Phytochemical Profiling and Pharmacognostic Evaluation of *Oldenlandia corymbosa* and *Ocimum sanctum* Leaves Hydroalcoholic Extracts: Comparative Study

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This work was carried out in collaboration among all authors. Author SC: supervision. Authors SD, PG and SC: conceptualization of current research idea. Author SC: resources. Authors PG, SC, AKH: methodology. Authors SD, PG, CG, MS, SC and AKH: investigation. Authors SD, PG and SC: data curation. Authors SD, PG, CG, MS and AKH: formal analysis of data. Authors SD, CG and MS: software use, graph preparation and statistical analysis. Authors SD, PG and CG: data validation. Authors SD and PG: drafting the manuscript. Authors SD, PG, CG, SC, MS and AKH: final review and editing. All authors have read and agreed to the published version of the manuscript.

ABSTRACT

Herbs are an important source of bioactive substances. These are widely used to treat several disorders for better function in the human body, minimum toxic effects, and widespread availability. A total of two medicinal herbs from West Bengal, India, such as *Oldenlandia corymbosa* (Diamond flower) and *Ocimum sanctum* (Holy basil), are being considered for inclusion in the current study. Hydroalcoholic extracts (70% ethanolic) of the two plants’ leaves were analyzed to detect and...
quantify important phytochemical substances and investigate in vitro antioxidant and pharmacological effects. Spectrophotometric and HPLC-DAD techniques were used for the quantitative estimation of different phytochemicals. In addition, in vitro antimicrobial properties were studied using the Kirby-Bauer paper disc diffusion method. Several assays have been performed on the medicinal plant *Oldenlandia corymbosa* (OC). The results have been compared to those obtained from a traditional medical plant, *Ocimum sanctum* (OS) for the first time to our knowledge. Results showed that OS contains a higher quantity of polyphenols, flavonoids, and has higher antioxidant potential with respect to OC. Similar trends were observed for polysaccharides contents. In contrast, OC contains a higher quantity of tannins, alkaloids, and protein and higher in vitro antibacterial and anti-diabetic properties. HPLC-DAD-based profiling of eight important phenolic constituents viz. Gallic acid, catechin hydrate, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, sinapic acid, coumarin, quercetin, and kaempferol, were performed. The current study concluded that *Oldenlandia corymbosa* has many bioactive phenolics in considerable amounts compared to the highly established medicinal herb OS leaves extracts. The current study demonstrates the pharmacological significance of *Oldenlandia corymbosa* that may generate enthusiasm among researchers and those working in the pharmaceutical industry.

**Keywords:** anti-diabetic; antimicrobial; antioxidants; HPLC-DAD; Oldenlandia corymbosa; Ocimum sanctum; phytochemicals; UV-Vis.

1. **INTRODUCTION**

Since ancient times, traditional medicinal herbs have been used to treat a wide range of human illnesses in the Indian healthcare system. They contain a diverse range of chemical compounds of high therapeutic value. These phytochemicals such as polyphenols, flavonoids, tannins, alkaloids, and other organic components belong to secondary metabolites of medicinal plants [1-6]. These compounds have a vital function in protecting us against infections and other diseases. Wild medicinal plants have been the primary source of human livelihood and medicines. The daily life food consumption pattern of the rural population does not depend only on the cultivated products, but also natural resources [7-15].

In the present scenario, caution should be taken in taking synthetic drugs as it has enormous side effects and high cost. But natural product-derived medicines were getting high popularity due to their cost-effectiveness and low side effects on the human body. Thus the demand for ethnomedical treatment has progressed towards prosperous and effective research for current and new pharmaceutical companies [7-10,12,13].

