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

ABSTRACT

Herbs are the important source of substances and these are widely used to treat several disorders for its better function in the human body, minimum toxic effects and for its availability. Two ethnomedicinally important and comparatively less characterized herbs of West Bengal, India. These herbs are *Oldenlandia corymbosa* (Rubiaceae) and *Ocimum sanctum* (Lamiaceae). These two plants were used for the present study. 70% ethanolic (hydro alcoholic) extracts of the two plants leaves were characterized and analyzed for detection and quantification of important phytochemical substances and to investigate *in vitro* antioxidant and pharmacological effects. For the estimation purposes spectrophotometric and HPLC-DAD techniques were used. *In vitro* antimicrobial properties were studied by using the paper disc diffusion method. As per our information's and knowledge, many assays of *Oldenlandia corymbosa* (OC) have been carried out in the study and comparative characterizations of these two ethnomedicinally used plants are reported first time. *Ocimum sanctum* (OS) results showed higher quantity of polyphenols, flavonoids and polysaccharides contents as well as inhibitory values in percentages for antioxidant tests whereas OC showed higher quantity of tannins, alkaloids and protein presence and higher *in vitro* antibacterial and anti-diabetic properties. HPLC-DAD profile analysis of OS and OC 70% ethanolic (hydro alcoholic) extracts helped us to detect and identify the ten and eight important phenolic constituents (Gallic acid, catechin hydrate, chlorogenic acid, caffeic acid, syringic acid, p coumaric acid, sinapic acid, coumarin, quercetin and kaempferol), respectively. The current study concluded that *Oldenlandia corymbosa* has good amount of medicinal substances and it exhibits promising *in vitro* pharmacological effects in comparison with the highly established medicinal herb OS leaves extracts.

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

INTRODUCTION

From ancient time in Indian healthcare system a large quantity of traditionally used medicinal herbs have utilized for treating many human disorders as they contain diverse range of chemical compounds of high therapeutic value. Different phytochemicals such as polyphenols, flavonoids, tannins, alkaloids and few other organic components are known as secondary metabolites of medicinal plants and these compounds has vital function in protection against microbes, insects and others organisms. The wild medicinal plants have been main source of human livelihood and medicines throughout human history. The daily life food consumption of rural population does not depend only on the cultivated products, but natural resources also plays major role on 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).

At present scenario caution should be taken for in taking synthetic drugs as it has huge side effects and high cost. But natural product derived medicines were getting high popularity due to its cost-effectiveness as well as better adjustment and low side effects in the human body. Thus the demand for ethno medicinal treatment has been progressing towards the prosperous and effective research steps for current as well as for new pharmaceutical companies (Kirtikar et al., 1991; Ghosh P & Biswas S et al., 2019; Ghosh P & Das C et al., 2020).

India is the enriched pool of ethnomedicinal plants. From that diverse pattern of flora we have chosen 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 and it is 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. Among these two herbs *Ocimum sanctum* is a very well known and established medicinal plant for its huge ethnomedicinal as well as modern medicinal uses. Another plant *Oldenlandia corymbosa* is a very less characterized and currently with reported several medicinal properties. *Oldenlandia corymbosa* is also known a weed for its nature and growth habitat. These two plants are generally grows in a similar pattern of habitat. Botanically *Ocimum sanctum* also shows its weedy nature and habit. These two medicinally important weeds have huge potentiality in the field of natural medication system as on folkloric information's (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 medicinal herbs different parts has showed the ability to inhibit or regulate the reactive oxygen species (ROS) that is basically the primary source of catalysts. It also initiate the oxidation *in vivo* and *in vitro* mechanism and to generate oxidative stress and cell damage that can results 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 or natural antioxidants has huge role to give protection against oxidative stress-related microbial pathogenesis. To protect the human body from these types pathogenesis intake of natural antioxidants are highly necessary and the medicinal plants are taking its share on that ground (Ghosh P & Das C et al., 2020; Malliga et al., 2014; Vinoth B et al., 2012; Sen et al., 2002).

For controlling the blood glucose level of diabetic patients, α-amylase enzyme inhibitors input are necessary. Evaluations of the in vitro anti-diabetic and free radical inhibition properties are important because it protects various vascular complications of diabetes and related diseases (Balaji et al., 2015; Srinivasan et al., 2016; Ghosh P & Das C et al., 2020).

