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Unveiling antibacterial, antioxidant, phytochemicals of *Saussurea obvallata* and exploring it's antidiabetic effects

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Abstract
Saussurea obvallata belong to family *Asteraceae* of flowering, Aromatic and Herbal plants this genus also encompasses of 490 species this species are utilized in various countries for their medicinal properties, contributing to diverse traditional health care practises found in mountain terrain regions. *Saussurea obvallata* holds diverse indigenous medicinal practices. The blossoms, foliage and rhizomes of *s.obvallata* serves various traditional, spiritual, healing, and decorative functions. Our motive of study was to identify the antimicrobial activity of *S. obvallata* extracts was assessed against a panel of pathogens, including bacteria such as *Escherichia Coli, Campylobacter jejuni, staphylococcus aureus, Clostridium Spp* and *Pseudomonas Spp.* using agar well diffusion. Phytocontitutinal activity Furthermore, extract demonstrated potent antioxidant activity, as evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. These assays revealed the extracts' ability to scavenge free radicals effectively, highlighting their potential in combating oxidative stress-related disorders. assays of *obvallata* blossoms extract. Moreove the antidiabetic potential of S. obvallata extracts was assessed through α-amylase inhibition assays. Both extracts showed promising inhibition of these enzymes, suggesting their potential in managing diabetes and related complications by regulating blood glucose levels.In conclusion, *S. obvallata* extracts possess a rich phytochemical profile with significant antimicrobial, antioxidant, and antidiabetic activities.These discoveries highlight the promise of S. obvallata as a valuable natural resource for developing therapeutic agents against microbial infections, oxidative stress, and diabetes. Further research is warranted to isolate and characterize the active constituents responsible for these bioactivities and elucidate their mechanisms of action for future drug development endeavors.

Keywords: *Saussurea obvallata*, Antimicrobial, phytocontitutinal, antioxidant, antidiabetic, α-amylase

Introduction

It holds botanical significance with approximately 490 known species. (Pant. M, *et al*; 2013) [10] This genus has gained attention due to its diverse applications in traditional medicine across various countries (Schultz. F *et al*;2020) [17]. Among these species, Saussurea obvallata stands out for its remarkable antimicrobial, antioxidant, and phytochemical properties. The plant is a valuable resource, with its flowers, leaves, and rhizomes being utilized for traditional, religious, therapeutic, and ornamental purposes., (Abhay P. Mishra *et al*; 2018).

In recent years, scientific interest has intensified in exploring the medicinal potential of *Saussurea obvallata*. Studies have highlighted its antimicrobial activity, showcasing effectiveness against various pathogens. (Semwal P *et al*; 2013) [4] The plant's antioxidant properties add another layer of significance, as antioxidants play a crucial role in neutralizing harmful free radicals in the body (Panovska TK *et al*; 2005) [16]. Additionally, *Saussurea obvallata* exhibits a rich profile of phytochemicals, compounds known for their potential health benefits.

Understanding the multifaceted properties of *Saussurea obvallata* is not only essential for validating its traditional uses but also for exploring new avenues in pharmaceutical and healthcare applications. This research aims to delve into the intricate interplay of antimicrobial (Sharifi-Rad *et al;* 2016) [22, 23, 24, 25], phytochemical (Mishra *et al*; 2018), antidiabetic (Sati SC, April 2014). Antioxidant (Prabhakar Semwal, *et al;* 2015) aspects, shedding light on the potential therapeutic contributions of *Saussurea obvallata* to modern medicine (Ponnusamy S *et al*; 2011) [42].

