ORIGINAL RESEARCH ARTICLE

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

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.

ORIGINAL RESEARCH ARTICLE

Keywords: *Oldenlandia corymbosa; Ocimum sanctum;* Phytochemicals, UV-Vis, HPLC-DAD, Antioxidants, Antimicrobial, Anti-diabetic.

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. Different phytochemicals such as polyphenols, flavonoids, tannins, alkaloids, and other organic components are known as secondary metabolites of medicinal plants. 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 of the rural population does not depend only on the cultivated products, but natural resources also play a major role in that cases (Ghosh P & Biswas S et al., 2019; Ghosh P & Chatterjee S, 2020; Ghosh P & Das C et al., 2020; Kirtikar, 1991; Begum et al., 2018; Horo et al., 2015).

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 ethnomedicinal treatment has progressed towards prosperous and effective research for current and new pharmaceutical companies (Kirtikar et al., 1991; Ghosh P & Biswas S et al., 2019; Ghosh P & Das C et al., 2020).

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 and has reported 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. The common features of these two medicinal plants are they are herb, weed, annual, growth habit is same, pollination pattern is similar, and they have the immense

uses in traditional medicine and high prospects towards modern pharmaceutical industries. 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 (Kirtikar et al., 1991; Ghosh P & Biswas S et al., 2019; Das S & Mondal N et al., 2019; Patel T et al., 2014; Noiarsa P et al., 2008; Hussain AZ et al., 2013; Sandip G et al., 2014; Kulkarni KV et al., 2018; Kalyan KP et al., 2012).

These two different medicinal herb have shown the ability to inhibit or regulate the reactive oxygen species (ROS) which initiates the oxidation *in vivo* and *in vitro* mechanism and generates oxidative stress and cell damage that can result in various physical disorders (Ghosh P & Biswas S et al., 2019; Ghosh P & Biswas M et al., 2019; Ghosh P & Das C et al., 2020; Sahoo et al., 2018).

Medicinal plant-derived phytochemical constituents of natural antioxidants have a considerable role in protecting against oxidative stress-related microbial pathogenesis. Therefore, to protect the human body from these types of pathogenesis, intake of natural antioxidants are essential, and the medicinal plants are taking their share on that ground (Ghosh P & Das C et al., 2020; Malliga et al., 2014; Vinoth B et al., 2012; Sen et al., 2002).

The use of α-amylase enzyme inhibitors is needed to regulate blood glucose levels in diabetic individuals. Therefore, evaluations of the *in vitro* anti-diabetic and free radical scavenging properties are essential because it protects against various vascular complications of diabetes and related diseases (Balaji et al., 2015; Srinivasan et al., 2016; Ghosh P & Das C et al., 2020).

The medicinal importance of the wild herbs can be evaluated by their phytochemicals and *in vitro* pharmacological assessments (Begum et al., 2018; Ghosh P & Chatterjee S, 2020). 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.

MATERIALS AND METHODS

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 stored at 4°C, and it was diluted as per the requirement for the particular test.

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.

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.

QUALITATIVE ASSAYS

Standard methodologies were employed for quantification of Polyphenols, Flavonoids, Carbohydrates, Reducing Sugar, Cardiac Glycosides, Tannins, Anthocyanin, Quinone,

Alkaloids, and Proteins (Ghosh P & Biswas S et al., 2019; Ghosh P & Biswas M et al., 2019; Ghosh P & Chatterjee S, 2020; Sahoo et al., 2018) 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 (Ghosh P & Kulavi S et al., 2019; Ghosh P & Saha M et al., 2020; Das S & Saha M et al., 2020).

QUANTITATIVE ANALYSIS

Quantification of Total Phenolic Contents

Total polyphenolic 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 (Singleton et al., 1999).

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 reagent. The result of this experiment was expressed as mg Quercetin equivalents/g of dry tissue (Zhishen et al., 1999).

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 quantities of the experiment were expressed as mg Tannic Acid Equivalent/g dry tissue (Broadhurst et al., 1978).

Quantification of Total Alkaloids Content

Total alkaloids content (TAC) was estimated by the standard method (Fazel et al., 2008) with some modification. The absorbance of the obtained complex in the chloroform solvent was read at 470 nm. Caffeine was used as standard. Total alkaloids content was expressed in mg Caffeine Equivalent/g dry weight (Fazel et al., 2008; Biju et al., 2014).

Quantification of Polysaccharides Content

The polysaccharide content (PC) was evaluated by using the standard method (Harshal et al., 2011) with slight modification (Harshal et al., 2011). 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.

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 (The Protein Protocols Handbook. JM Walker © Humana Press Inc.).

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 (Hanfer et al., 2019; Shamili G et al., 2019; Ghosh P & Das C et al., 2020).

DETERMINATION OF ANTIOXIDANT PROPERTY (*IN VITRO*)

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 (Shen et al., 2010).

%DPPH radical scavenged = Control OD- Sample OD/ Control OD *100

Hydrogen Peroxide Scavenging Assay

A standard protocol was used to determine the experimental samples' hydrogen peroxide (H_2O_2) radical scavenging potential. The absorbance of the experiment was taken at 230 nm. In the experiment, Gallic acid was used as standard. %H_2O_2 radical scavenged was calculated by the formula mentioned below (Ruch et al., 1989; Patel et al., 2010).

%H_2O_2 radical scavenged = Control OD- Sample OD/ Control OD *100

DETERMINATION OF ANTIMICROBIAL ACTIVITY (*IN VITRO*)

Antimicrobial activity study was carried out by using Kirby-Bauer disc diffusion assay. In the study, plates were kept in incubation condition at 37ºC 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* (Sen et al., 2002; Ghosh P & Biswas M et al., 2019).

DETERMINATION OF ANTI-DIABETIC ACTIVITY (*IN VITRO*)

Alpha-amylase Inhibition Assay

Alpha-amylase inhibitory activity was measured by measuring the reducing sugars produced by soluble starch hydrolysis by an 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: (Balaji et al., 2015; Srinivasan et al., 2016; Ghosh P & Das C et al., 2020).

% Inhibition = (OD of Control-OD of Sample)/OD of Control*100

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, IC_{50}, and one-way ANOVA were performed in MS Excel Software. Statistical significance level was accepted at $P<0.05$.

