hot de-ionized water until the solution became free from chloride. The residue was transferred to the previously weighted watch glass. The watch glass along with the residue was placed in water bath and was dried in the oven at 100 °C until a constant weight was obtained [22].

Estimation of Total Phenols

5g of ground sample was taken in a 250ml titration flask and was added 100ml n-hexane twice for 4 hrs each. The filtrate was discarded for fat free sample preparation. Then 50ml diethyl ether was added twice, was heated for 15min each and was cooled up at room temperature then filtered into a separating funnel. For separating the aqueous layer from the organic layer about 50ml of the 10% NaOH solution was added twice and was shaken well. The 25ml de-ionized water was used to wash three times then was added 10% HCl solution and 50ml dichloro methane (DCM) twice to acidify up to pH 4 in the separating flask. Then the organic layer was collected, dried and weighted [23].

Estimation of Steroid Contents

Distilled waster 50ml was added to 2.5g of ground sample. Then filtered and extract solution was washed with 3ml of 0.1M NaOH pH 9 and later was mixed with 2ml of chloroform and 3ml of acetic anhydride and also was added two or three drops of concentrated H_2SO_4 . The absorbance was measured in a visible spectrophotometer at 240nm [24].

Functional group determination with FTIR Infra-red spectroscopy

Infra-red spectra of the crude samples were recorded to detect various functional groups responsible for biological activities. Perfectly dried powder/paste of the extracts was placed on the sample chamber of FTIR spectrophotometer and the spectra were recorded in the range of 3600-600 cm^{-1} on Nicolet Avatar330. FTIR spectrometer. Important absorption frequencies appeared in functional group region as well as fingerprint region of the spectra was noted down.

Statistical analysis

The experimental data were measured as mean \pm Standard Error of Mean (SEM).

Three measurements of each protocol were calculated and data were analyzed using one-way ANOVA of “Graph pad prism”. Results were recorded as significant if P-values is <0.05.

Result and Discussion

Chemical composition of *J. excelsa* berries was studied for the first time its nutritional contents such as protein, carbohydrate and vitamins (A, B1, B3 and C) were estimated in (Table 1). The berries were found to be rich in vitamin B1 and vitamin A while vitamin B3 and C were also found in good quantity this might be the reason Juniper berries have been used for many health benefits and United States Department of Agriculture, 2012 reported vitamin A and C are found in carrots, in 73g of carrot contain 160% vitamin A and 4% vitamin C. These are more in quantity than reported in *Cymbopogon citratus* [25]. Berries are used in intestinal infections, circulatory problems, edema, bladder and kidney disorders and various kinds of inflammation and also used in several medications. *J. excelsa* berries were checked for the presence of alkaloids, flavonoids, terpenoids, saponins, pectin, total phenols and steroid contents as anti-nutritional studies. In Table 2 showed the high contents of flavonoids were recorded 21.8 ± 7.85 . This amount was higher than that reported from *Cymbopogon citratus* 4.76 ± 0.02 [25]. Flavonoids are the secondary class of metabolites with significant antioxidant and chelating properties. Antioxidant depends on the structure and substitution pattern of hydroxyl groups [25]. Many studies reported that flavonoid inhibits varieties of cancers in animals. According to Linus Pauling institute Cornell university researchers, 2017 reported that flavonoids provide strong anti-proliferation effects against liver and colon cancer cells. Flavonoids also helped in lower blood pressure and cholesterol, helped to reduce heart diseases. Berries extracts are demonstrated (Figure 1) which shows the various functional groups. It was observed that 3274.90 cm^{-1} and 3251.76 cm^{-1} peak represents Amines (-NH) functional groups. The peaks at 2923.88 were recorded showing the presence of CH and OH bonds. The presences of broad peak show carboxylic acid (C=O,C-O) between $1689.53-779.19 \text{ cm}^{-1}$ wavenumber. The presence of the amine and carboxylic acid shows the presence of protein in the sample as zwitter ion amino acids. The broadness of peak also suggested the high amount of proteins in the sample. The CH and OH groups can be the interruption of methanolic solvent used for separation or can be an integrated alcoholic groups present in the sample. These fundamental functional groups were recorded in the FTIR result. FTIR spectra of methanolic extract of berries of *J. excelsa* are showed various functional groups which indicate the presences of antioxidant properties [26-30].

Table 1: Estimation of Nutritional Contents of berries of *J. excelsa*.

S.No.	Nutritional Contents/ Phytochemicals	Mean± SD	Std. Error SEM
1	Proteins	3.80±1.22	0.223
2	Carbohydrates	1.21±0.16	0.0965
3	Vit-A	7.34±0.57	0.329
4	Vit-B1	15.27±3.9	2.257
5	Vit-B3	0.62±0.04	0.0265
6	Vit-C	0.20±0.006	0.0035

Values were mean ± standard deviation n=3.

Table 2: Estimation of Antinutritional Contents of berries of *J. excelsa*.

S.No.	Antinutritional Contents/ Phytochemicals	Mean± SD	Std Error (SEM)
1	Alkaloids	4.90±2.75	1.377
2	Flavonoids	21.8±7.85	4.536
3	Terpenoids	8.00±5.44	2.722
4	Saponins	17.3±2.51	1.453
5	Pectin	2.43±1.54	0.7731
6	Total Phenols	8.33±1.52	0.8819
7	Steroid contents	2.76±0.53	0.3062

Values were mean ± standard deviation, n=3.

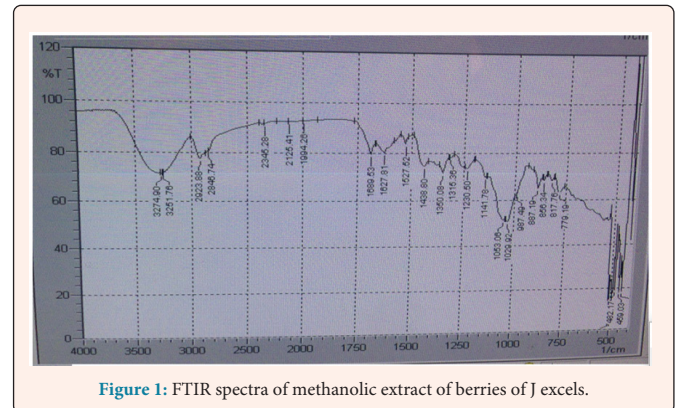


Figure 1: FTIR spectra of methanolic extract of berries of *J. excelsa*.

Conclusion

Concluded that the berries of *J. excelsa* have potential sources of nutrients and antinutrients that mean berries can be used as good physiological state of man and animals.

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