Material methodology

Sample Collection

The whole flower sample *Saussurea obvallata* was collected from botanical garden of K.J. SOMAIYA COLLAGE KOPARGOAN, Taluka-Kopargoan, District-Ahmadnagar, Maharashtra India. Latitude of Kopargaon is 19.8751^0N and longitude is 74.4825^0E. and the collected sample was identified and authenticated by department of botany K.J. SOMAIYA COLLAGE KOPARGOAN

Materials

Instumemention: Spectrophotometer, incubator, Hot air oven, Water bath, Vortex, Weighing balance, Well borer

Chemicals: HCL, H2SO4, Chloroform Ferric chloride, Zinc chloride, Glacial acetic acid, Pottasiun iodine, Iodine, Sodium carbonate DPPH powder Amphicillin Amylase enzyme Phosphate buffer Alcohol

Reagents: Dragendorffs reagent, Mayers reagent, Molish reagent, FC reagent

Media
Muller hinton agar
Starch media

Methodology
Preparation of plant extract
The collected plant material was bought to laboratory and was shade dried in order to maintain their compositions and therapeutic activity. Then it was dried for 15 days till there is no water activity in flower petals. (James Redfern *et al;*2014) Then this dried sample was grinded with the help of Electronic grinder and was grinded till powder form and this powder was used for further use of bioactive compound extraction. (Eze *et al*; 2013) [39] Powder obtained for above procedure was use in solvent extraction method with the help of soxhlet extraction assembly where with the help of suitable solvent such as Ethanol the bioactive compound was separated from powder of *Saussurea obvallata* dried flower
(Das.S *et al;* 2014) [20]. Then the extracted bioactive compound was stored in 7-15^{0}celcius in refrigerator and percentage of yield was calculated by using following formula.

Phytochemical analysis
Test for Alkaloids
Take 15 mg of extract and incorporate it with 6ml 1% Hydrochloric Acid and introduce it to boiling water bath for 5minutes and filter after it with filter paper and distribute it in two separate tubes for reagent testing. (Zhu Gy *et al;* 2015)

Mayer's Test
Mayer's reagent preparation: Incorporation of 1.36 gm of mercuric chloride and 5grm Potassium Iodide in 100ml sterile distilled water.
Test protocol (Chen QL *et al;* 2016) [9]
Addition of traces of Mayer's reagent in extract filtrate formation of cream colour precipitation ensures presence of alkaloids

Wagner
Incorporation of Potassium iodide (2g) and iodide (1.27g) in 5ml distilled water and diluted in 100 ml distilled water and traces of this solution is introduced in filtrate and formation of brown color precipitation ensures presence of alkaloids. (Semwal.p *et al;* 2014) [11]

Test of Terpenoids
Salkowski test
The crude extract 100mg is vigorously mixed with chloroform 2ml followed by introduction of 2ml concentration H_2SO_4 and formation of reddish brown colour ensures presence of Terpenoids.

Test for saponins
Plant extract 0.5 g is introduced in 10 ml distilled water in test tube and placed in boiling water bath for 5 min and observation of foaming in test tube ensures presence of saponins.
(Anand.J *et al;*2015) [14]

Test for glycosidase
Plant Test for saponins
The few fractions of flower extract is incorporated with distilled water and shaken with the help of Continuous stirring and formation of foaming layer at top ensures presence of saponins. (Noumedm.jak *et al;* 2013)

Antimicrobial Activity
The bioactive compound extracted from *Saussurea obvallata* was prepared in various concentrations with sterile distilled water and it was tested against different gram positive and gram negative species namely *Escherichia coli, Campylobacter jejuni, staphylococcus aureus, Clostridium Spp* and *Pseudomonas Spp* (Sayed E *et al;* 2022) [27]. The method Employed for this technique was agar diffusion where the sterile mular Hinton agar plate were used and three wells were made with the help of well borer. (Sharifi-Rad J *et al;*2016) [22, 23, 24, 25]