RESULTS

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% ethanol decoctions. The results (Table 1) show that Polyphenols, Flavonoids, Carbohydrate, Reducing Sugars, Tannins, and Proteins were detected, and 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

Test Name	OC	OS
Polyphenols	+	+
Flavonoids	+	+
Carbohydrate	+	+
Reducing Sugars	+	+
Cardiac Glycosides	-	-
Tannins	+	+
Anthocyanin	+	-
Quinones	-	+
Alkaloids	-	+
Proteins	+	+

Where "+" presence "-" absent

UV-Vis Absorption Spectrum Characterization

The UV-Vis absorption spectrum profiling of 70% ethanolic plants leave decoctions 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

Plant Name	70% Ethanolic Extracts	
	Peak (nm)	**Absorbance**
OC	241.6 659.4	2.891 0.251
OS	238 668.5	2.981 0.153

Quantitative Assay

Polyphenols

Total polyphenolic content was determined with respect to the Gallic acid standard curve (R^2=0.999). It was observed that the total polyphenolic content (Figure 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).

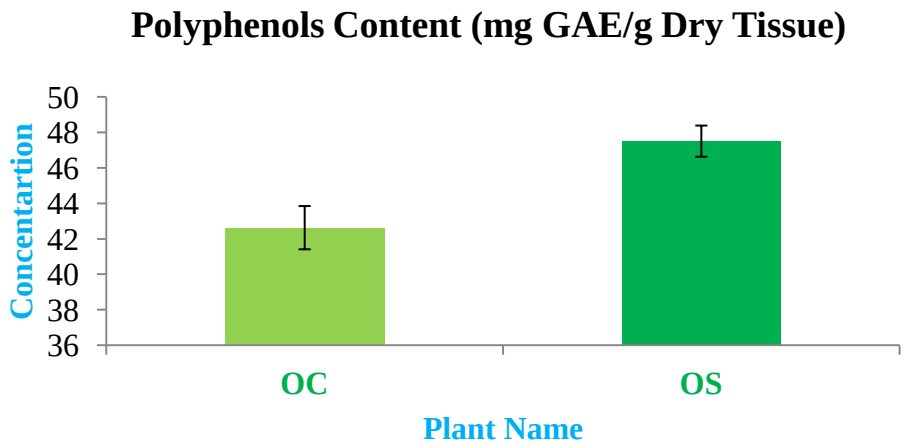

Figure 1: Total Polyphenol Contents (mg GAE/g Dry Tissue)

Flavonoids

Total flavonoids content was determined with respect to the Quercetin standard curve (R^2=0.994).

It was observed that the total flavonoids content (Figure 2) in OC leaves showed the lowest amount (22.60±0.48 mg QE/g dry tissue), and OS leaves exhibited the highest amount (27.29±1.21 mg QE/g DW).

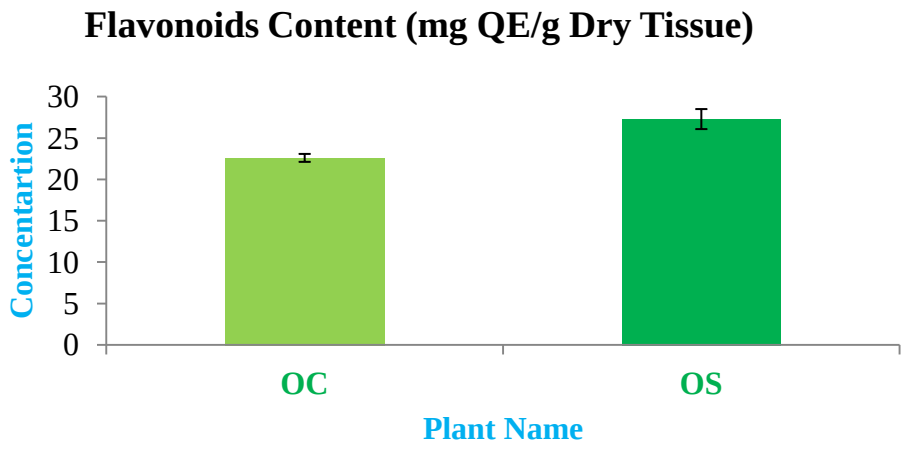

Figure 2: Total Flavonoids Content (mg QE/g Dry Tissue)

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 (Figure 3) in OC leaves showed the highest amount (19.30±1.51 mg TAE/g dry tissue), and OS leaves exhibit the lowest amount (8.65±0.69 mg TAE/g dry tissue).

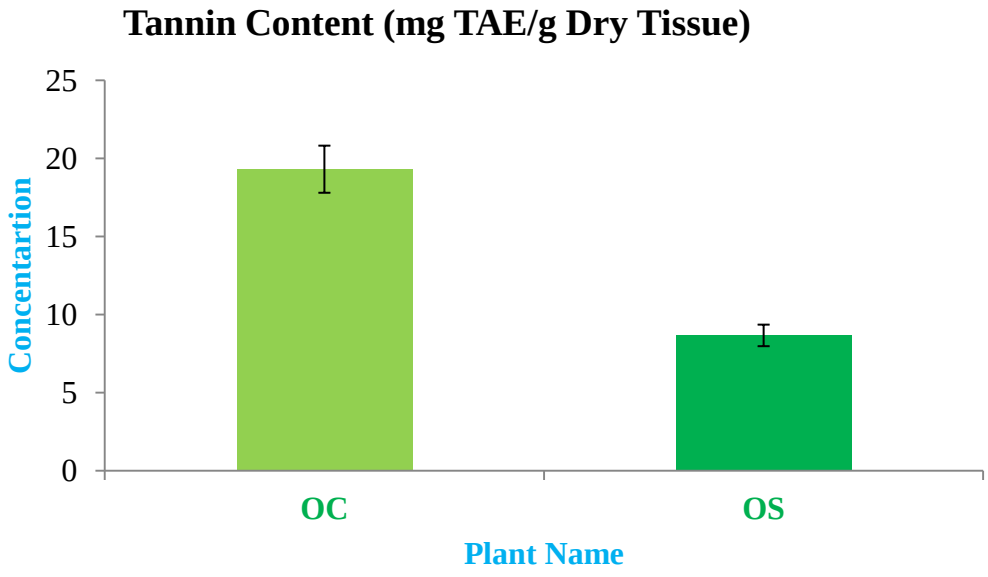

Figure 3: Total Tannin Content (mg TAE/g Dry Tissue)

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 (Figure 4) in OC leaves showed the highest amount (0.046±0.007 mg CE/g dry tissue), and OS leaves exhibit the lowest amount (0.022±0.003 mg CE/g dry tissue).

