WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 1, 1270-1284. Research Article ISSN 2277–7105

EVALUATION OF IN VITRO ANTI-INFLAMAMTORY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF SAUSSUREA OBVALLATA

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Article Received on
21 Nov. 2024,

Revised on 11 Dec. 2024,
Accepted on 01 Jan. 2025

DOI: 10.20959/wjpr20251-34913

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ABSTRACT

Plants are essential to the conventional medicinal framework. Saussurea obvallata is a medicinal plant local to Uttarakhand that has extraordinary cultural and medicinal value. It may be found in the Himalayan zone at high elevations of 3000- 4800 meters.[1] Various research on phytochemical components and their traditional utilize have been distributed, illustrating their therapeutic benefits. All of the accessible information approximately the traditional and regional utilization of Brahma Kamal are included in this manuscript.[1,2] To assess anti inflammatory activity of Saussurea obvallata by in- vitro methods. Phytochemical screening where perform according to standard convention. The anti inflammatory activity of extract (methanol) where inspected using by utilizing protein denaturation test and HRBC membrane stabilizing test. According to WHO report, expanding request of medicinal plants is expected to reach 5 trillion by the year 2050 in international market. So there are different opportunities for pharmaceutical industry. Inflammation is a biological response of the immune system that can be activated by a assortment of variables, counting pathogens, damaged cells and harmful compounds. These factors may initiate acute and/or chronic inflammatory reactions in the heart, pancreas, liver, kidney, lung, brain, intestinal tract and reproductive system, potentially leading to tissue damage or disease.

INTRODUCTION

A very significant cultural plant, Saussurea obvallata is an imperiled medicinal herb found in the Himalayan region's high altitude good countries. The inhabitants of Garhwal and Tibet broadly produce Brahma Kamal blossoms, leaves, and rhizome for religious, enriching, traditional, and medical purposes.[3] They have been utilized to treat bone pain, wounds,

mental health issues, stomach related diseases, colds, and coughs. Among the most important and imperiled varieties of this kind is Brahma Kamal, which is utilized for traditional, strict, and ornamental uses.[4] The objective of this consider is to survey and get it the consistent investigate conducted hence far on Saussurea obvallata , taking into account its substantial protective point, phytochemistry, pharmacology, ethno-medicinal uses, pharmacogenetic illustration, and scaled down proliferation.

Bestowed with rich botanical wealth, the individuals of India have likely the oldest, wealthiest and most assorted cultural traditions in the utilize of medicinal plants.[5] At higher altitudes of the Western Himalayan ranges (expanding from ca. 500 m to snow-clad peaks), a assortment of vital and endemic medicinal plants are found, making it a biodiversity-rich region.[6] Records show that these medicinal herbs have been in utilize for treating diseases since ancient times. The advent of herbal renaissance in recent times has led to heavy extraction of these medicinal plants from the wild. This, helped by loss of habitat by deforestation and intemperate grazing pressure in high-altitude pastures in the whole Himalayan locale threatens the survival of a few vital medicinal plants, one of them being Brahma Kamal.

Additionally, it is utilized to remedy heart disorders (with roots and leaves), mental disorders (using seeds), and wounds, cuts, and boils (using dried leaves). It is utilized to treat cerebral ischemia and limb paralysis in the Tibetan medical system.[7] Since the rhizospheric microflora is significant for the germination, advancement, and survival of plants in their particular specialties, This work was carried out to separate the fungal community of the Saussurea obvallata rhizospheric zone in arrange to decide the part of rhizospheric microflora in Saussurea obvallata growth. Saussurea is a very imperative genus of the family Asteraceae due to a variety of reasons, and most species of this genus are well investigated at the universal level.[8–11] Be that as it may, small is known almost S. obvallata. It is an endemic herb of the Himalayan locale and dispersed between 3000 and 4800m amsl. Commonly it is known as Brahma Kamal and is the state flower of Uttarakhand (India). It is a well known plant over Uttarakhand due to traditional, medicinal, ornamental and religious purposes.[12] It is utilized for the treatment of different diseases or disorders like paralysis, cerebral ischemia, wounds, cardiac disorders and mental disorders; a few individuals too utilize it as antiseptic, in healing cuts, etc.[12] Preliminary investigations around the phytochemicals found in S. obvallata (qualitative) have been detailed[13], and its mineral composition has been

described.[14] The present study, deals with the estimation of its total phenolic and flavonoid contents, as well as antioxidant and antimicrobial activities related with its extracts. Gas Chromatography-Mass Spectrometry (GC-MS) based analyses of crude methanolic extracts of S. obvallata leaves and flowers have been carried out for the identification of active component.