Antidiabetic Activity
Testing antibiotics in plant extracts with amylase enzyme involves assessing the potential antimicrobial properties of these extracts (Ahmed. M.F *et al;* 2010) [19]. Amylase, an enzyme that catalyzes the breakdown of starch into sugars, is commonly used in such tests due to its susceptibility to microbial degradation (Luitel.S *et al*; 2019) [28]. Researchers typically employ disc diffusion or well diffusion methods, where filter paper discs or wells impregnated with plant extracts are placed on agar plates inoculated with bacterial or fungal cultures
(Alotaibi *et al*; 2021) [30]. To verify the antibiotic activity of extracted *Saussurea obvallata* is done by preparation of starch media plates and simultaneously 3 test tubes of different extract concentrations 20µg/ml,40 µg/ml and60µg/ml were prepared with addition of 40µlit of amylase enzyme with addition of phosphate buffer in first two concentration tubes with 40µlit and 20µlit.
After preparation of tubes 3 wells are prepared with the help of well borer on starch agar plate and this wells are labelled after this the wells are filled with test samples of different concentration and incubated at room temperature for period of 24 hours. After completion of incubation period Zone on plates are visualized with help of phosphate buffer incorporated with iodine and after visualization zones are measured with zone measuring scale

Antioxidant Activity Assessment:
DPPH Radical Scavenging Assay The DPPH assay, developed by Blois in (1958), aims to determine antioxidant activity by utilizing the stable free radical a, a-diphenyl-ß-picrylhydrazyl (DPPH). (Vinson. J.A *et al;*1995) [21] This method relies on measuring the scavenging capacity of antioxidants towards DPPH. When antioxidants react with DPPH, the odd electron of the nitrogen atom in DPPH is reduced by accepting a hydrogen atom from the antioxidants, leading to the formation of the corresponding hydrazine. (Brand-Williams, *et al*; 1995) [35] This reaction results in the discoloration of the DPPH solution, which can be quantitatively measured to assess the antioxidant activity of the tested compounds. (The antioxidant potential of the Saussurea obvallata extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging

assay. (Prabhakar semwal *et al*; 2019) First the DPPH solution was prepared with suitable solvent ethanol to the concentration of 0.1 Mm. Then the test sample with solvent Ethanol with different concentrations of the extract were prepared, and and each concentration was mixed with the 100µl DPPH solution. And all sample tubes where incubated in dark conditions for 30 min to 1 Hours period, then the absorbance was measured at [specific wavelength]., (Sachindra *et al*; 2010) Ascorbic acid was used as the reference standard.

Observation

Table 1: percentage of yield

Before drying weight (mg)	After drying weight (mg)
80.21	18.04

[Percentage of yield (%) = $C_x/C_y \times 100$]
Where C_x - Weight after extraction
C_y - Weight before extraction
=18.04/80.21 X 100
=22.49%

Identification of secondary metabolite

Table 2: Phytochemical activity

Sr no	Name of test	Result
1)	Test for Alkaloids (Wagner's test)	Positive
2)	Test for Terpenoides (Salkowaski test)	Positive
3)	Test for Saponins	Negative
4)	Test for Resins	Positive
5)	Test for flavonoids	Positive
6)	Carbohydrate	Negative
7)	Glycosidase	Positive
8)	Tannin	Negative
9)	Amino acid	Negative
10)	Phenol	Positive

Fig 1.1: Alkaloid Wagner's Test

Fig 1.2: Terpenoids Salkowaski Test

Fig 1.3: Saponins Test

Fig 1.4: Resins Test

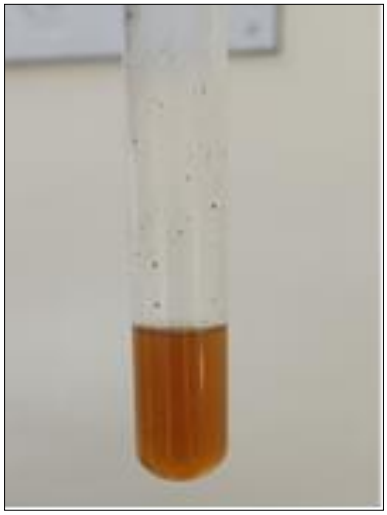


Fig 1.5: Flavonoids Test

Fig 1.6: Carbohydrate Test

Fig 1.7: Glycosidase Test

Fig 1.8: Tannins Test

Fig1.9: Amino acid Test

Antimicrobial activity

Table 3: Antimicrobial activity

SR No	Bacterial strains	Test (mm)	% of inhibition
1)	Campylobacter jejuni	09 ± 0.5	18.2
2)	Escherichia coli	22 ± 0.3	45
3)	Staphylococcus aureus	11 ± 0.7	74
4)	Clostridium	07 ± 0.5	22.2
5)	Pseudomonas	22 ± 0.4	48.8

