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Research Paper

PHYTOCHEMICAL INVESTIGATION, CHARACTERIZATION AND ISOLATION OF MEDICINAL PLANT: *SAUSSUREA OBVALLATA*

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Saussurea is a genus of about 300 species of flowering plants in the Asteraceae family, with the highest diversity in the alpine regions of the Himalayas. Saussurea obvallata, also known as Brahmakamal in Sanskrit, is named after Brahma, the Hindu God of creation. The species is the state flower of Uttarakhand. The objective of the study was to isolate phytoconstituent from S. Obvallata flower ethanol extract by column chromatography and characterization by various spectroscopic techniques such as ultraviolet-visible, infrared, nuclear magnetic resonance, and mass. The S. Obvallata flower ethanol extract was prepared by successive solvent extraction using Soxhlet apparatus. The ethanol extract was found rich in phytoconstituents with the help of chemical tests, and also it was found effective against pain, inflammation, and pyrexia in experimental animal studies. Hence, ethanol extract was selected for isolation of important plant constituents by column chromatography. The column was carried out with the different solvent system used in particular ratios. It justified the effect of flower extract in the treatment of pain, swelling, and inflammation and also as an antioxidant because flavonoids are a group of therapeutic active compounds due to their supreme antioxidant action. It was concluded that S. Obvallata flower ethanol extract was rich in plant constituents and also has a number of therapeutic active constituents which suggest the plant used for other activities.

Key Words: Brahmakamal, saussurea obvallata, Asteraceae, phytochemistry.

INTRODUCTION

The natural products, either in the form of pure compounds or as standardized plant extracts, provide infinite opportunities for drug discoveries. However, only 10% of the world's biodiversity has only been explored, and it represents wide scope for the discovery of new molecules from plant sources.[1] With this view, we have selected saussurea obvallata plant for our research. It belongs to asteraceae family. Commonly it is named as brahmakamal. The requirement for plant based medicines and other herbal healthcare products, including pharmaceutical active agents, functional foods as dietary supplements, cosmetics substances etc. are increasing constantly in both developing and developed countries. During the last twenty or thirty years, rapid rise in the rate of infectious diseases, antibiotic resistances in microorganisms and untoward effects of synthetic antibiotics are on the rise. The evaluation in phytochemistry and identification of new bioactive compounds from plants has renewed the popularity of herbal medicines [2]. Saussurea obvallata is an endemic herb of the Himalayan region (encompassing the Indian Himalayan Region, northern Burma and Southwest China). Ethanobotanically it is a hermaphrodite herb which achieves an average height ranging from 5–10 cm. Flowers bloom in season of mid-monsoon in between the rocks and grasses of the hillside. Flower heads are purple in color, hidden from view in layers of yellowish green papery bracts, which provide protection from the cold mountain environment. The flowers can be seen till the season of mid-October, after which the plant perishes, becoming visible again in April. In

Uttarakhand, Saussurea obvallata is found in the regions of Kedarnath, the Valley of Flowers, Hemkund Sahib and in Tungnath [3-6]. Besides religious value, the plant is extensively used by local people for preparation of traditional ayurvedic medicines by cultivating. The flowers, rhizomes and leaves are very important part for treatment of bone ache, intestinal ailments, cough/cold and urinary tract problems. The rhizomes in particular are utilized as antiseptic and for healing cuts and in treatment of bruises [7-10]. In the Tibetan system of medicine, the plant is used in the treatment of Hemiplegia and cerebral ischaema [11]. Almost all the phytochemical constituents like alkaloids, carbohydrates saponins, coumarin, flavonoids, glycosides, steroids, and tannins were reported to be present in Saussurea obvallata [12].

The aim of this study was to explore the presence of phytochemical constituents present in different solvent extracts of flower samples of Saussurea obvallata collected from Dehradun, Uttarakhand State, India.

Material and methods

Collection and identification of plant: Whole plants were collected from Dehradun, Uttarakhand, India, during the month of June 2017. Further authentication specimen forwarding Jodhpur. Taxonomic and Ethnomedicinal identification of the collected plant has been done by Mr. S.L. Meena [Deputy Director of Botanical Survey of India, near khem ka kuaon, nandan van, Jodhpur- (324008)]. The shade dried flower samples were cleaned, washed, dried and pulverized to coarse powder using an electric grinder.

Chemicals: The solvents for the extraction process were of analytical grade chemicals were used from college laboratory.