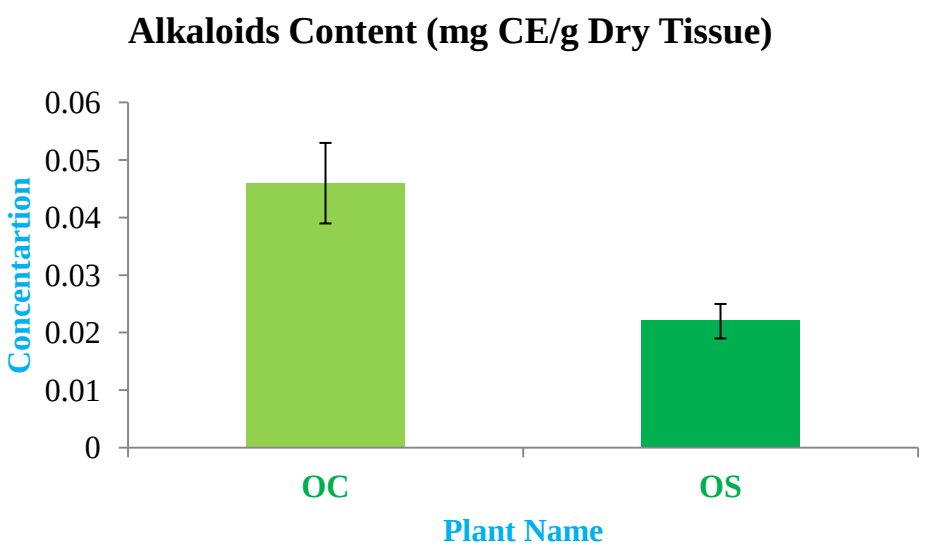

Figure 4: Total Alkaloids Content (mg CE/g Dry Tissue)

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 (Figure 5) in OC leaves showed the lowest amount (53.44±1.37 mg DE/g dry tissue), and OS leaves exhibit the highest amount (64.76±2.65 mg DE/g dry tissue).

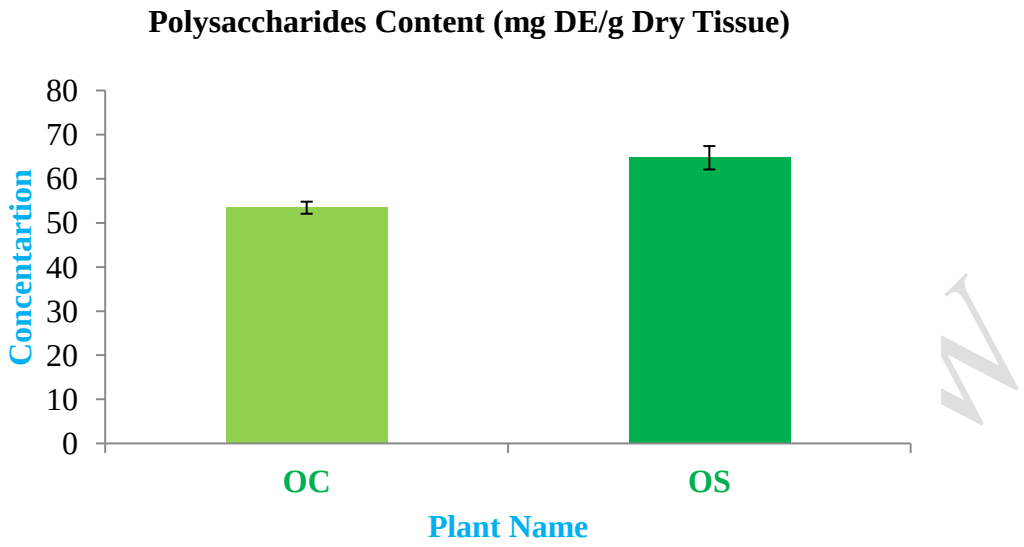

Figure 5: Total Polysaccharides Content (mg DE/g Dry Tissue)

Protein

Total soluble protein content was determined with respect to the BSA standard curve (R^2=0.997). The total protein contents of both OC and OS were quantified. It was observed that the protein content (Figure 6) in OC leaves showed the highest amount (4.70±0.15 mg BSAE/g dry tissue), and OS leaves exhibited the lowest amount (4.25±0.28 mg BSAE/g dry tissue). In the experiment, the p-value>0.05, which did not, showed the significant presence of total protein content in both the plant extracts.

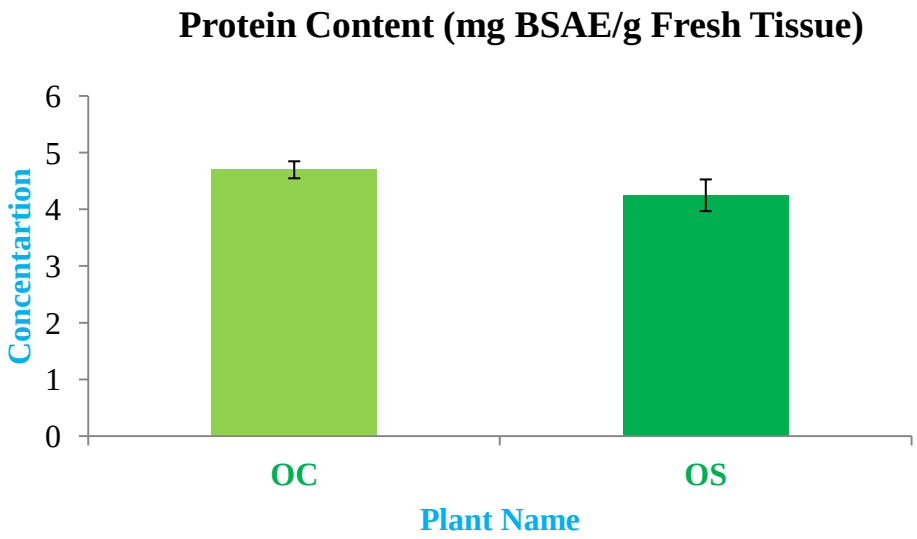

Figure 6: Total Protein content (mg BSAE/g Fresh Tissue)

HPLC-DAD Analysis

A simple, accurate, and productive HPLC online method has been used and validated to identify and estimate phenolics. HPLC profile analysis obtained from the experimental plants 70% ethanol extract identified nine phenolics: Gallic acid, chlorogenic acid, caffeic acid, syringic acid, p coumaric acid, sinapic acid, coumarin, quercetin, and kaempferol (Figure 7 & 8). Among these, eight compounds were present in both the plant extracts. In OS, highest ten compounds were identified. In OC lowest eight compounds were identified. The HPLC-DAD Chromatograms are represented in figures (Figure 7 and Figure 8).