India is an enriched pool of ethnomedicinal plants. From that diverse pattern of flora, we have chosen the two most important medicinal plants. These two plants are *Ocimum sanctum* Linn. (Family: Lamiaceae) and *Oldenlandia corymbosa* (Family: Rubiaceae). *Oldenlandia corymbosa* is an annually born weed, commonly termed as White Diamond. In West Bengal, this herb is called Khetpapra. *Ocimum sanctum* is an annually or perennially born weed, and it is commonly termed Holy Basil. In Bengali, it is known as Tulsi. The *Ocimum sanctum* is a very well-known and established medicinal plant for its immense ethnomedicinal and modern medicinal uses among these two herbs. Another plant, *Oldenlandia corymbosa*, is significantly less characterized but it is well known for its several medicinal properties. *Oldenlandia corymbosa* is also known as weed for its nature and growth habitat. Botanically *Ocimum sanctum* also shows its weedy nature and habit. These two medicinal plants share several characteristics: they are herb, weed, annual, have the same growing habit, pollination pattern, and have extensive usage in traditional medicine as well as great prospects in modern pharmaceutical companies. These two herbal leaves are readily available throughout the year. In a few cases, their leaves are also eaten as a vegetable in different areas of the world. These two medicinally important weeds have huge potentiality in natural medication systems as on folkloric information. In India as well as West Bengal districts, the Holly Basil and White Diamond herb been widely used for the ethnomedicinal purpose from ancient times [13,7-10,16-22].

These two different medicinal herb have shown the ability to inhibit or regulate the reactive oxygen species (ROS) which are responsible for the oxidation in in vivo and in vitro mechanism.
and generates oxidative stress and cell damage that can result in various physical disorders [7-10,12,23].

Medicinal plant-derived natural antioxidants have a considerable role in protecting against oxidative stress and related microbial pathogenesis. To protect the human body from these sorts of diseases, natural antioxidants must be consumed, and medicinal herbs are playing an important role in this regard. [12,24-26].

The use of α-amylase enzyme inhibitors is needed to regulate blood glucose levels in diabetic individuals. Therefore, continuous search for the in vitro and in vivo anti-diabetic and free radical scavenging properties of the natural inhibitors are essential because it can protects against various vascular complications of diabetes and related diseases [12,27,28].

The medicinal importance of the wild herbs can be evaluated by their phytochemicals constituents and In vitro pharmacological assessments [14,11]. So, it is crucial to take a scientific approach for detection, quantification, and analyze the phytochemical substances of these two medicinal herb leaves, which are mainly responsible for healing various diseases and complications. After considering the parameters such as botanical, ethnomedicinal, and other characteristics, we have chosen these two medicinal plants for the current research work to know their phytochemical and biological importance and to highlight the future drug formulation prospects. The current research study was designed to detect, quantify, and analyze phytochemical substances in the leaves hydroalcoholic extracts of these two ethnomedicinal herbs, as well as to determine their in vitro antioxidant capacity, in vitro antimicrobial and anti-diabetic properties, using a variety of standard assays. In this course of study very well-known medicinal plant Ocimum sanctum was taken as standard, and the less characterized Oldenlandia corymbosa was compared with it.

2. MATERIALS AND METHODS

2.1 Collection, Identification, and Extraction of Plant Samples

The fresh medicinal plants' materials were collected from Kolkata, West Bengal, India, and authenticated by the Botanical Survey of India, Howrah. Leaves of the medicinal herbs were washed with distilled water, and it was dried in the ambient environment for one month under the shaded condition. Leaves of the plants were grinded to make powder with the help of mortar and pestle, and then extracted by the 70% ethanol as solvent. 50 ml ethanol was taken for 1 g of dried powder. The extracts were clarified and stored at 4°C, and it was diluted as per the requirement for the particular test.

2.2 Collection of Bacterial Strains

Gram-negative (Escherichia coli) and Gram-positive (Staphylococcus aureus) bacterial strains were used for the evaluation of the antimicrobial property of the hydroalcoholic extracts of the two experimental herbs. These bacterial strains were collected from Calcutta University, Microbiology Department, West Bengal, and India.

2.3 Chemicals and Reagents

Chemicals and necessary reagents were of analytical grade quality. Folin-Ciocalteu, Aluminum chloride, and Ascorbic acid were purchased from Merck Life Science, Mumbai. Sodium carbonate and Sodium nitrite were purchased from RFCL Limited, New Delhi. Gallic acid, Sodium hydroxide, and Hydrogen peroxide were procured from SD Fine-Chem Limited, Mumbai. Quercetin and Tannic acid were obtained from SRL Pvt. Ltd.

2.4 Qualitative Assays

Standard methodologies were employed to know the presence of Polyphenols, Flavonoids, Carbohydrates, Reducing Sugar, Cardiac Glycosides, Tannins, Anthocyanin, Quinone, Alkaloids, and Proteins [7-10,12,23] in plant extracts.