Physicochemical Study: Physicochemical parameters like foreign matter, moisture content, PH value, Ash value, Extractive values were recorded for different samples.

Determination of percentage extractive: Leaf powder (5 gm) was macerated with 50 ml of respective seven solvents in closed flasks for 24 hr and was frequently shaken with 6 hr time intervals and was allowed to stand for 24 hr. After filtration, 25 ml of the filtrate was evaporated to dryness and dried at 105 °C and weighed. Percentage of soluble extractive was calculated with reference to the air dried drug.

The percent extractive of each solvent extract of S. Obvallata was calculated by

Percent extractive = (weight of dried extract÷weight of dried plant material) × 100

Phytochemical analysis: The phytochemical constituents present in S. Obvallata flower were carried out with different solvent extracts (i.e. hexane, chloroform, Ethyl Acetate, ethanol and water) as mentioned using standard methods. Preparation of plant extract: The dried flower powder of S. Obvallata flower was extracted separately in different solvents (i.e. hexane, chloroform, Ethyl Acetate, ethanol and water) by keeping them in respective solvents for 24 hours and was then filtered using Whatman filter.

Extraction and fractionation:

1 kilogram of powdered drug was packed in soxhlet apparatus and continuously extracted with petroleum ether (60-80°C) to defat the drug. Petroleum ether was removed from the powdered defatted drug, which was then extracted with ethanol (95%). The alcoholic extract thus obtained was further fractioned with hexane and ethanol. The solvents were removed from each extract and fraction by distillation and the last traces of solvent being removed under reduced pressure. The extracts and fractions were weighed and their % value was recorded and also the physical appearance, color and odor was evaluated and recorded and thereafter, were stored in refrigerator for further experimental work.

Packing of column: Wet packing method was adopted for packing the column. Slurry of activated silica gel (neutral) was prepared in solvent system and was poured into the column with the help of a hollow glass cylinder. The column was previously filled with solvent system. While pouring the slurry the column was continuously tapped with a rubber cork so that a compact column was formed devoid of any air bubble. The solvent was eluted thereafter at a steady rate till solvent head remained about 2-3 cm above the column.

Preparation of sample: About 10 grams of extract was mixed with 50 grams of silica gel for CC (60-120 mesh) and a very small amount of an appropriate solvent. This mixture was triturated in a pestle till a homogenous and dry free flowing mixture was obtained.

TLC study of column fractions

The column fractions were analyzed by TLC method on pre-coated silica gel plates using solvent system "toluene:ethyl acetate:methanol:water in ratio 7:6:5:2," detection by sunlight and iodine vapours.

RESULTS AND DISCUSSION

Preliminary Pharmacognostic Studies:

The physicochemical characters of this species has not been studied earlier, therefore, the purpose was investigate these properties. The observations presented here will be helpful for future reference and distinguishing this species from other related species.

The preliminary phytochemical screening was carried out to assess the qualitative chemical composition of crude extract from *saussurea obvallata* using precipitation and coloration reaction to identify the major natural chemical groups. General reactions in this analysis revealed the presence or absence of these compounds in the crude extracts tested. Summary of preliminary phytochemical screening of different extract is depicted in Table-1.

Table 1: physical properties of *Saussurea obvallata:*

Studied parameters	Observation (% w/w)
Loss on drying	12%
pH	5.8
Total ash value	5.5%
Acid insoluble ash value	2.64%
Water soluble ash value	4.44%
Alcohol extractive value	4.87%
Water extractive value	7.67%

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Table 2: characteristics of *Saussurea obvallata* Extract:

S.NO.	EXTRACT	PHYSICAL APPERANCE	COLOUR	ODOUR
1.	Hexane extract	Syrupy liquid	Light green	Aromatic
2.	Chloroform extract	Viscous mass	Dark green	Slightly aromatic
3.	Ethylacetate extract	Semisolid	Dark brownish	Aromatic
4.	Ethenolic extract	semisolid	Light greenish brown	Slightly aromatic
5.	Aqueous extract	Viscous mass	Light yellowish	Non aromatic

Table 3: Phytochemical profile of extract and of *Saussurea obvallata*:

Test for	Hexane fraction	Ethanolic fraction
Alkaloid	+	+
Carbohydrate	+	+
Steroids	+	+
Phenol	_	+
Flavonoids	+	+
Glycosides	_	+

(+) = POSITIVE, (-) = NEGATIVE

Qualitative phytochemicals tests showed presence of alkaloids, carbohydrate, glycosides, flavonoids, steroids, phenols, were identified in various extract.