Figure 7: OC Chromatograms

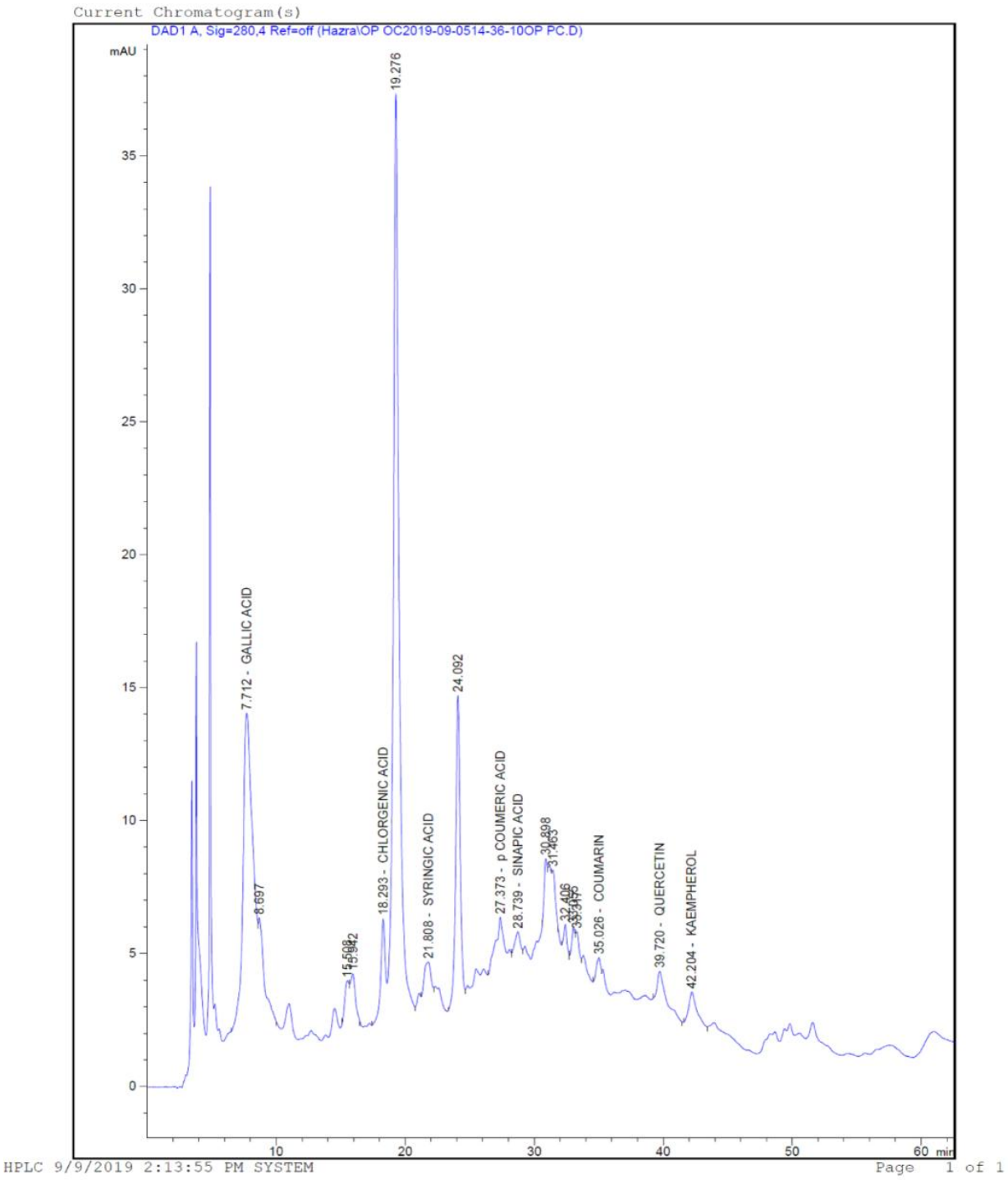

Figure 8: OS Chromatograms

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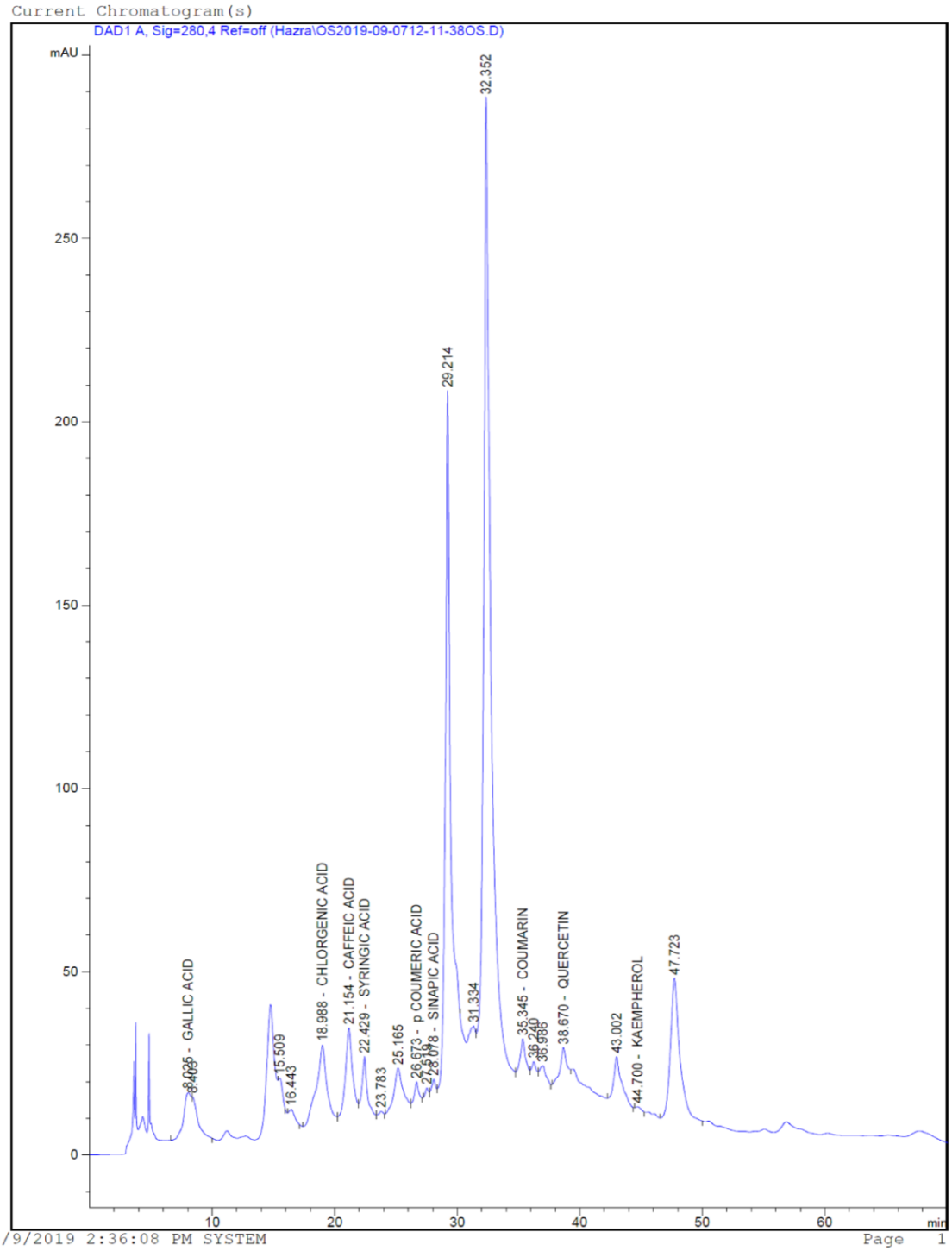

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DPPH radical scavenging activity

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 (Figure 9).