Fig.no. 1 flower of *S. obvallata*

Inflammation ordinarily happens when infectious microorganisms such as bacteria, viruses or organisms invade the body, reside in specific tissues and/or circulate in the blood.[15–17] Inflammation may too happen in response to forms such as tissue damage, cell death, cancer, ischemia and degeneration.[15,18–23] Mostly, both the innate immune response as well as the versatile safe reaction are included in the arrangement of aggravation.[15,19,23] The natural safe framework is the first defense mechanism against attacking microorganisms and cancer cells, including the action of various cells including macrophages, mast cells and dendritic cells. The adaptive immune systems includes the action of more specialized cells such as B and T cells who are responsible for eradicating invading pathogens and cancer cells by producing specific receptors and antibodies Various inflammatory mediators are synthetized and discharged during inflammatory responses of different types. Inflammatory substances are ordinarily separated to two primary categories: pro- and anti-inflammatory mediators. Nevertheless, a few mediators such as interleukin (IL)-12 have both pro and anti-inflammatory properties.[24]

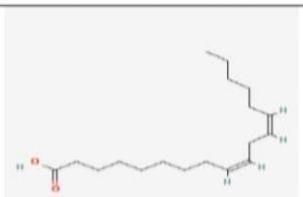
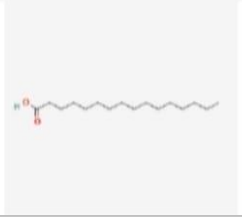
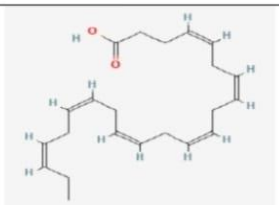
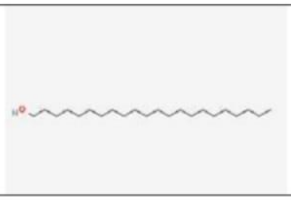
Among the inflammatory mediators and cellular pathways that have been extensively studied in affiliation with human pathological conditions are cytokines (e.g., interferons, interleukins and tumor necrosis factor), chemokines (e.g., monocyte chemo attractant protein), eicosanoids (e.g., prostaglandins and leukotrienes) and the potent inflammation-modulating transcription factor nuclear factor B. Inflammation is the immune system's reaction to

harmful stimuli, such as pathogens, damaged cells, toxic compounds, or irradiation[25], and acts by removing damaging stimuli and initiating the healing process.[26] Inflammation is therefore a defense mechanism that is imperative to health.[27] Usually, during acute inflammatory responses, cellular and molecular events and interactions efficiently minimize impending injury or infection. This relief process contributes to restoration of tissue homeostasis and determination of the acute irritation. Be that as it may, uncontrolled intense inflammation may become chronic, contributing to a variety of chronic inflammatory diseases.[28] Nowadays it is accepted that inflammation is part of the non specific immune response that occurs in response to any type of real injury and that the cardinal signs of inflammation can be explained by expanded blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular influx. In a few disorders the inflammatory process, which beneath ordinary conditions is self-limiting, gets to be continuous and chronic inflammatory diseases develop subsequently.

Inflammation (from Latin: inflammation) is part of the natural response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[29,30] The five cardinal signs are warm, pain, redness, swelling, and loss of function (Latin calor, dolor, rubor, tumor, and function malaesa). Inflammation is a nonexclusive response, and hence is considered a mechanism of intrinsic immunity, while adaptive immunity is specific to each pathogen Inflammation is a protective reaction including immune cells, blood vessels, and molecular mediators. The work of inflammation is to eliminate the initial cause of cell injury, clear out damaged cells and tissues, and initiate tissue repair. As well little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the living being. Be that as it may inflammation can moreover have negative effects.[31] As well much inflammation, in the form of chronic inflammation, is related with various diseases, such as feed fever, periodontal illness, atherosclerosis, and osteoarthritis. Aggravation can be classified as intense or constant. Acute irritation is the starting reaction of the body to hurtful jolts, and is accomplished by the expanded development of plasma and leukocytes (in specific granulocytes) from the blood into the harmed tissues.[31] A arrangement of biochemical occasions engenders and matures the inflammatory response, including the local vascular system, the immune system, and various cells in the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation, such as mononuclear cells, and involves simultaneous destruction and healing of the tissue.[32]

CHEMICAL COMPONENTS

Alkaloids, calcium, chromium, copper, ferrous ions, iron, lead, magnesium, manganese, minerals, nickel, phenol, proteins, saponins, steroids, strontium, tannins, terpenoids, zinc phenolics, flavonoids, sesquiterpenes, and lactones.[33,34]

Compounds	Molecular Formula	PubChem CID	Chemical Structure
Linoleic acid	C18H32O2	5280450	
Palmitic acid	C16H32O2	985	
Doconexent	C22H32O2	445580	
Methyl palmitate	C17H34O2	8181	
Stearic acid	C18H36O2	5281	
1-Docosanol	C22H46O	12620	
Gondoic acid	C20H38O2	5282768	
Henicosanoicacid	C21H4202	16898	
Methyl oleate	C19H3602	5364509	

METHODS

Collection of plant material

Plant material (leaves) was collected from Chandoli forest in karad, Maharashtra india, during in months of April. The new material was kept in perforated polybags and quickly brought to laboratory.