Medicinal importance of the wild herbs can be evaluated by its phytochemicals and *in vitro* pharmacological assessments (Begum et al., 2018; Ghosh P & Chatterjee S, 2020). So, it is important to take 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. The present research study was framed to detect, quantify and

analyze the phytochemical substances and to determine the *in vitro* antioxidant capacity and *in vitro* antimicrobial and anti-diabetic properties from the leaves hydro alcoholic extracts of these two ethno medicinal herbs by the help of different standard assays and comparison has been done with each other. In this research course very well known medicinal plant *Ocimum sanctum* taken as a standard medicinal herb and this plant compared with the less characterized *Oldenlandia corymbosa*. As per our latest information's, the comparative determinations of these two herbs are reported first. In the study, extracts and powdered herbs leaves were investigated for UV-Vis and HPLC-DAD analysis.

MATERIALS AND METHODS

Collection, Identification and Extraction of Plant Samples

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

Collection of Bacterial Strains

The bacterial strains used for the antimicrobial property evaluation study were taken from Calcutta University, Microbiology Department, West Bengal and India.

Chemicals and Reagents

Various types Chemicals and important reagents were of analytical grade. Folin-ciocalteu, aluminum chloride and ascorbic acid were taken from Merck Life Science, Mumbai. Sodium carbonate and Sodium nitrite both were taken from RFCL Limited, New Delhi. Gallic acid, sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2) were supplied by SD Fine-Chem Limited, Mumbai. Quercetin, Tannic acid was obtained from SRL Pvt. Ltd.

QUALITATIVE ASSAYS

Test for 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) were done by using standard protocol.

UV-Vis Absorption Spectroscopic Screening

In the UV-Vis absorption spectroscopic techniques the medicinal herbs extracts were used for analysis of the peaks. In this technique standard method was utilized. Centrifugation was done of the extracts and then filtered. It was diluted to 1:10 ratio with the respective solvent for spectrophotometer analysis. The extracts were scanned in the 200-800 nm wavelength range by using Spectrophotometer (Model: Systronics117) and the significant peak was taken (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 quantities were tested by applying the Folin-Ciocalteu reagent. The standard curve of the experiment was made by using Gallic acid as standard reagent. The absorbance in this method was taken at 765 nm. The results of the study 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 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 determination was carried out by applying a standardized protocol. Tannic acid was utilized as a standard reagent. The absorbance in this method 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 was estimated by Fazel et al., 2008 method. The absorbance's 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 polysaccharides content was evaluated by using standard method. Dextrose was used as standard reagent. The absorbance in this method was measured at 488 nm. The quantity of polysaccharides was measured in mg Dextrose equivalent/g of dry tissue (Harshal et al., 2011).

Quantification of Total Protein Content

Total protein content was determined according to Lowry method. Bovine serum albumin (BSA) was utilized as standard reagent. The absorbance in this method was taken at 660 nm. The content of protein of the samples was measured in mg BSA Equivalent/g dry tissue (The Protein Protocols Handbook. JM Walker © Humana Press Inc.).

HPLC-DAD Profile of the 70% Ethanol Extracts

The High Performance Liquid Chromatography analysis was carried out by using Agilent Technologies 1260 Infinity liquid chromatography. 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 was consisting 3% aqueous acetic acid and acetonitrile. The solutions were freed in an ultrasonic bath and filtered through 0.22μm membranes. In gradient condition 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 by the standard protocol. The standard curve of the experiment was made by using ascorbic acid. Absorbance of the experiment was taken at 517 nm. The DPPH radical scavenging capacity was measured by Ascorbic Acid Equivalent, as inhibition percentage of the assay was expressed in the below mentioned formula (Shen et al., 2010).

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

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide (H_2O_2) radical scavenging effect was determined by utilizing a standard protocol. Absorbance of the experiment was taken at 230 nm. In the experiment Gallic acid reagent was used. H_2O_2 radical scavenging effect was measured by Gallic Acid Equivalent, inhibition percentage was measured by the formula (Ruch et al., 1989; Patel et al., 2010).

Inhibition of H_2O_2 (%) = Control OD- Sample OD/ Control OD *100

DETERMINATION OF ANTIMICROBIAL ACTIVITY (*IN VITRO*)

Antimicrobial activity study was carried out by using disc diffusion assay. In the study plates kept in incubation condition at 37°C for 16-18 hrs. After incubation period the zone of inhibition was measured. Antimicrobial activity was done against one gram positive i.e. J =*Staphylococcus aureus* and 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 carried out by the investigating the reducing groups generated by hydrolysis of soluble starch with the help of isolated α-amylase. Absorbance of the experiment was taken at 540 nm. In the study acarbose reagent was utilized as positive reaction control at a concentration of 10 mg/ml. The percentage of α-amylase inhibition in this study was measured by 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 and it is showed as the average ± standard deviations. The magnitude of obtained statistical variables like means, standard curve, standard deviations, IC_{50} and one way ANOVA were measured and analyzed by applying MS Excel Software. Statistical significance level was accepted at $P<0.05$.