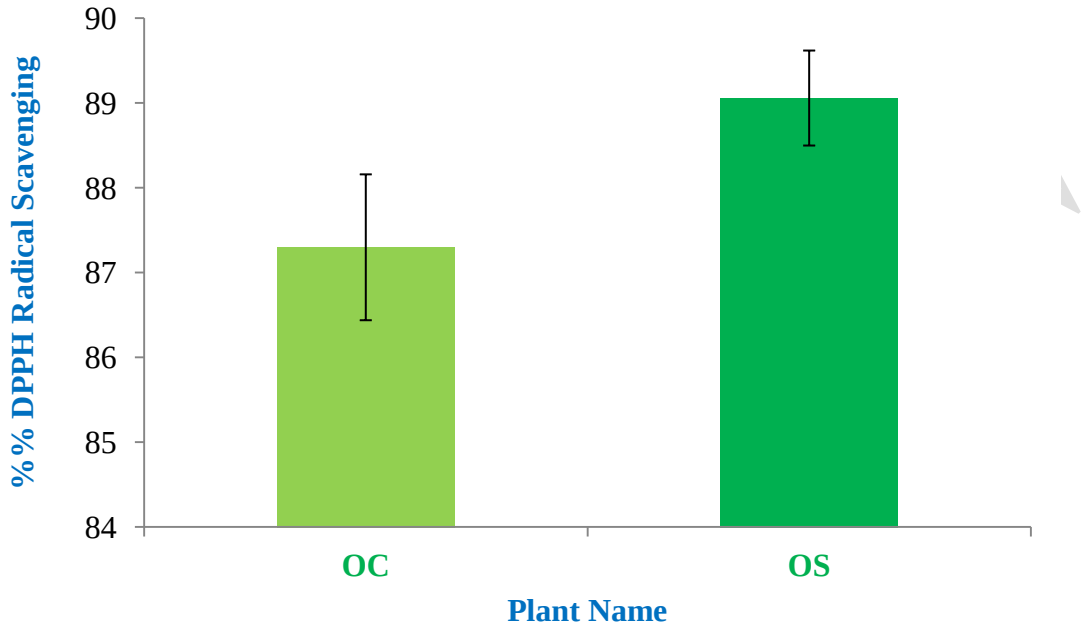

Figure 9: % DPPH Free Radical Scavenging Assay

Leaf hydroalcoholic extracts of OC and OS showed 77.63±0.67% and 82.15±0.68% H_2O_2 free radical scavenging activity, respectively, whereas the standard Gallic acid showed at the same concentration 92.96% H_2O_2 free radical scavenging activity (Figure 10).

In both assays, OS leaf shows a higher antioxidant potential than OC leaf extract.

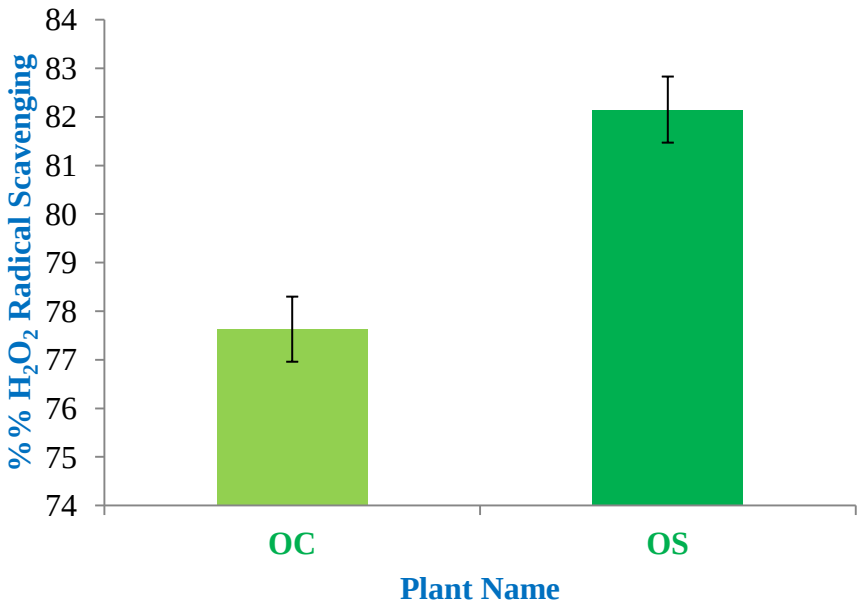

Figure 10: % H_2O_2 Radical Scavenging Assay

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 (Figure 11 & 12). Furthermore, it gives the highest zone of inhibition against EC (Figure 13 & 14). On the other hand, OS shows a comparatively less zone of inhibition against these two strains.

Antimicrobial Activity of OC:

Figure 11: SA

Figure 12: EC

Antimicrobial Activity of OS:

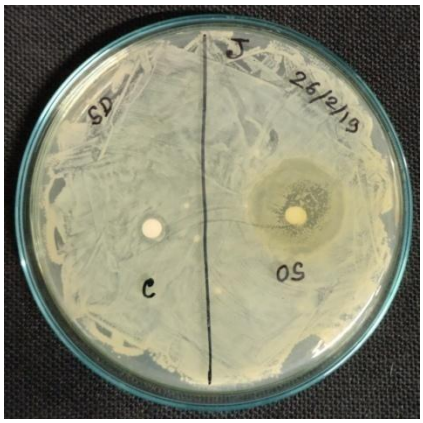

Figure 13: SA

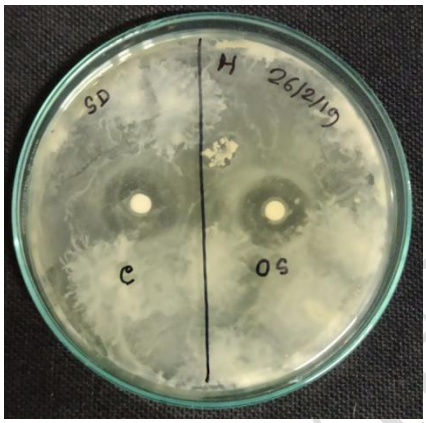

Figure 14: EC

Table 3: Zone of Inhibition (mm)