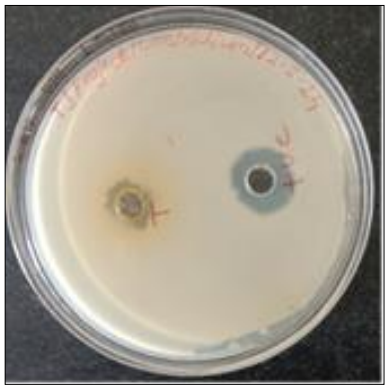


Fig 2.1: *Clostridium spp*

Fig 2.2: *Staphylococcus. Aureus*

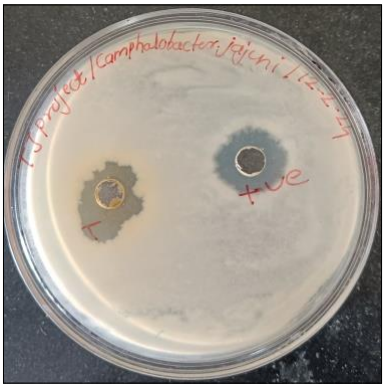


Fig 2.3: *Campylobacter jejuni*

Fig 2.4: *Escherichia coli.*

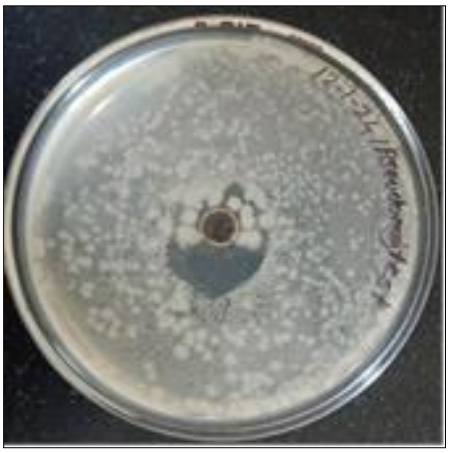
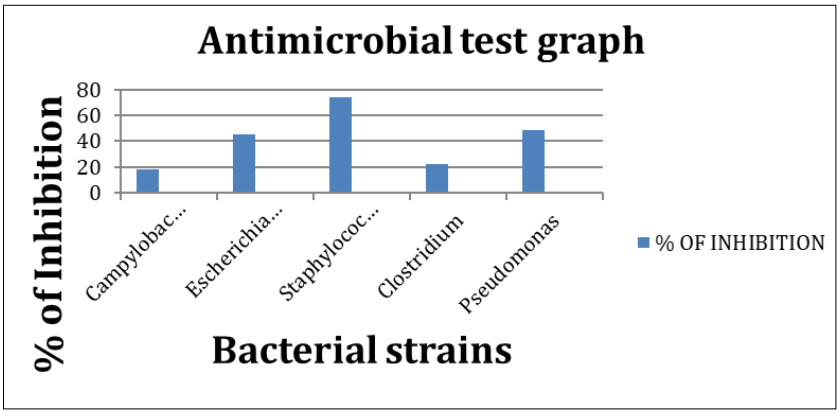


Fig 2.5: *Pseudomonas spp*



Graph 3.1: Antimicrobial activity

Antidibetic activity

Table 4: Antidibetic activity

Conc. Of extract (mg/ml)	% of inhibition (mm)	Size of Zone (mm)
20	23.81	16
40	14.29	18
60	9.52	19