RESULTS

Qualitative Assay

In these biochemical assays total 10 qualitative experiments were done to detect various phytomolecules that are present in the OC and OS 70% ethanol decoctions. From the results it is observed that six phytochemicals are present in both the samples and one phytochemicals are absent in both the samples (Table 1).

Table 1: Results of Phytochemical Screening

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

Where, "+" presence "-" absent

UV-Vis Absorption Spectrum Characterization

The UV-Vis absorption spectrum profiling of 70% ethanolic plants leave decoctions was characterized at a wavelength from 200 to 800 nm with the obtained peak values and exact

baselines. UV spectrum analysis observed the peaks at UV and Visible range both on 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

It was observed that the total polyphenolic content (Figure 1) in OC leaves showed lowest amount (42.63±1.22 mg GAE/g of dry tissue) and OS leaves exhibit highest amount (47.51±0.88 mg GAE/g of dry tissue). Standard curve was prepared (R^2=0.999). OC plant having more polyphenolic compound indicates significant antioxidant property.

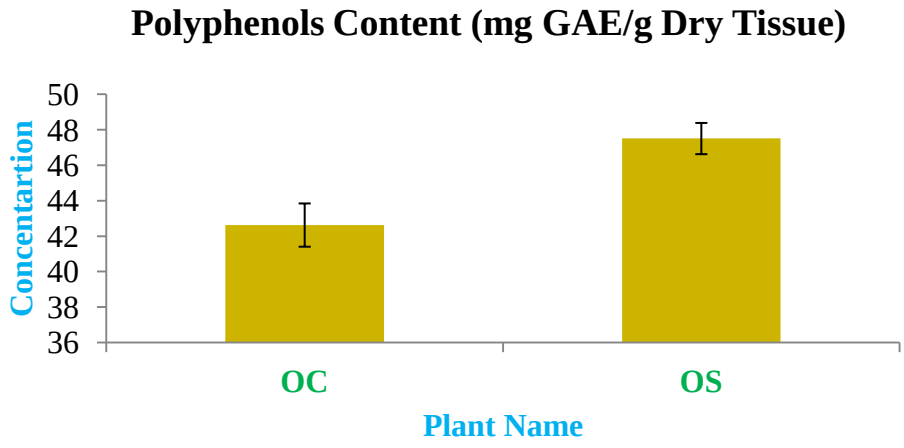

Figure 1: Total Polyphenol Contents (mg GAE/g Dry Tissue)

Flavonoids

It was observed that the total flavonoids content (Figure 2) in OC leaves showed lowest amount (22.60±0.48 mg QE/g dry tissue) and OS leaves exhibit highest amount (27.29±1.21 mg QE/g DW). Standard curve was prepared (R^2=0.994).

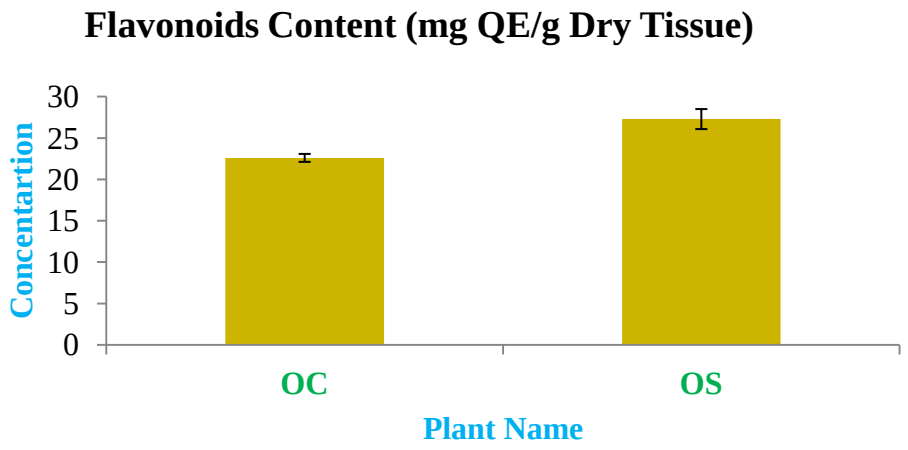

Figure 2: Total Flavonoids Content (mg QE/g Dry Tissue)

Tannins

It was observed that the total tannin content (Figure 3) in OC leaves showed highest amount (19.30±1.51 mg TAE/g dry tissue) and OS leaves exhibit lowest amount (8.65±0.69 mg TAE/g dry tissue). Standard curve was prepared (R^2=0.993).