Organisms Name	Zone of Inhibition (mm)					
	OC	**Control**	**Net zone**	**OS**	**Control**	**Net Zone**
EC	24	16	8	16	14	2
SA	16	10	6	6	14	8

***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 (Figure 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.

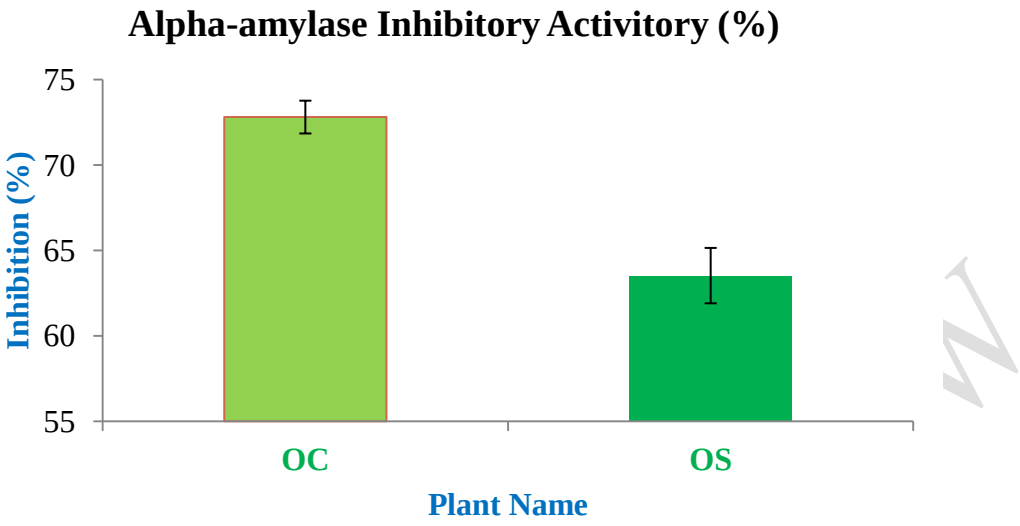

Figure 15: *In Vitro* Anti-diabetic Activity by Alpha-amylase Inhibition (%)

Table 4: IC_{50} of Standard Drug Acarbose and Plant Extracts in Alpha-amylase Inhibition

Sample	**IC_{50} Values (mg/ml)**
Acarbose	3.55
OC	4.50
OS	5.21

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 (Ghosh et al., 2020). 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.*).

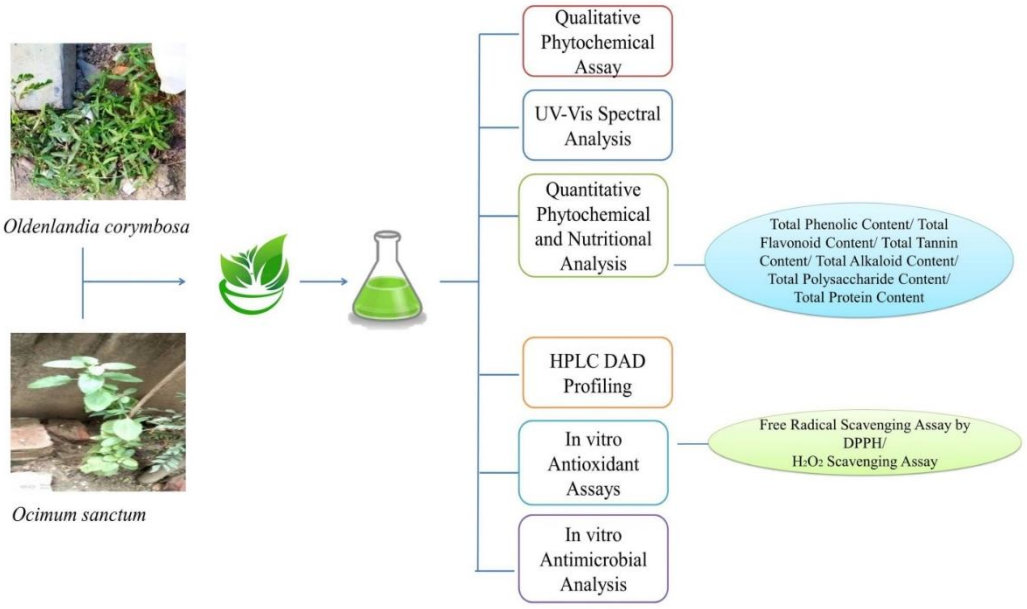

Figure: 16 Overall Work flow of the current research work

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. (Sahoo et al., 2018; Malliga et al., 2014). 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 [Dhivya S. M., and Kalaichelvi K., 2017]. 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 (Chandha et al., 2009; Fukumoto et al., 2000). 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.

Next, we measured the TFC of the leaf hydroalcoholic extracts of the two plant's leaves because flavonoids are a family of polyphenolic compounds with tremendous therapeutic importance. Vegetables and fruits are rich sources of flavonoids and are consumed as 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. Tannins categories of compounds precipitate proteins and organic substances like amino acids, alkaloids, and nitrogenous components. Tannin-protein complex can provide persistent pharmacological properties (Cowan MM, 1999; Chandha et al., 2009). Alkaloids are necessary secondary metabolites, and it has enormous applications in phytomedicine. In addition, it is observed that alkaloids have extensive uses in antimicrobial treatment purposes (Fazel et al., 2008; Biju et al., 2014). 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 highly applied in preparing many industrial drugs (Harshal et al., 2011). In addition, polysaccharides can be easily oxidized to produce instant energy, and their polymers also can act as storage molecules (Begum et al., 2018; Horo et al., 2015). Protein is an essential nutrient component, and it is also a primary metabolite needed for human body functions (Ghosh P & Chatterjee S, 2020). 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.