UV-Vis absorption spectra of the medicinal herb extracts were analyzed to detect the characteristic peaks. In brief, clarified herb extracts were diluted to a 1:10 ratio with the respective solvent for spectrophotometer analysis. The extracts were scanned in the 200-800 nm wavelength range by using a Spectrophotometer (Model: Systronics117), and the significant peaks were documented [29-31].
2.5 Quantitative Analysis

2.5.1 Quantification of total phenolic contents

Total polyphenol content (TPC) was quantified using the standard Folin-Ciocalteu assay. The standard curve of the experiment was prepared by using Gallic acid. The absorbance was taken at 765 nm. The results were expressed as mg Gallic acid equivalents/g of dry tissue [32].

2.5.2 Quantification of total flavonoids content

The quantification of total flavonoids content (TFC) was investigated by aluminium chloride (AlCl$_3$) colorimetric assay. The absorbance in this method was taken at 510 nm. The standard curve of the experiment was made by using Quercetin as a standard. The result of this experiment was expressed as mg Quercetin equivalents/g of dry tissue [33].

2.5.3 Quantification of total tannins content

Total tannins content (TTC) determination was carried out by applying a standardized protocol. Tannic acid was used as standard. The absorbance was taken at 500 nm. Total tannin content were expressed as mg Tannic Acid Equivalent/g dry tissue [34].

2.5.4 Quantification of total alkaloids content

Total alkaloids content (TAC) was estimated by the standard method [35] with some modification. The absorbance was read at 470 nm. Caffeine was used as standard. Total alkaloids content was expressed in mg Caffeine Equivalent/g dry weight [35,36].

2.5.5 Quantification of polysaccharides content

The polysaccharide content (PC) was evaluated by using the standard method [37] with slight modification [38]. Dextrose was used as a standard. The absorbance in this method was measured at 488 nm. The quantity of polysaccharides was expressed in mg Dextrose equivalent/g of dry tissue.

2.5.6 Quantification of total soluble protein content

Total soluble protein content (TSPC) was determined according to the Lowry method. Bovine serum albumin (BSA) was utilized as a standard reagent. The absorbance in this method was taken at 660 nm. The protein content of the samples was measured and expressed in mg BSA Equivalent/g dry tissue [38].

2.5.7 HPLC-DAD profile of the 70% ethanol extracts

Profiling of polyphenolics was performed using the High-Performance Liquid Chromatography system with Diode Array Detector (Agilent Technologies 1260 Infinity liquid chromatography system). The phenolics were separated under the following conditions: Phenomenex-C18 (2)-column (250 mm×4.6 mm i.d.; Luna 5 μm particle diameter 100 Å), the Detector of HPLC profiling was fixed at 280 nm; mobile phase of the solution consisted of 3% aqueous acetic acid and acetonitrile. The solutions were freed in an ultrasonic bath and filtered through 0.22μm membranes. In gradient conditions, the flow rate was 0.9 ml/min. 20 μl of sample injected. All the separations are done at 25°C temperature [12,39,40].

2.6 Determination of Antioxidant Property (In vitro)

2.6.1 DPPH radical scavenging assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging property was investigated by using the standard protocol. The standard curve of the experiment was made by using ascorbic acid. The absorbance of was taken at 517 nm. The DPPH radical scavenging capacity was measured in the below-mentioned formula [41].

\[
\%\text{DPPH radical scavenged} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]

2.6.2 Hydrogen peroxide scavenging assay

A standard protocol was used to determine the experimental samples’ hydrogen peroxide ($\text{H}_2\text{O}_2$) radical scavenging potential. The absorbance of the experiment was taken at 230 nm. In the experiment, Gallic acid was used as standard. %$\text{H}_2\text{O}_2$ radical scavenged was calculated by the formula mentioned below [42,43].

\[
\%\text{$\text{H}_2\text{O}_2$ radical scavenged} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]
2.7 Determination of Antimicrobial Activity (In vitro)

Antimicrobial activity study was carried out by using Kirby-Bauer disc diffusion assay. In the study, plates were incubated at 37°C for 16-18 h, the zone of inhibition was measured. Antimicrobial activity was done against one Gram-positive (i.e., J = Staphylococcus aureus) and the other was Gram-negative bacteria (i.e., H= Escherichia coli) [7-10,26].