Preparation of extract

The leaf sample of S. Obvallata were washed, dried, & powdered utilizing an electric blender. Two solvents methanol & water were utilized for the extraction of phyto-constituent from dried powdered.

Methanolic extracts of leaves are prepared by the maceration procedure. In this method, the granular powdered of S. obvallata along with the solvent is kept in a stoppered container. This is permitted to remain at the room temperature for approximately 3–7 days with repeated blending till the solvent matter is dispersed. The blend is filtered to obtain the extract. At that point the extract was dried by utilizing shedding dry. This strategy is appropriate and convenient for the plant material that is thermolabile.[35,36]

PHYTOCHEMICAL SCREENING OF S.OBVALLATA

Chemical considers taking after established procedures are utilized to screen for and distinguish the active ingredients in the flower extract. Each solvent extract was analyzed in 100µl for each test.

Test for Saponins: A test tube containing the extract was filled and shook briskly. It was accepted that the production of stable foam indicated the presence of saponins.

Test for Phenols: Extract combined with 2 milliliters of a 2% FeCl3 solution. Phenols were denoted by a blue-green color.

Test for Tannins: Extract combined with 2 milliliters of a 2% FeCl3 solution. Tannins were signified by the color black.

Test for Terpenoids: Chloroform (2 ml) was combined with the extract. After that, 2 milliliters of concentrated sulfuric acid were included and gently shaken. The interphase's reddish-brown tones mean the presence of terpenoids.

Test for Flavonoids: A few drops of sodium hydroxide arrangement were included to the extract, causing a bright yellow color to show up. It turns colorless when diluted acid is included, demonstrating the presence of flavonoids.

Test for Glycosides: Two milliliters of glacial acetic acid with a few drops of 2% FeCl3 were combined with the extract and at that point exchanged into a second tube that held two milliliters of concentrated sulfuric acid. Glycoside is present at the interphase when a brown ring is present.

Test for Protein: When a small amount of strong nitric acid is included to the extract, proteins are present since a yellow tint forms.

Test for Alkaloids: After each extract was separately dissolved in diluted HCl and filtered through saturated picric acids, the presence of alkaloids was shown by the production of a brown precipitate.

Test for Steroids: When extract is combined with two milliliters of chloroform and cautiously added H2SO4, a reddish-brown tone shapes, implying the presence of steroids.[37]

Extracts	Saponin	Phenol	Tannins	Terpenoids	Flavonoids	Glycosides	Proteins	Alkaloids	Steroids
Cloroform	+	-	+	+	+	-	+	+	+
Methanol	+	+	-	+	+	+	+	+	+
Ethanol	+	-	-	+	+	+	-	+	-
Distilledd water	+	+	-	+	+	+	+	+	+

NOTE - Positive (+) show the presence of constituents; whenever negative (-) show the absence of constituents in the flower extract

ANTI -Inflammatory Activity BY Using IN VITRO TEST

1. Membrane stabilizing test

The HRBC method utilized was utilized for the estimation of anti-inflammatory activity in vitro and procedure adopted with slight modifications. Blood was collected from healthy human volunteers who did not expended NSAID for two weeks was obtained. At that point it was blended with rise to volume of sterilized Alsever solution. This blood solution was centrifuged at 3000 rpm and the pressed cells isolated. The stuffed cells were washed with isosaline solution and 10%v/v suspension was with isosaline. The reaction mixtures 4.5ml comprises of 2ml hypotonic saline (0.25% NaCl) + 1ml 0.15M phosphate buffer (pH 7.4) + 100 µl test solution (1000µg/ml)+ 0.5ml of 10% rat RBC in normal saline. The mixture was incubated at 56^{o}c for 30 minutes. The tubes were cooled beneath running tap water for 20 minutes. The mixture was centrifuged for 3000rpm for 10min and the absorbance of the supernatant was measured by spectrophotometer (Equiptronics) at 560nm. Diclofenac sodium was utilized as standard drug. The percentage hemolysis was evaluated by the assuming the hemolysis produced for in the control as 100%.[47]

Percentage Stabilization = 100−(O.D. of Test − O.D. of Sample) O.D. of Control X100

Table no. 1 Anti-inflammatory activity of different Concentration Extract by membrane stabilizing study.