In the current research study, a simple, authentic, and reproducible online HPLC-DAD technique has been applied to identification and profiling of phenolic acids. Phenolic acids and flavonoids class of compounds are available in a plant leaf, stem, roots, etc., as well as plant-derived natural products in high concentration. Phenolic acids are well known for their active scavenging properties to Reactive Oxygen Species (ROS). Phenolics are sub-categorized as benzoic acid and

cinnamic acid backbone structure, which contains seven and nine carbon atoms, respectively. 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, OC contains a higher amount of Gallic acid and Kaempferol with respect to OS, as evident from chromatograms. Like other phenolic acids, Gallic acid and Kaempferol can help cure oxidative stress-related diseases like diabetes and microbial pathogenesis (Hazra et al., 2018). They have anticancer activity. Kaempferol have estrogen modulatory activity (Wang et al., 2009). A previous study showed that caffeic acid is the primary factor for antioxidant activities. Sinapic acid, phenolic compound are also known for their antioxidant and antimicrobial activities (Ghosh P & Das C et al., 2020).

Since both the plants contain high TPC and TFC, then 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 (Ghosh P & Biswas S et al., 2018; Chandha et al., 2009; Cowan MM, 1999). 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 (Ghosh P & Biswas S et al., 2018; Chandha et al., 2009; Fukumoto et al., 2000; Hazra et al., 2018; Sahoo et al., 2018; Ruch et al., 1989; Patel et al., 2010).

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 (Ghosh P & Das C et al., 2020). Phenolic acids and other bioactive compounds create barriers in the synthesis of nucleic acids of both Gram-negative and Gram-positive bacteria

(Malliga et al., 2014; Cowan MM, 1999; Vinoth B et al., 2012; Sen et al., 2002; Ghosh P & Biswas M et al., 2019).

The *in vitro* anti-diabetic property determination of two experimental plant leaves extracts was done by the α-amylase enzyme inhibition method. 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, (Figure 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 phenolics, alkaloids, flavonoids, and tannins. 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 (Balaji et al., 2015; Srinivasan et al., 2016; Ghosh P & Das C et al., 2020).

Traditional medicinal plants are the prime source of clinically applied plant-derived polyphenol or other bioactive substances that act as antioxidants (Jagadeesan et al., 2011). 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.

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 utilized medicinal herbs becomes essential for industrial product development. In this study, 70% ethanol extracts of OS and OC 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 phenolics, flavonoids, tannins, and alkaloids are present in OC compared to 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 H_2O_2 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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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 "herbal" which 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.

AUTHORS CONTRIBUTIONS:

Conceptualization, SD, PG, SC; **Data Curation**, SD, PG, SC; **Formal Analysis**, SD, PG, CG, MS, AH; **Investigation**, SD, PG, CG, MS, SC, A, H; **Methodology**, PG, SC, AH; **Resources**, SC; **Software**, SD, CG, MS; **Supervision**, SC; **Validation**, SD, PG, CG, MS; **Visualization**, SD, CG, MS; **Writing-Original Drafting**, SD, PG; **Writing- review and editing**, SD, PG, CG, SC; MS, A. H;

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REFERENCES

Balaji RM, Chitra J, Sundaram KM, Ramasamy MS. Studies on anti-diabetic activity of Indian medicinal plants using alpha-amylase and alpha-glucosidase inhibitory activity- A pathway to anti-diabetic drugs. World Journal of Medical Sciences. 2015; 12 (3): 207-212.

Begum HA, Hamayun M, Shad N, Yaseen T, Asad F. Nutritional analysis of some selected medicinal plants of Khyber Pakhtunkhwa, Pakistan. Pure and Applied Biolog. 2018; 7 (3): 955-964.

Biju J, Sulaiman CT, Satheesh G, Reddy VRK. Spectrophotometric estimation of total alkaloids in selected *Justicia* species. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6 (5): 647-648.

Broadhurst RB, Jones WT. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture. 1978; 29 (9): 788-794.

Chandha S, Dave R. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research. 2009; 3 (13): 981-996.

Cowan MM. Plant products as antimicrobial agents. Clinical Microbio. Reviews. 1999; 12: 564-582.

Das S, Mondal N, Mondal S, Ghosh P, Ghosh C, Das C, Chatterjee S. Botanical features,

phytochemical and pharmacological overviews of *Oldenlandia corymbosa* Linn.: A brief review. The Pharma Innovation Journal. 2019; 8 (2): 464-68.

Das S, Saha M, Ghosh P et al. Green synthesis of silver nano-particles using *Oldenlandia corymbosa* and *Ocimum sanctum* leaf aqueous extract. International Journal of Pharmaceutical Sciences and Nanotechnology. 2020, 13 (3): 4959-4964.

Dhivya S. M., Kalaichelvi K. UV-Vis spectroscopic and FTIR analysis of Sarcostemma brevistigma, wight. and arn. *International Journal of Herbal Medicine.* 2017; 9(3):46–49.

Fazel S, Hamidreza M, Rouhollah G, Mohammadreza V. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Journal of Applied Horticulture. 2008; 32 (1): 17-20.

Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activity of phenolic compounds. J Agric Food Chem. 2000; 48 (8): 3597-3604.

Ghosh P, Biswas M, Biswas S, Dutta A, Hazra L, Nag SK, Sil S, Chatterjee S. Phytochemical screening, antioxidant and antimicrobial activity of leaves of *Cleome rutidosperma* DC. (Cleomaceae). Journal of Pharmaceutical Sciences and Research. 2019; 11 (5): 1790-1795.

Ghosh P, Biswas S, Biswas M, Dutta A, Sil S, Chatterjee S. Morphological, ethno biological and phytopharmacological attributes of *Tridax procumbens* Linn. (Asteraceae): A review. International Journal of Scientific Research in Biological Sciences. 2019; 6 (2): 182-191.

Ghosh P, Biswas S, Dutta A, Biswas M, Das S, Das C, Ghosh C, Chatterjee S. Evaluation of phytochemical constituents and antioxidant property of leaf acetone extracts of five herbaceous medicinal weeds. Journal of Pharmaceutical Sciences and Research. 2019; 11 (8): 2806-2813.

Ghosh P, Chatterjee S, Das P, Karmakar S, Mahapatra S. Natural habitat, phytochemistry and pharmacological properties of a medicinal weed-Cleome Rutidosperma DC. (Cleomaceae): A comprehensive review. International Journal of Pharmaceutical Sciences and Research. 2019; 10 (4): 1605-12.

ORIGINAL RESEARCH ARTICLE

Ghosh P, Chatterjee S. Evaluation of organoleptic, proximate parameters and analysis of nutritional composition of five wild weeds: A search for low-cost nutraceuticals. International Journal of Pharmaceutical Sciences and Research. 2020, 11 (10): 5170-5181.

Ghosh P, Das C, Biswas S et al. Phytochemical composition analysis and evaluation of *In Vitro* medicinal properties and cytotoxicity of five wild weeds: A comparative study. F1000Research (Taylor & Francis). 2020, 9: 493.