2.8 Determination of Anti-diabetic Activity (In vitro)

2.8.1 Alpha-amylase inhibition assay

Alpha-amylase inhibitory activity was measured by measuring the reducing sugars produced by soluble starch hydrolysis by α-amylase enzyme with or without the presence of inhibitors (Acarbose or plant extracts). Acarbose was utilized as positive reaction control at a 10 mg/ml concentration in the study. The absorbance of the experiment was taken at 540 nm. The percentage of α-amylase inhibition in this study was measured using the following formula: [12,27,28].

\[
\% \text{ Inhibition} = \frac{(\text{OD of Control} - \text{OD of Sample})}{\text{OD of Control}} \times 100
\]

2.9 Statistical Analysis

All the measurements of this experimental study (except antimicrobial activity assay and HPLC-DAD analysis) were carried out in triplicate sets, represented as (average ± standard deviation). All the statistical analyses like means, standard curve, standard deviations, IC50, and one-way ANOVA were performed in MS Excel Software. Statistical significance level was accepted at P<0.05.

3. RESULTS

3.1 Qualitative Assay

Ten qualitative biochemical assays were done to detect the presence of different major classes of natural products present in the OC and OS 70% hydroethanolic extracts. The results (Table 1) show that Polyphenols, Flavonoids, Carbohydrate, Reducing Sugars, Tannins, and Proteins were present in both the samples, but Cardiac Glycosides was not detected in both the samples. In addition, anthocyanin was only detected in OC, whereas Quinones and Alkaloids were only detected in OS.

### Table 1. Results of phytochemical screening

<table>
<thead>
<tr>
<th>Test Name</th>
<th>OC</th>
<th>OS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anthocyanin</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Where "+" presence "-" absent

3.2 UV-Vis Absorption Spectrum Characterization

The UV-Vis absorption spectrum profiling of 70% ethanolic decoctions of the experimental plants leave were carried out at 200 to 800 nm with the obtained peak values and exact baselines. UV spectrum analysis reveals that the peaks came are in between 241 nm to 669 nm (Table 2).

### Table 2. UV-Vis spectrum peak values (nm) and absorbance of leaves extracts

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>70% Ethanolic extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peak (nm)</td>
</tr>
<tr>
<td>OC</td>
<td>241.6</td>
</tr>
<tr>
<td></td>
<td>659.4</td>
</tr>
<tr>
<td>OS</td>
<td>238</td>
</tr>
<tr>
<td></td>
<td>668.5</td>
</tr>
</tbody>
</table>

3.3 Quantitative Assay

3.3.1 Polyphenols

Total polyphenol content was determined with respect to the Gallic acid standard curve (R²=0.999). It was observed that the total polyphenol content (Fig. 1) in OC leaves (42.63±1.22 mg GAE/g of dry tissue) is lower than OS leaves (47.51±0.88 mg GAE/g of dry tissue).

3.3.2 Flavonoids

Total flavonoids content was determined with respect to the Quercetin standard curve (R²=0.994).
It was observed that the total flavonoids content (Fig. 2) in OC leaves (22.60±0.48 mg QE/g dry tissue) is lower than the OS leaves (27.29±1.21 mg QE/g DW).

3.3.3 Tannins

Total tannin content was determined with respect to the tannic acid standard curve ($R^2=0.993$). It was observed that the total tannin content (Fig. 3) in OC leaves (19.30±1.51 mg TAE/g dry tissue) is significantly higher than the OS leaves (8.65±0.69 mg TAE/g dry tissue).

3.3.4 Alkaloids

Total alkaloid content was determined with respect to the Caffeine standard curve ($R^2=0.996$).
The total alkaloids contents of both OC and OS were quantified. It was observed that the total alkaloids content (Fig. 4) in OC leaves is significantly higher (0.046±0.007 mg CE/g dry tissue), than OS leaves (0.022±0.003 mg CE/g dry tissue).

3.3.5 Polysaccharides

Total polysaccharides content was determined with respect to Dextrose standard curve ($R^2=0.999$).

The polysaccharides contents of both OC and OS were quantified. It was observed that the polysaccharides content (Fig. 5) in OC leaves is lower (53.44±1.37 mg DE/g dry tissue) than OS leaves (64.76±2.65 mg DE/g dry tissue).

3.3.6 Protein

Total soluble protein content was determined with respect to the BSA standard curve ($R^2=0.997$).
Fig. 5. Total Polysaccharides Content (mg DE/g Dry Tissue)

Fig. 6. Total protein content (mg BSAE/g Fresh Tissue)
The total protein contents of both OC and OS were quantified. It was observed that the protein content (Fig. 6) in OC leaves is higher (4.70±0.15 mg BSAE/g dry tissue) than OS leaves (4.25±0.28 mg BSAE/g dry tissue). However p-value˃0.05 depicts that the difference is insignificant.

