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Phytochemical screening an antioxidant activity of seeds of *Saussurea costus* (Lalc.) Lipsch: an indigenous medicinal plant of Neelum Valley Azad Kashmir

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Abstract:The purified ingredients of medicinal plants have advantageous curative potentials. These constituents may be different in different parts of the plants. *Saussurea costus* (Falc.) Lipsch. is well known wild medicinal plant. The root of this plant has been used in indigenous medicine to cure various diseases particularly in Pakistan, China and India. The aim of this study was to analyze the seeds of *Saussurea costus* for physicochemical, phytochemical substances and antioxidant activity. Many phytochemicals (alkaloids, phenols, glycosides, terpenoids, saponins, flavonoids, carbohydrates, saponins and protein) were screened out in water and chloroform extracts while acetone and methanol were not fittest solvents for extraction. Protein was present in a high amount of 10.9 mg/g whereas carbohydrate and phenolic compounds were detected in a low amount, due to the low amount of phenolic compounds seeds showed poor antioxidant activity. The phytochemicals screening may form the basis for drug development in the future.

Keywords: *Saussurea costus*, Pharmacognosy, Phytochemical, Antioxidant, Seed, Neelam valley

INTRODUCTION

Saussurea costus (Falc.) Lipsch belonging to the family Compositae, the species is known by various synonyms *Saussurea lappa* (Decne.) Sch.Bip., *Aplotaxis lappa* Decne., *Theodorea costus* Kuntze and *Saussurea lappa* Decne in the literature (TPL, 2013) found throughout the Himalaya region, in Pakistan (Kashmir), India (Utter Khand, Himachal Pradesh) (Fig. 1) and widely cultivated in China for medicinal purposes. Root has diverse uses and medicinally important. Due to the unsystematic collection of its root, this species has been enlisted in an endangered category (FOP, 1971-2020). The root of *Saussurea costus* has been broadly studied worldwide; the study covers ethnomedicinal uses, phytochemicals screening and pharmacology. Due to the presence of different active compounds and good pharmacology, the plant has great therapeutic potential used against tumor, dyspepsia, diarrhea, fever, asthma, leprosy, and rheumatism and skin diseases. possesses a range of bioactivities such as antidiabetic, antifungal, hepatoprotective, antiparasitic, anthelmintic and antimicrobial,

immunostimulant, anti-inflammatory and antihepatotoxic (Khalid et al, 2011; Yashvanth et al, 2010; Zahara et al, 2014). Phytochemical compounds are natural plant-based compounds present in leaves, roots, bark, stem, flowers, fruits, and seeds depending on the nature of the species. These compounds are the chief source of new drug discovery (Newman & Cragg, 2012) considered as therapeutic agents to overcome health troubles categorized into different classes alkaloids, phenolic compounds, and terpenoids have good pharmacological effects such as anti-viral, anticancer, antibacterial, anti-inflammatory, antimalarial, antioxidant and inhibition of cholesterol production (Lallianrawna et al, 2013). The compounds like polyphenols phenolic acid and flavonoid which are good antioxidant scavenge free radicals (hydroperoxide, peroxide and lipid peroxyl) and inhibit the oxidative mechanism. Medicinal herbs are considered as a good antioxidant and chief source of antioxidant compounds (Mahdi-Pour et al, 2012). Herbal medicinal products have authenticated as ordinary medicines by European legislators. The concurrent appliance of different scientific disciplines with the purpose of getting information on drugs from all aspects functional science that deals with the biochemical, biologic, and fiscal features of natural drugs and their components" (Tyler et al, 1988). The existence of a phytochemical of curiosity may guide to its further isolation, cleansing and categorization unless it gets attention as a new pharmaceutical product (Lalitha and Jayanthi, 2012). The seeds of *Saussurea costus* have not been studied yet in the same way as the root has been studied. The purpose of the present study was to screen out phytoconstituents and pharmacognostic features in the seeds of *Saussurea costus* because of some seeds seemed to have potential as a source of constructive drugs.

MATERIAL AND METHODS

Seeds Collection and Plant Identification: An adequate quantity of seeds of *Saussurea costus* was collected from Lawat village, Neelum valley Azad Kashmir and plant specimen was identified through searching Flora and online database (FOP, 1971-2020; TPL, 2013) Plant specimen was submitted in karachi university Herbarium (KUH), Centre for Plant Conservation, University of Karachi, Karachi

Physicochemical study: Extractive value under different solvents (acetone, chloroform, methanol and water) moisture content and ash value was studied as per standard techniques (Vermani *et al*, 2010; Perveen *et al*, 2020).Extract preparation Collected seeds were cleaned and shade dried at room temperature then grind till obtained fine powder. 10 gm seed powder was separately dissolved in acetone, chloroform, ethanol and water solvents then were placed on stirrer for 48 hours. After 48 hours solutions were sieve with the help of filter paper and put on an open place unless completely dry, the dried extract was used for further tests.