1. In case of hexane fraction steroids, alkaloids, carbohydrates and flavonoids were present.
2. In case of ethanolic fraction alkaloids, carbohydrates, phenols, flavonoids, and glycoside, were present.

Table 4: Percentage of phytoconstituents present in extract of *Saussurea obvallata*:

Constituents present	Quantity of phytoconstituents (%)
Alkaloids	9.31%
Phenols	4.32%
Flavonoids	7.58%
Tannins	3.62%

Charecterization and identification of compounds:
COMPOUND 41

GC-MS analysis Total ion chromatogram of Sample 41 fraction was acquired at the condition reported below. It revealed 4 peaks showing the following retention times (tR): 01.40 min, 01.80 min, 01.91 min, and 2.20 min. Chromatographic analysis of the main peak (tR 1.91 min) revealed a molecular ion at m/z 680 (M+) that can be proposed for a molecular formula $C_{24}H_{24}$. Indicative ions were also revealed at m/z 757(M+ -C_{40} H_{71}), 842(M+ - C_{36} H_{74}). Fragment ion at m/z 680 agrees to the loss of ion (C_{40} H_{71}) of 757 mass. Ion peak at

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m/z 718 showed the loss of $C_{36}H_{74}$. In general, this mass ionization pattern indicates a 680 molecular mass compound of $C_{24}H_{24}$ formula, putatively biological amines.

TLC Studies of *saussurea obvallata*

Table 5: TLC Studies of Ethanolic extract and Ethanolic fraction of *S. obvallata.*

Fraction/ Extract	Solvent system	No. of spots	TLC profile	
			R_f value	Color
Ethanolic extract	Toluene:Ethylacetate:Methanol: Water(7:6:5:2)	8	0.94;0.90;0.81; 0.74;0.69;0.61; 0.59;0.45	Dark green, green, green, faint green ,pale green, yellow , light yellow
Ethanolic Fraction	Ethylacetate: methanol: toluene: water (5:4:6:5)	5	0.90;0.86;0.73; 0.54;0.32	Dark green, green, light green, light yellow, brown

Table 6: TLC studies of Hexane fraction of *S. obvallata.*

Fraction/ Extract	Solvent system	No. of spots	TLC profile	
			R_f value	Color
Hexane fraction	Hexane:DCM: Ethyl acetate: Methanol (10:5:2:3)	6	0.76;047;0.41 0.29;0.27;0.22	Dark yellow, Dark brown, Dark brown, Very light green. Light yellow, light brown

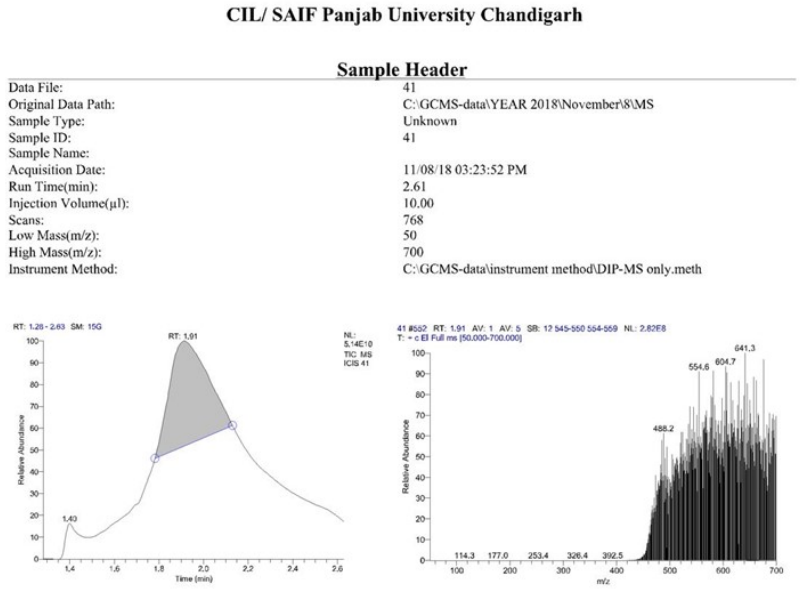

Fig. 1 – mass spectrum of compound no (41)