3.4 HPLC-DAD Analysis

A simple, accurate, and productive HPLC online method has been used and validated to identify and estimate different phenolic constituents. HPLC profile analysis obtained from the experimental plants 70% ethanol extract identified nine phenolic constituents: Gallic acid, Chlorogenic acid, Caffeic acid, Syringic acid, p-Coumaric acid, Sinapic acid, Coumarin, Quercetin, and Kaempferol (Fig. 7 and Fig. 8).

Among these, eight compounds were present in both the plant extracts. In OS, highest ten compounds were identified. The HPLC-DAD Chromatograms are represented in figures (Fig. 7 and Fig. 8).

3.5 DPPH Radical Scavenging Activity

Leaf hydroalcoholic extracts of OC and OS showed 87.30±0.86% and 89.06±0.56% DPPH free radical scavenging activity. In contrast, the standard ascorbic acid showed 93.57% DPPH free radical scavenging activity (Fig. 9).

Leaf hydroalcoholic extracts of OC and OS showed 77.63±0.67% and 82.15±0.68% H₂O₂ free radical scavenging activity, respectively, whereas the standard Gallic acid showed at the same concentration 92.96% H₂O₂ free radical scavenging activity (Fig. 10).

Fig. 7. OC Chromatogram
Fig. 8. OS Chromatogram

Fig. 9. % DPPH free radical scavenging assay
In both assays, OS leaf shows a higher antioxidant potential than OC leaf extract.

### 3.6 Antimicrobial Activity

70% Ethanolic extracts of the leaves showed inhibitory activity against these two bacteria, shown in Table 3. OC shows the zone of inhibition against two strains, EC and SA (Figs. 11 & 12). Furthermore, it gives the highest zone of inhibition against EC (Fig. 13 and Fig. 14). On the other hand, OS shows a comparatively less zone of inhibition against these two strains.

![Graph showing % H$_2$O$_2$ Radical Scavenging](image)

**Fig. 10. % H$_2$O$_2$ Radical Scavenging Assay**

### 3.7 Antimicrobial Activity of OC

![Images of petri dishes showing antimicrobial activity](image)

**Fig. 11. SA**

**Fig. 12. EC**
3.8 Antimicrobial Activity of OS

![Fig. 13. SA](image)

![Fig. 14. EC](image)

**Table 3. Zone of Inhibition (mm)**

<table>
<thead>
<tr>
<th>Organisms Name</th>
<th>Zone of Inhibition (mm)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OC Control</td>
<td>Net zone</td>
<td>OS Control</td>
<td>Net Zone</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>24</td>
<td>16</td>
<td>8</td>
<td>16</td>
<td>14</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>SA</td>
<td>16</td>
<td>10</td>
<td>6</td>
<td>6</td>
<td>14</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

**Alpha-amylase Inhibitory Activity (%)**

![Graph](image)

**Fig. 15. In vitro anti-diabetic activity by alpha-amylase inhibition (%)**

**Table 4. IC_{50} of standard drug acarbose and plant extracts in alpha-amylase inhibition**

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC_{50} Values (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acarbose</td>
<td>3.55</td>
</tr>
<tr>
<td>OC</td>
<td>4.50</td>
</tr>
<tr>
<td>OS</td>
<td>5.21</td>
</tr>
</tbody>
</table>
3.9 *In vitro* Anti-diabetic Activity (Alpha-amylase Inhibition)

The different leaf extract concentrations (between 0.0195 mg/ml to 10 mg/ml) of OC and OS were selected for the Alpha-amylase inhibition assay. The study revealed that OC and OS show 72.81±0.96% and 63.53±1.62% α-amylase inhibition in their highest concentration, respectively (Fig. 15). In contrast, the inhibitory percentage for standard Acarbose was 98.69% at 10 mg/ml of concentration. IC$_{50}$ values of standards Acarbose, OC and OS leaf extracts are 3.55 mg/ml, 4.50 mg/ml, and 5.21 mg/ml (Table 4) respectively.