Qualitative Phytochemical screening: For phytochemical assessment standard methods were applied to find out the presence or the absence of plant metabolites in the seeds. Alkaloids were checked by Mayer' s test, Hager's test and Wagner's test, Carbohydrate by benedict's test, Protein and Amino acid by Biuret test and Millon's test (Resch and Swift, 1960). Phenolic compound by Lead acetate test and Ferric chloride test. Fixed oil by spot test and Saponin by Foam test, Glycoside by Boorntrager's test (Evans, 1997; Raaman, 2006; Perveen *et al*, 2020; Ali *et al*, 2018).

Quantitative Phytochemical analysis: Quantitative phytochemicals estimation was studied by applying standard methods. Such as Proteinby Bradford (Bradford, 1976), Carbohydrate by Anthron reagent and Phenolic compounds by Folin reagent (Singleton *et al.,* 1965; Ali *et al.*, 2018). DPPH Radical Scavenging Activity

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2, 2-diphenyl-1-picrylhydrazl (DPPH) solution (95µl, 300µM) in Ethanol is mixed with test solution (5µl, 500µM). The reaction is allowed to develop for 0.5 hours at 37ºC. By using multiple reader Spectra Max 340 at 517 nm absorbance is observed. Where color changes from violet to pale yellow. The percentage of Radical Scavenging Activity is determined by contrast with a DMSO holding control. The decreasing in concentration that foundation in the early DPPH concentration by 50% is defined as IC_{50} value. The EZ-Fit Enzyme kinetics software program was used to calculate the IC_{50} values of compounds. (Perrella Scientific Inc. Amherst, MA, USA). Ascorbic acid and N-acetylcysteine and BHA was taken as the reference compounds (Lee *et al*, 1998)

RESULTS

Seed morphology of Saussurea costus (Falc.) Lipsch: Brown with black spots, cylindric, 6-8 mm, apically wrinkled, apex with a crenulate or dentate crown. Pappus straw-colored, 1.2-1.7 cm.

Physicochemical analysis Saussurea costus (Falc.) Lipsch: Physicochemical analysis of the seeds of *Saussurea costus* (Falc.) Lipsch revealed the total ash value as 20.09%, water soluble ash was 32%, acid insoluble as 60% Moisture content and extracting values in different solvent also evaluated and results shown in Table 1.

Phytochemical Screening of Seeds of Saussurea costus (Falc.) Lipsch: Phytochemical analysis of seed extracts of *Saussurea costus* showed that alkaloid, carbohydrates, protein, phenolic compounds terpenoids and fixed oil were present in chloroform and methanol extract, flavonoids and glycoside were present in all solvent extracts. Results are shown in Table 2. The highest percentage of protein in the sample of seeds (10.9 ± 0.13 mg/g) was observed followed by carbohydrate (0.380 ± 0.07) phenolic compound (0.320 ±0.05).

Table. 1 Physicochemical Analysis of seeds of *Saussurea costus* (Falc.) Lipsch.

S.No	Physicochemical parameters	*Saussurea costus* seeds
1.	Physical state of ash	Powder
2.	Colour of ash	Greyish white
3.	% of loss on drying	8%
4.	% of ash content	20.9%
5.	Water-soluble ash	32%
6.	Water-insoluble ash	68%
7.	Acid soluble ash	40%
8.	Acid insoluble ash	60%
9.	Extractive value in acetone	1.4%
10.	Extractive value in chloroform	5.%
11.	Extractive value in methanol	2.8%
12.	Extractive value in water	7%

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Table. 2 Phytochemical screening of seeds of *Saussurea costus* (Falc.) Lipsch.

S.No	Phytochemical tests	Solvents			
		Acetone	Chloroform	Methanol	Water
1.	Alkaloids Hager's test Wagner's reagent Mayer's reagent	 _ _ _	 + _ _	 _ _ _	 + _ +
2.	Carbohydrate Benedict's test Fehling test	 _ _	 + +	 _ _	 + +
3.	Protein and amino acid Biuret test Millions test	 _ _	 + +	 _ _	 _ +
4.	Phenolic compounds Lead acetate Ferric chloride test	 _ _	 + +	 + +	 + +
5.	Flavonoids	+	+	+	+
6.	Glycosids Salkowsk's test Keller-kilani test	 + +	 + +	 + +	 _ +
7.	Terpenoids	_	+	_	+
8.	Saponins Foam test	_	+	_	+
9.	Fixed oil Spot test	+			

Key: + = Present - = Absent

Table. 3 Quantitive phytochemical estimation of seeds of *Saussurea costus* (Falc.) Lipsch.