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1H NMR (CDCl3): δ 3.80 (2H, d, J = 10.4 Hz, H2-1), 2.11 (2H, m, CH2), 1.50 (2H, m, CH2), 1.46 (2 H, m, CH2) 1.38 (2H, m, CH2), 1.24 (26H, brs, 13 x CH2), 0.87 (3H, t, J= 6.5 Hz, Me-19)

The ^{1}H NMR spectrum of **41** was consistent with the proposed structure and clearly showed a one-proton downfield multiplet at d 6.58 and a one-proton downfield doublet at d 5.85 (*J* = 7. 69 Hz) assigned to vinylic H-22 and H-24, respectively. A one-proton broad signal at d 3.31 was due to 3 a-carbinol proton.

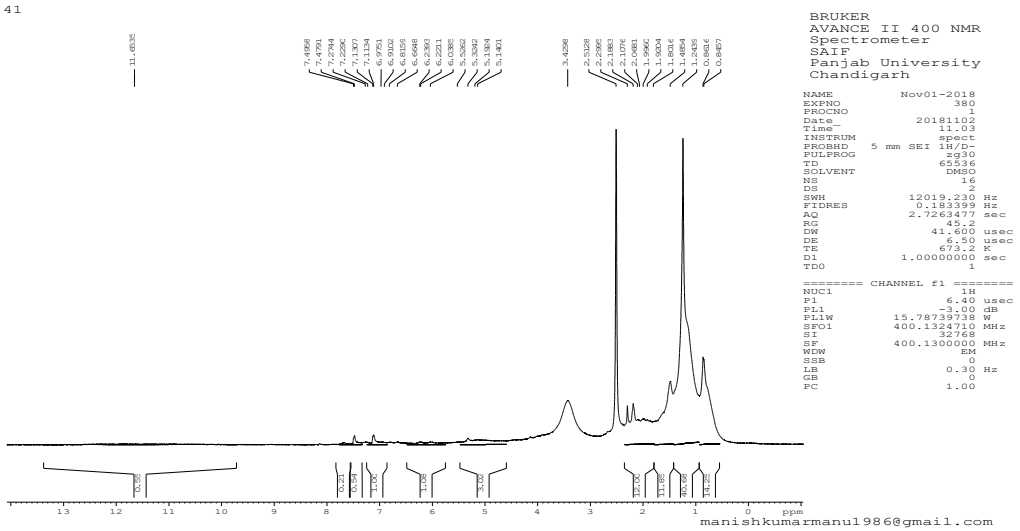

Fig. 2– NMR spectrum of compound no (41)

Table 7: spectrum data of compound no. 41

IR (KBr) ν_{max}cm $^{-1}$	^{1}H NMR (in ppm)	Mass Spectra	Ultra Violet Wave Length
3435.3 (-OH) Stretch 1712.7 (-C=O) stretch 1378.6 (-NH) Stretch 2850 (Ar-CH) Stretch 2919.2 (Ar-CH_3) Stretch	CH_3 (3H, t 1.2439-1.8016) CH_2 (2H, d 2.0681-2.1079) NH (1H, S 3.4298) Ar-H (5H.m 6.0385-6.8159) Ar-H (4H, m 6.9102-7.4958) -COOH (1H, S 11.6535) Ar-OH (1H, d 5.3242-5.5262)	Base Peak= 641.3	Λ max= 259 Absorbance=0.813

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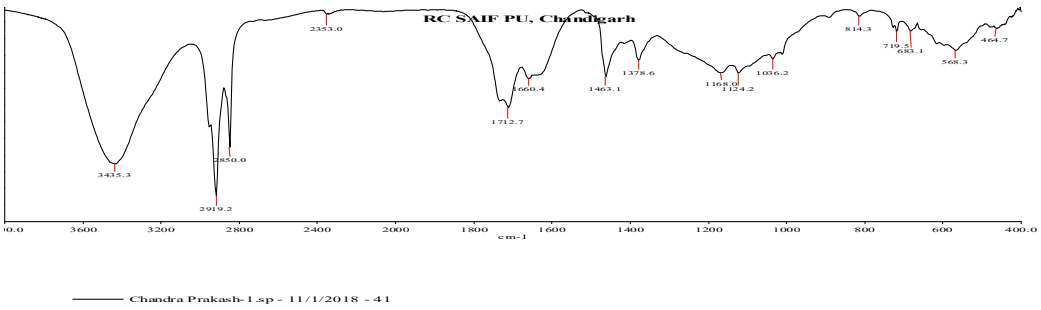