4. DISCUSSIONS

Medicinal weeds are rich sources of different bioactive phytochemicals useful in drug development against several diseases. Many weeds have already been explored to know their medicinal importance [12]. However, a literature search reveals that many weeds are still not explored completely to know their medicinal attributes. Therefore, in the present course of study, we have chosen a comparatively less characterized garden weed, namely Old World Diamond flower (*Oldenlandia corymbosa*), for phytochemical composition analysis and evaluation of the medicinal property (Fig. 16) and compared with a well established medicinal herb Tulsi or Holy Basil (*Ocimum sanctum*).

Qualitative assessments of the leaf ethanolic extract of the two experimental herbaceous medicinal plants reveal that they harbor different important classes of natural products (Polyphenols, Flavonoids, Carbohydrate, Reducing Sugars, Tannins, and Proteins). Hence, they can be utilized for the development of herbal formulations [23,24]. Spectroscopic studies confirmed the presence of flavonoids and their derivatives. The UV-Vis spectra of the 70% ethanolic extract of leaf of OC and OS had absorption maxima at 241.6 nm and 238 nm, respectively, within the range of characteristic absorption for flavonoids [44]. Peaks at 659.4 and 668.5 are the characteristic peaks of chlorophyll.

Next, a quantitative estimation of the phytochemicals was performed. Phytochemical composition analysis evaluates the concentration of the specific bioactive constituents in the plants' extracts. Polyphenolic components are natural antioxidants, and hence they can scavenge free radicals generated during cellular stress, which are responsible for oxidative stress-related physical ailments like diabetes, high blood pressure, arthritis, and cancer. Moreover, polyphenols act in a process that can have substantial pharmacological uses in the industrial sector [45,46]. Results highlighted that the TPC content in OC leaves is lower than OS leaves. However, the differences between the TPC are not high between OS and OC. Hence, OC also can be used in oxidative stress like the standard medicinal herb OS.

![Fig. 16. Overall work flow of the current research work](image-url)
Next, we measured the TFC of the leaf hydroalcoholic extracts of the two plant because flavonoids belong to polyphenols family with tremendous therapeutic importance. Vegetables and fruits are rich sources of flavonoids and are consumed as a part of the human diet in significant amounts. The current study reveals that OC harbors less TFC with respect to OS.

Tannins are primarily found in barks of plants. Tannins are astringent, bitter polymeric substances that can be used for tanning leather. Tannin-protein complex can produce persistent pharmacological properties [1,45]. Alkaloids are necessary secondary metabolites, and it has enormous applications in phytotherapy. In addition, it is observed that alkaloids have extensive uses in antimicrobial treatment purposes [35,36]. The present study shows that TTC and TAC are respectively very high in OC compared to OS. Hence it can be apprehended that OC would be a potent medicinal herb.

Next, we determined polysaccharides and soluble protein content in the extracts to understand their nutrition status. Polysaccharides function as the main binder, suspension, emulsifier, stabilizer, and water capturing agents for pharmaceutical products. These properties are utilized for the generation of pharmaceutical and drug release processes. Polysaccharides are inexpensive, non-toxic, and is biologically degradable. For these reasons, it is widely used in encapsulation of drugs in many pharma industries [37]. In addition, polysaccharides can be easily oxidized to produce instant energy, and their polymers also can act as storage molecules [14,15]. Protein is an essential nutrient component, and it is also a primary metabolite needed for human body functions [12]. Our current findings highlighted that polysaccharides content is relatively high in OS. However, OC content higher amount of protein with respect to OS. The result signifies that OC is a medicinal herb and can also be used as a nutritional supplement for protein and carbohydrates.

Phenolic acids are well known for their active scavenging properties to Reactive Oxygen Species (ROS). Phenolic acids are subcategorized as benzoic acid and cinnamic acid backbone structure. The study analysis reveals the presence of hydroxybenzoic acid and cinnamic acid derivatives of phenolic substances in the experimental samples. Among the different phenolic acids and flavonoids, OC contains a higher amount of Gallic acid and Kaempferol with respect to OS, as evident from chromatograms. Like other polyphenols, Gallic acid and Kaempferol can help to cure oxidative stress-related diseases like diabetes and microbial pathogenesis [2]. They have anticancer activity. Kaempferol have estrogen modulatory activity [3]. A previous study showed that caffeic acid is the primary factor for antioxidant activities. Sinapic acid, is also known for their antioxidant and antimicrobial activities [12].