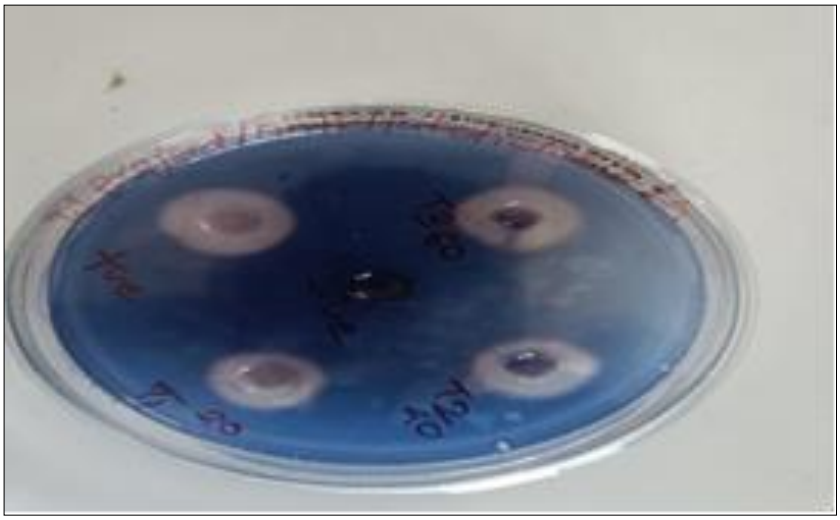
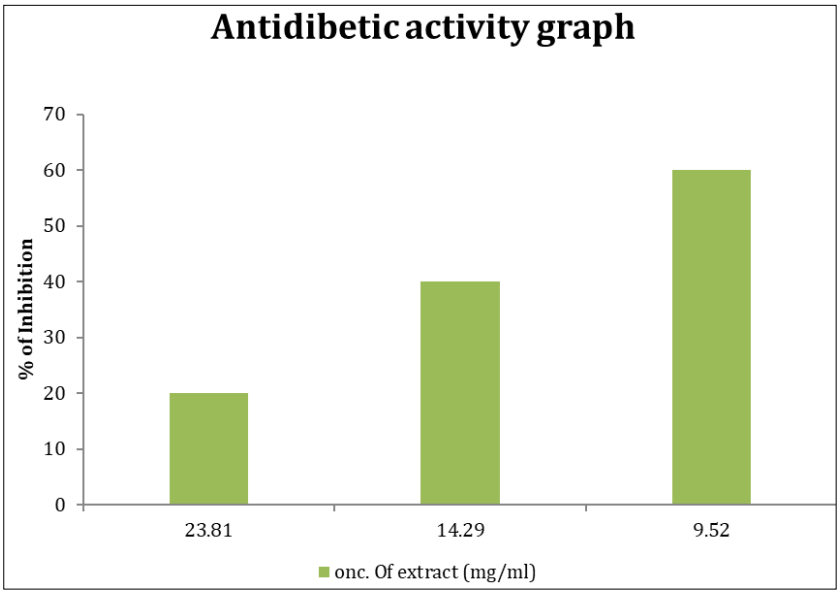
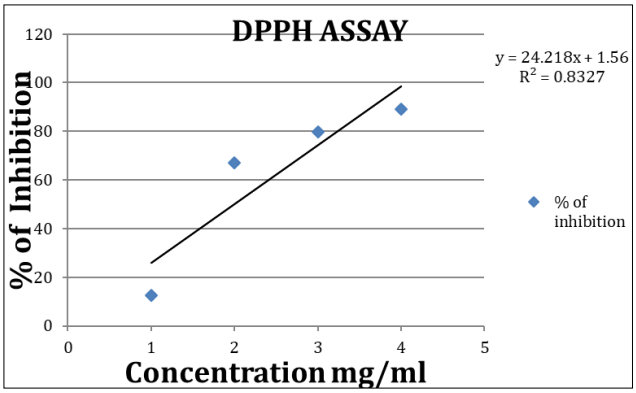


Fig 2.6: Anti diabetic test



Graph 3.2: Antidibetic activity

Antioxidant activity



Graph 3.3: antioxidant activity (DPPH)

Results

This study focused on identifying the bioactive compound *Saussurea Obvallata* and exploring its pharmacological potential, including assessments of total phenolic content, total flavonoid content, antidibetic assay, and antimicrobial activity. The findings from the investigation are organized and presented in this chapter under the respective headings.