Fig. 3-IR spectrum of compound no (41)

COMPOUND 42

GC-MS analysis Total ion chromatogram of Sample 42 fraction was acquired at the condition reported below. It revealed 3 peaks showing the following retention times (tR): 01.29 min, 01.48 min and 1.66 min (Figure 1). Chromatographic analysis of the main peak (tR 1.48 min) revealed a molecular ion at m/z 206 (M+) that can be proposed for a molecular formula C_9H_9. Indicative ions were also revealed at m/z 308(M+ -C_{14} H_{20}), 355(M+ - C_{20} H_{31}). Fragment ion at m/z 206 agrees to the loss of ion (C_{14} H_{20}) of 308 mass. Ion peak at m/z 308 showed the loss of $C_{14}H_{20}$. In general, this mass ionization pattern indicates a 206 molecular mass compound of C_9H_9 formula, putatively biological amines.

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CIL/ SAIF Panjab University Chandigarh

Sample Header

Data File:	42
Original Data Path:	C:\GCMS-data\YEAR 2018\November\8\MS
Sample Type:	Unknown
Sample ID:	42
Sample Name:	
Acquisition Date:	11/08/18 03:31:29 PM
Run Time(min):	2.69
Injection Volume(µl):	10.00
Scans:	793
Low Mass(m/z):	50
High Mass(m/z):	700
Instrument Method:	C:\GCMS-data\instrument method\DIP-MS only.meth

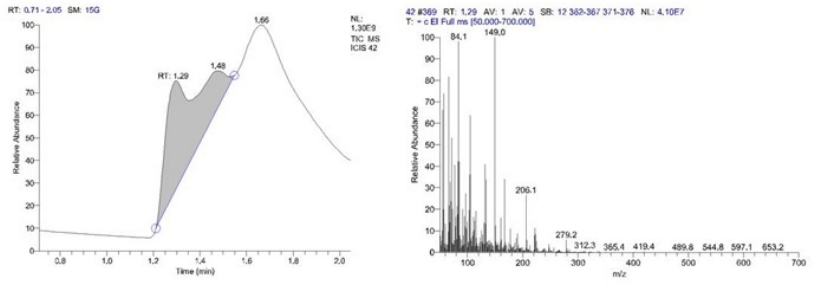

Fig. 4– mass spectrum of compound no (42)

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1H NMR (CDCl3): δ 3.63 (2H, m, H2-1), 2.53 (2H, m, CH2), 1.98 (2H, m, CH2), 1.56 (2H, m, CH2), 1H-NMR (CDCl3) δ 1.371–0.97 (4H, m, H-4, 8), 2.122−.58 (5H, m, H-5, 7, 9), 2506 10 of 14 2.842−.95 (2H, m, H-3, 11), 4.82 (1H, s, 6-CH2b), 4.90 (1H, s, 10-CH2b), 5.27 (1H, d, 1.72 Hz, 10-CH2a), 5.07 (1H, d, 1.56 Hz, 6-C=CH2a), 5.49 (1H, s, 2-CH2b), 6.22 (1H, s, 2-CH2a).

The ^{1}H NMR spectrum of **42** was consistent with the proposed structure and clearly showed a one-proton downfield multiplet at d 6.58 and a one-proton downfield doublet at d 1.98 (*J* = 7. 69 Hz) assigned to vinylic H-22 and H-24, respectively. A one-proton broad signal at d 3.63 was due to 3 a-carbinol proton.

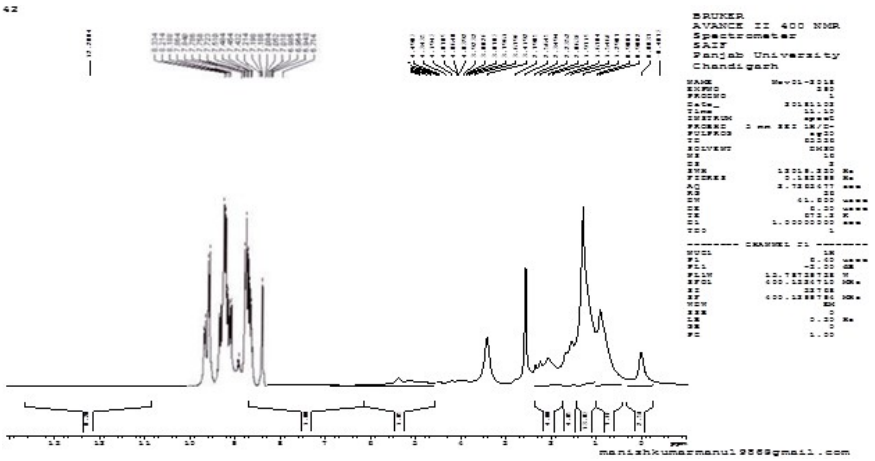

Fig. 2– NMR spectrum of compound no (42)