Since both the plants contain high TPC and TFC, they can be used in oxidative stress-related disorders. Antioxidant effects of polyphenols and flavonoids are due to the presence of hydroxyl groups in their structure. Flavonoids’ antioxidant properties can be observed in various reaction processes. It acts in regulating free radicals, metal ions chelating, and the regulatory activity of enzymes. Earlier investigations showed the protective capacity of flavonoids classes against several microbial pathogeneses [45,1]. Present research deciphered the antioxidative potentials in terms of free radical scavenging activity. It is observed that OS has strong free radical scavenging property (DPPH free radical and peroxide radical) with respect to OC. Phytochemical substances have a massive importance in antioxidant activity and inhibit free radicals [2,23,37,44-46].

The results of the Kirby-Bauer disk diffusion assay indicated that both the leaves’ ethanolic extracts had shown antimicrobial properties. OC showed a greater zone of inhibition against Gram-negative bacteria E. coli than Gram-positive bacteria S. aureus. Even the inhibitory activity is greater than OS. The main bioactive substances present in the extracts, such as polyphenols, flavonoids, tannins, and alkaloids, are responsible for antimicrobial activity. Previous studies concluded that plants with caffeic acid had shown a prominent antimicrobial property [12]. Phenolic acids and other bioactive compounds create barriers in the synthesis of nucleic acids of both Gram-negative and Gram-positive bacteria [1,7-10,24-26].
The in vitro anti-diabetic property determination of two experimental plant leaves extracts was done by the α-amylase enzyme inhibition assay. The study revealed that OC and OS show 72.81±0.96% and 63.53±1.62% α-amylase inhibition respectively in their highest concentration, (Fig. 15). In contrast, the inhibitory percentage for standard Acarbose was 98.69% at 10 mg/ml of concentration. This Inhibitory activity happens due to the presence of phytomolecules substances such as phenolic acids, flavonoids, flavonols, tannins, and alkaloids. The 3, 5-dinitrosalicylic acid reagents (DNSR) test is a biochemical technique to measure the quantity of reducing sugars generated after treating a specific solution by α-amylase and plant extracts. Bioactive molecules mainly act as inhibitors of the α-amylase enzyme as well as preventing the β-cells destruction and diabetes-induced ROS formation [12,27,28].

Traditional medicinal plants are the prime source of clinically applied plant-derived polyphenols or other bioactive substances that act as antioxidants [4]. The current study results reveal that the OC harbors plenty of antioxidants and other bioactive compounds like polyphenolics, flavonoids, tannins, alkaloids, etc. Hence, OC may find its application as potent antioxidants in stress-related disorders, infection, cancer, diabetes, etc.

5. CONCLUSIONS

Herbal medicines find enormous applications for the management of various diseases due to their effectiveness, availability, low cost, and fewer side effects. Therefore, identifying wild and ethnobotanically important medicinal herbs becomes essential for industrial product development. In this study, 70% ethanol extracts of OS and OC plant leaves were observed with a sufficient amount of phytomolecules responsible for the antioxidant property and therapeutic potential. The study showed that the higher content of phytomolecules like phenolic acids, flavonoids, tannins, and alkaloids are present in OC like well-established medicinal plant OS. It is also observed that OC harbors a considerable amount of two important nutritional components, such as carbohydrates, and proteins like OS. DPPH and H₂O₂ radical scavenging assay of the extracts were also investigated. The study concludes that the folkloric plant OC may serve as naturally occurring antioxidants. OS has the highest quantity of polyphenols and flavonoid contents among these two medicinal herbs and the highest antioxidant properties.

The antimicrobial activity was moderate in the case of both medicinal herbs. However, OC performed better in the Kirby-Bauer disk diffusion assay with respect to OS.

The therapeutic standpoint had focused on decreasing hyperglycemia, which can be achieved through the inhibition of α-amylase. The current study concluded that 70% ethanol extracts of the OC and OS leaves can inhibit the α-amylase enzyme. Furthermore, OC showed better inhibition capacity than OS.

The present study indicates that OC possesses a considerable amount of phytochemicals which is comparable with the standard medicinal herb OS, which has huge herbal applications worldwide. Hence, OC can also be included in the list of potential medicinal herbs and can be utilized to separate, detect and identify active components for the proper therapeutic purposes.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

NOTE

The study highlights the efficacy of "medicinal herbs" that is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS
Authors have declared that no competing interests exist.

REFERENCES


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