Extract	Amount mg/g
Total carbohydrate	0.380 ±0.07
Total protein	10.9 ±0.13
Total phenols	0.320 ±0.05

Table 4: Antioxidant assays (IC_{50} values) of different extracts of seeds of *Saussurea costus* (Falc.) Lipsch.

Extract	Conc. Mg	% Inhibition	IC_{50}+SEM mg/ml	Result
Std N-acetylcysteine	0.5	95.2	0.018 ±0.002	Poor
Std Ascorbic acid	0.5	91.2	0.0042 ±0.001	Poor

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Fig.1: A & B Seeds of *Saussurea costus* (Falc.) Lipsch. C. plant

DISCUSSION

Phytopharmacopoeial appraisals for any plant resources should be developed to sanction the quality manage chemists to confirm and approve of the materials (Govil and Singh, 2009). Successful detection of bioactive phytochemicals from any part of plant part is mainly reliant on the kind of the solvent used in the extraction procedure. Thus we need to attempt as many solvents as feasible during the screening of plant parts for phytochemicals analysis (Lalitha and Jayanthi, 2012). The residual ash amount and composition after combustion of plant part/material vary significantly according to the part of the plant and age etc. The amount of the ash also varies with time and from organ to organ. Generally, Ash represents the inorganic part (phosphates, carbonates, silica and silicates) present in the crude powder of the plant, while water and acid solubility is also checked for this purpose. Lowest moisture value is beneficial for any plant material against pathogen if it is conserved (Vermani *et al*, 2010; Chanda, 2014). DPPH is a free radical compound which has a scavenging skill for antioxidants samples and confirms fine absorbance at 517nm. The ascorbic acid (Vitamin C) is generally taken as a standard antioxidant and it has a strong DPPH scavenging potential. In the plant extracts, numerous studies confirmed a linear relationship among phenolic compounds and antioxidant activity (Juan and Chou, 2010; Tona *et al.,* 1998). The seeds extract was measured for antioxidant activity by the ability to scavenge DPPH free radicals comparing with N-acetylcysteine and vitamin C Table 4. Lesser IC_{50} value reveals higher DPPH radical scavenging activity. Our results revealed that the extract showed no considerable DPPH activity with the percentage of inhibition 95.2 and 91.2 which are high values. It reduced the IC_{50} value of 0.0042, 0.018mg/ml N-acetylcysteine and ascorbic acid, respectively.

Seeds of *Saussurea costus* were subjected to physiochemical and phytochemical screening (qualitative and quantitative) which help to identify the plant material and status of the mixture present in the crude powder. We were used four different solvents (acetone, chloroform, methanol and water) for the extraction. In this study the physiochemical results showed with different value such as moisture content 8%, extractive value by different solvents (Acetone 1.4%, chloroform 5%, methanol 2% and water 7%) and ash 31% shown in (Table 1). Many phytochemical compounds such as Alkaloids, carbohydrate, protein, phenols, glycosides, terpenoids and saponins were detected in the seeds powder of *Saussurea costus* in chloroform and water extracts. Flavonoids were detected in all used solvents, whereas acetone and methanol are not good solvents for the extraction for other compounds present in the seeds of *Saussurea costus* because the extractive value in such solvents was very low. Similarly percentage of water-soluble ash 50% was high in water and it was low 20% in acid.
All the results of aqueous and chloroform extracts show maximum compounds with a high amount of soluble extractable constituents compared to acetone and methanol. Alkaloid was only detected in chloroform and water extract by Hager's test. The presence of protein and amino acid was conformed through the Millions test in the chloroform and water extracts. Majority phytochemicals except glycosides and phenolic compounds were absent in acetone and methanol extract all results showed in Table. 2. Preliminary quantitative phytochemical test results revealed the amount of some phytochemicals like carbohydrate, protein and phenolic compound shown in Table 3. The highest percentage of protein in the sample of seeds (10.9 ± 0.13 mg/g) was achieved followed by carbohydrate (0.380 ± 0.07) phenolic compound (0.320 ±0.05). Carbohydrate and phenolic compounds were found in a very low amount. Qualitative analysis revealed their appearance it gives approximate idea for their quantity present.

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

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