Ghosh P, Kulavi S, Nandi S, Sengupta T, Biswas M, Das P, Das C, Chatterjee S. Green synthesis and characterization silver nano-conjugates using *Heliotropium indicum* and *Glycosmis pentaphylla* leaf aqueous extracts. Journal of Nanoscience, Nanoengineering & Applications. 2019; 9 (2): 22-30.

Dhivya S. M., Kalaichelvi K. UV-Vis spectroscopic and FTIR analysis of Sarcostemma brevistigma, wight. and arn. *International Journal of Herbal Medicine.* 2017;9(3):46–49.

Ghosh P, Saha M, Nandi S, Sengupta T, Kulavi S, Das S, Chatterjee S. Green synthesis and characterization of silver nano-conjugates using some common medicinal weeds leaf aqueous extracts. International Journal of Pharmaceutical Sciences and Nanotechnology. 2020; 13 (1): 4752-4758.

Ghosh, P.; Biswas, M.; Biswas, S.; Dutta, A.; Hazra, L.; Nag, SK; Sil, S.; Chatterjee, S. Phytochemical screening, antioxidant and antimicrobial activity of leaves of *Cleome rutidosperma* DC. (Cleomaceae). JPSR. 2019, 11 (5), 1790-1795.

Hanfer M, Cheriet T, Menad A, Seghiri R, Ameddah S. HPLC profile and *in vitro* antioxidant properties of the *n*-butanol extract of *Linaria tingitana* Boiss. and Reut. JCPR. 2019; 11 (3): 50-58.

Harshal AP, Priscilla MD. Spectrophotometric estimation of total polysaccharides in *Cassia tora* gum. Journal of Applied Pharmaceutical Science. 2011; 01 (03): 93-95.

Hazra AK, Sur TK, Chakraborty B, Seal T. HPLC analysis of phenolic acids and antioxidant activity of some classical ayurvedic Guggulu formulations. Int. J. Res. Ayurveda Pharm. 2018; 9 (1): 112-117.

ORIGINAL RESEARCH ARTICLE

Horo S, Topno S. Study and analysis of nutritional value of some wild and semi wild edible plants consumed by "HO" tribes of W. Singhbhum district, Jharkhand, India. International Journal of Herbal Medicine. 2015; 3 (5): 25-32.

Hussain AZ, Kumaresan S. Phytochemical and Antimicrobial Evaluation of *Oldenlandia corymbosa*. Asian Journal of Plant Science and Research. 2013, 3(4): 155-158.

Jagadeesan P, Prasad DA, Pandikumar P, Ignacimuthu S. Antioxidant and free radical scavenging activities of common wild greens from Tiruvallur District of Tamil Nadu, India. IJNPR. 2011; 2 (2): 156-163.

Kalyan KP, Rupesh Kumar M, Kavitha K et al. Pharmacological Actions of *Ocimum sanctum*: Review Article. International Journal of Advances in Pharmacy, Biology and Chemistry. 2012; 1 (3): 406-414.

Kirtikar KR, Basu BD. Indian Medicinal Plants. Periodical Experts Books Agency. 2nd Edition. New Delhi. 1991; 3: 47-49.

Kulkarni KV, Adavirao BV. A review on: Indian traditional shrub Tulsi (*Ocimum sanctum*): The unique medicinal plant. Journal of Medicinal Plants Studies. 2018; 6 (2): 106-110.

Malliga, E.; Dhanarajan, M.S.; Rajalakshmi, A.; Jayachitra, A.; Pardhasaradhi, M.; Narasimharao, B. Analysis of phytochemicals, antibacterial and antioxidant activities of *Moringa oleifera* Lam. leaf extract- An in vitro study. Int. J. Drug Dev. & Res. 2014, 6 (4), 173-180.

Noiarsa P, Ruchirawat S, Otsuka H, Kanchanapoom T. Chemical constituents from *Oldenlandia corymbosa* L. of Thai origin. Journal of Natural Medicines. 2008; 62: 249-250.

Patel A, Patel A, Patel A, Patel NM. Estmation of flavonoids, polyphenolic content and *in vitro* antioxidant capacity of leaves of *Tephrosia purpurea* Linn. (Leguminosae). International Journal of Pharma Sciences and Research. 2010; 1 (1): 66-77.

Patel T, Jain V, Rajesh Dodia R. *Oldenlandia corymbosa* L.: A Phytopharmacological review; International Journal of Phytopharmacy. 2014; 4 (3): 79-82.

Rani N., Sharma S., Sharma M. Phytochemical analysis of *Meizotropis pellita* by FTIR and UV-VIS spectrophotometer. *Indian Journal of Science and Technology*. 2016;9(31):1–4.

Ruch RJ, Cheng S, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10 (6): 1003-8.

Sahoo A, Marar T. Phytochemical analysis, antioxidant assay and antimicrobial activity in leaves extracts of *Cerbera odollam* Gaertn. Pharmacog J. 2018; 10 (2): 285-92.

Sandip G. Buddhadev, Sheetal S. Buddhadev, Niraj D Mehta. A Review Article on *Ocimum Sanctum* Linn. 2014; 2 (1): 1-6.

Sen A, Batra A. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia Azedarach L*. Intl J Current Pharmaceutical Res. 2002; 4 (2): 67-73.

Shamili G, Santhi G. Identification and characterization of bioactive compounds of leaves of *Justicia Gendarussa* Burm. F. Int. J. Sci. Res. in Biological Sciences. 2019; 6 (1): 145-153.

Shen Q, Zhang B, Xu R, Wang Y, Ding X, Li P. Antioxidant activity in vitro of Selenium-contained protein from the Se-enriched *Bifodobacterium animalis* 01. Anaerobe. 2010; 16: 380-386.

Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Methods in Enzymology. 1999; 299: 152-78.

Srinivasan R, Dhanalekshmi MU, Gowri T, Duarah S. *Terminalia paniculata* bark extract for anti-diabetic activity. International Journal of Pharmaceutical Sciences and Research. 2016; 7 (3): 1331-1337.

The Protein Protocols Handbook. 2nd Edition. Edited by: J. M. Walker © Humana Press Inc., Totowa, New Jersy.

ORIGINAL RESEARCH ARTICLE

Vinoth B, Manivasagaperumal R, Balamurugan S. Phytochemical analysis and antibacterial activity of *Moringa Oleifera* LAM. International Journal of Scientific Research in Biological Sciences. 2012; 2 (3): 98-102.

Wang J, Fang F, Huang Z, Wang Y, Wong C. Kaempferol is an estrogen-related receptor α and γ inverse agonist. FEBS Letters. 2009; 583(4): 643-647.

Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999; 64 (4): 555-559.