Percentage of yield

The fresh flower weight of *Saussurea Obvallata* was 80.21 and 18.04 was weight after drying of flower and percentage of yield was calculated was 22.49%

Flower sample	Flower fresh weight (gm)	Powder weight (gm)	% of yield
Saussurea obvallata	80.21	18.04	22.49%

Phytochemical analysis

Phytochemical screening of *Saussurea obvallata* extracts revealed the presence of various bioactive compounds, including alkaloids, flavonoids, resins, phenols, glycosides, and terpenoides. These constituents are known for their diverse pharmacological properties and contribute to the medicinal significance of *S. obvallata*.

Antimicrobial activity

The antimicrobial activity of *S. obvallata* extracts was evaluated against a panel of pathogenic microorganisms, including both Gram-positive and Gram-negative bacterial strains such as *Escherichia coli*, *Campylobacter jejuni*, *staphylococcus aureus*, *Clostridium Spp* and *Pseudomonas Spp*. The ethanol extract exhibited significant antimicrobial activity, as evidenced by the formation of clear inhibition zones in agar well diffusion assays. Notably, S. obvallata extracts demonstrated potent activity against both bacterial strains, suggesting broad-spectrum antimicrobial potential.

Antidibetic activity

The antidiabetic potential of *S. obvallata* extracts was evaluated through α-amylase inhibition assay. Extract showed promising inhibitory effects against these enzymes, which are involved in carbohydrate digestion and glucose absorption. The inhibition of α-amylase indicates the extracts' ability to regulate blood glucose levels by slowing down the breakdown of complex carbohydrates into glucose and reducing glucose absorption in the intestine. These findings suggest that *S. obvallata* extracts hold potential as natural antidiabetic agents for managing diabetes and its associated complications

Antioxidant Activity

The antioxidant potential of S. obvallata extracts was assessed using DPPH assay. The ethanol extract exhibited considerable antioxidant activity, as indicated by their ability to scavenge free radicals. The scavenging activity was concentration-dependent, with higher concentrations of the extracts demonstrating increased radical scavenging capacity. These findings suggest that *S.obvallata* extracts possess significant antioxidant properties, which could contribute to their therapeutic efficacy in combating oxidative stress-related diseases.

Discussion

The findings of this study underscore the significant antimicrobial and antidibetic potential of S. obvallata flowers. Various researchers have documented the antibacterial activity of compounds isolated from these flowers, emphasizing their effectiveness compared to standard drugs. Moreover, all parts of the plant exhibit activity against at least one of the tested microbial strains, indicating the broad-spectrum antimicrobial properties of S. obvallata. (Azzahra LF *et al*; 2012) [15]. Notably, the flower extract shows superior efficacy compared to other plant parts, suggesting its potential as a potent antimicrobial agent. (Mahady. G *et al*; 2005) [5]. Phytochemical analysis reveals the presence of several bioactive compounds, including carbohydrates, flavonoids, alkaloids, and terpenoids, which could contribute to the observed antimicrobial and antidibetic properties. The absence of saponins further highlights the specificity of the phytoconstituents present in S. obvallata flowers. Confirmation of the antidibetic properties through *in vitro* tests strengthens the therapeutic potential of these flowers in managing diabetes (Vieira RF *et al*; 1993) [1]. Furthermore, the discussion emphasizes the importance of medicinal plants in drug discovery and underscores their potential role in disease resistance (Li WL *et al*; 2004). The information presented in this study provides valuable insights into the medicinal properties of S. obvallata flowers and lays the groundwork for further research in the field of diabetes management. Future studies should focus on elucidating the mechanisms of action of specific bioactive compounds and exploring their potential for drug development in the treatment of diabetes (Jung M *et al*; 2006) [47].

Conclusion

The obtained findings not only validate the traditional assertions but also serve as a valuable foundational dataset for the pharmacological utilization of Saussurea obvallata. Further, more sophisticated research is imperative to isolate and identify the precise active components responsible for the plant's pharmacological properties, thereby advancing our understanding and potential applications in medicine.

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