Compound	Concentration	O.D.	Mean	% Inhibition
blank		0.56 0.58 0.62	0.58	
Standard (diclofenac sodium)	1000µg /ml	0.32 0.30 0.31	0.31	46.55%
	2000µg/ml	0.28 0.25 0.22	0.25	56.89%
	3000µg/ml	0.20 0.18 017	0.18	68.96%
S.obvallata (extract)	1000µg/ml	0.50 0.52 0.54	0.52	10.34%
	2000µg/ml	0.51 0.47 0.48	0.48	17.24%
	3000µg/ml	0.35 0.38 0.34	0.35	39.65%

2. Protein denaturation method

The reaction mixture (10 mL) comprised of 0.4 mL of egg albumin (from fresh hen's egg), 5.6 mL of phosphate buffered saline (PBS, pH 6.4) and 100 µL of distinctive concentration test. Comparable volume of double-distilled water served as control. At that point the mixtures were incubated at (37 0c ±2) in a incubator for 15 min and at that point heated at 70^{o}c for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the concentration was utilized as reference drug and treated additionally for determination of absorbance.

The percentage inhibition of protein denaturation was calculated by utilizing the following formula,

% Inhibition = C –T/C

Where, T = absorbance of test sample C = absorbance of control

Table no. 2 Anti-inflammatory activity of different concentration Extract by protein denaturation method

SR.NO	Sample code	Concentration (µg/ml)	Protein denaturation assay					
			Absorbance at 660nm					
			Test 1	Test 2	Test 3	Mean	% Inhibition	IC50 (µg/ml)
1	Control	-	1.54	1.54	1.54	**1.54**	-	77.11
2	Standard(Diclofenac Sodium)	20	1.43	1.44	1.43	**1.43**	**7.14%**	
		40	1.20	1.20	1.22	**1.20**	**22.07%**	
		60	0.96	0.97	0.98	**0.97**	**37.01%**	
		80	0.72	0.72	0.71	**0.71**	**53.89%**	
		100	0.41	0.42	0.41	**0.41**	**73.37%**	
3	*S. obvallata* (extract)	20	1.42	1.44	1.40	**1.42**	**7.79%**	NE
		40	1.26	1.28	1.24	**1.26**	**18.18%**	
		60	1.02	1.02	1.04	**1.02**	**33.76%**	
		80	0.94	0.92	0.96	**0.94**	**38.96%**	
		100	0.83	0.81	0.85	**0.83**	**46.10%**	

RESULT AND DISCUSSION

An immune response that causes redness and swelling of an area on the body or conceivably tissues within the body are called inflammation. anti-inflammatory or antiphlogistic is the property of a substance or treatment that decreases inflammation or swelling. Anti

inflammatory drugs, moreover called anti-inflammatories. Phytochemical screening of extract showed the presence of Alkaloid, Flavonoid, Tannins, Saponin, Steroid. The extract at conc. 3000ug/ml appeared the highest % of inhibition of RBC lysis which was found to be 39.65% in HRBC membrane stabilization method. By utilizing the protein denaturation method, the extract showed 46.10%) of inhibition of protein denaturation at 100 ug / ml concentration. In this way, the extract of S. obvallata showed the anti inflammatory activity. Extract were proved anti-inflammatory action by utilizing membrane stabilizing activity that was compared to standard drug (diclofenac sodium) [By membrane stabilizing method]. since human red blood cell membranes similar to lysosomal membrane components, the avoidance of hypotonicity initiated HRBC membrane lysis was taken as a degree of anti-inflammatory movement of drugs. The results obtained illustrated that Methanolic extract of leaves of s. obvallata. At the distinctive concentrations of Extract appears the direct anti inflammatory activity as compared to standard Diclofinac Sodium. [by Protein denaturation method]

CONCLUSION

The scientific literature generally points to the phenols (flavonoids) and/or terpenoids as the fundamental anti-inflammatory agents. Of the two, the flavonoids have recently picked up noted interest in medicinal plants. Given all their anti-inflammatory properties, including their ability to associate with sugars to frame glycosides, which renders them water soluble**,** their wide run of activity, and their ability to in terpene at different levels of the immune response, phenols (primarily the flavonoids and the phenolic acids) show themselves as the main anti-inflammatory operators in crude plant extracts.

In conclusion, Extract were demonstrated anti-inflammatory activity by utilizing membrane stabilizing activity that was compared to standard drug (diclofenac sodium) [By membrane stabilizing method] At the different concentrations of Extract appears the moderate anti inflammatory action as compared to standard Diclofinac Sodium. [by Protein denaturation method].

These results may provide a strong basis for traditionally known medicinal properties of S. obvallata, numerous of which are too mentioned in India holy books. Screening and identification of components and systematic evaluation of their medicinal properties may provide clues for a potential drug, and its other bioactivities may give ground for these to be suggested for advance investigations.

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