Table 8: spectrum data of compound no. 42

IR (KBr) ν_{max}cm $^{-1}$	^{1}H NMR (in ppm)	Mass Spectra	Ultra Violet Wave Length
3446.3 (-OH) Stretch 1734 (-C=O) stretch 1095 (-CN) Stretch 2850.1 (-CH) Stretch 2919.5 (-CH_3) Stretch 2331.1 (-C=C) Stretch	CH_3 (3H, d 1.279-1.321), CH_2 (2H, d 2.019-2.127), Ar-H (4H, m 6.7746-7.8217) Ar-H (5H, m-7.9172-8.217) SH (1H, S 4.8641) COOH (1H, 511.3814)	Base Peak= 149.0 M+ Peak=206.1	Λ max= 279 Absorbance=0.913

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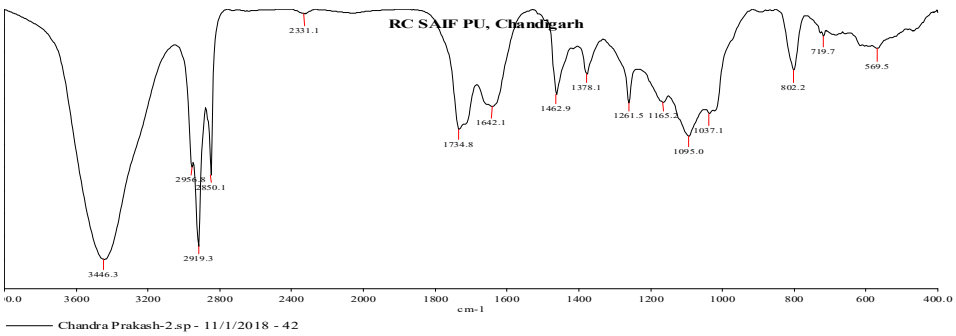

Fig. 6– IR spectrum of compound no (42)

CONCLUSION

The plant *Saussurea obvallata* have been found to be source of medicinal agents based on their use in traditional medicines In present work the taxonomic identification of collected plant by Mr. S.L. Meena. In brief preparation of plant material by different techniques such as drying, grinding, sieving.

Extraction of plant material by using different solvents ethanol, hexane, chloroform, ethylacetate and water and isolation of compound by chromatography has been done. Purification of isolated compounds has been done and characterization of isolated compounds has been done through spectral techniques like IR, NMR, Mass spectrum. This chapter deals with description, review of literature and finding of phytochemicals and pharmacological studies of *Saussurea obvallata.*

Physical parameters were- Loss on drying 12%(w/w), Total ash value 5.5%(w/w), acid in soluble ash value 2.64%(w/w), alcoholic extractive value 4.87%(w/w) and water extractive value 7.67 %(w/w) and quantitative estimation value is obtained By method alkaloid (9.31%), phenols (4.32%), flavonoids (7.58%), tannins (3.62%).

The TLC study of extract and fractions represents that ethanolic extract in the solvent system Toluene: ethyl acetate: methenol: water (7:6:5:2) gave 8 spot (the rf values were 0.94, 0.90, 0.81, 0.74, 0.69, 0.61,0.59 and 0.45) and Ethenolic fraction in the solvent system Ethyl acetate : methanol : toluene: water (5:4:6:5) gave 5 spot (0.90,0.86,0.73,0.54 and 0.32) ,Hexane fraction in the solvent system Hexane: DCM :ethyl acetate: methanol (10:5:2:3) gave Sunlight 6 spots (0.75, 0.47, 0.41, 0.29, 0.27, and 0.22).

Two compounds (41 and 42) were isolated by silica gel column chromatography from ethenolic extract of *Saussurea obvallata.*

On the behalf of characterization of isolated compounds I concluded that the alkaloids and steroids moiety were present in ethanolic and hexane fraction respectively.

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