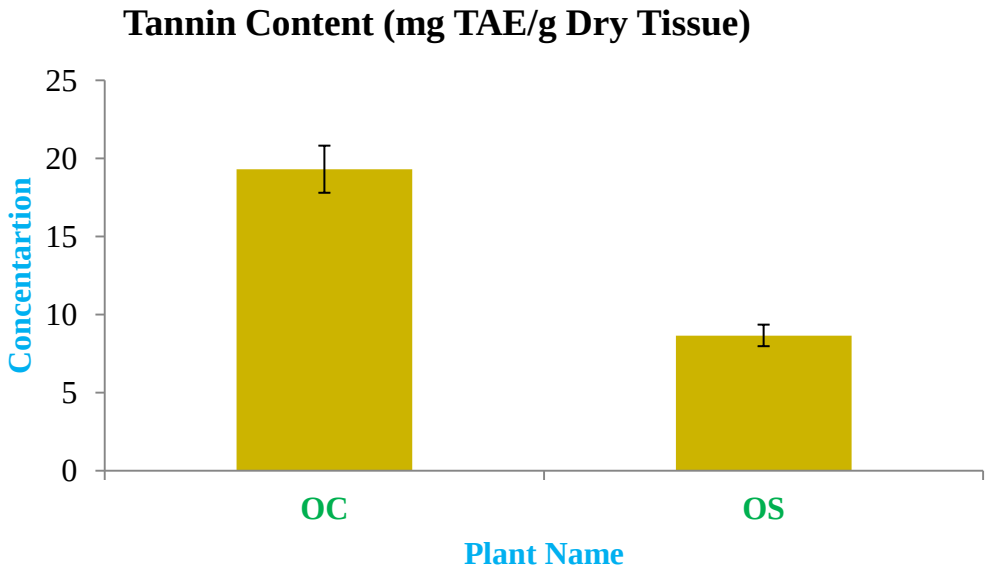

Figure 3: Total Tannin Content (mg TAE/g Dry Tissue)

Alkaloids

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 highest amount (0.046±0.007 mg CE/g dry tissue) and OS leaves exhibit lowest amount (0.022±0.003 mg CE/g dry tissue). Standard curve was prepared (R^2=0.996).

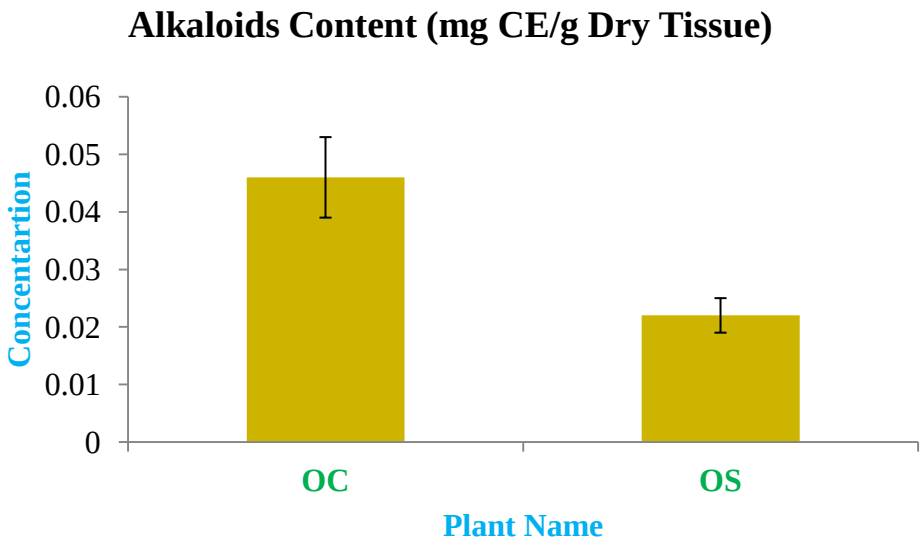

Figure 4: Total Alkaloids Content (mg CE/g Dry Tissue)

Polysaccharides

The polysaccharides contents of both OC and OS were quantified. It was observed that the polysaccharides content (Figure 5) in OC leaves showed lowest amount (53.44±1.37 mg DE/g dry tissue) and OS leaves exhibit highest amount (64.76±2.65 mg DE/g dry tissue). Standard curve was prepared (R^2=0.999).

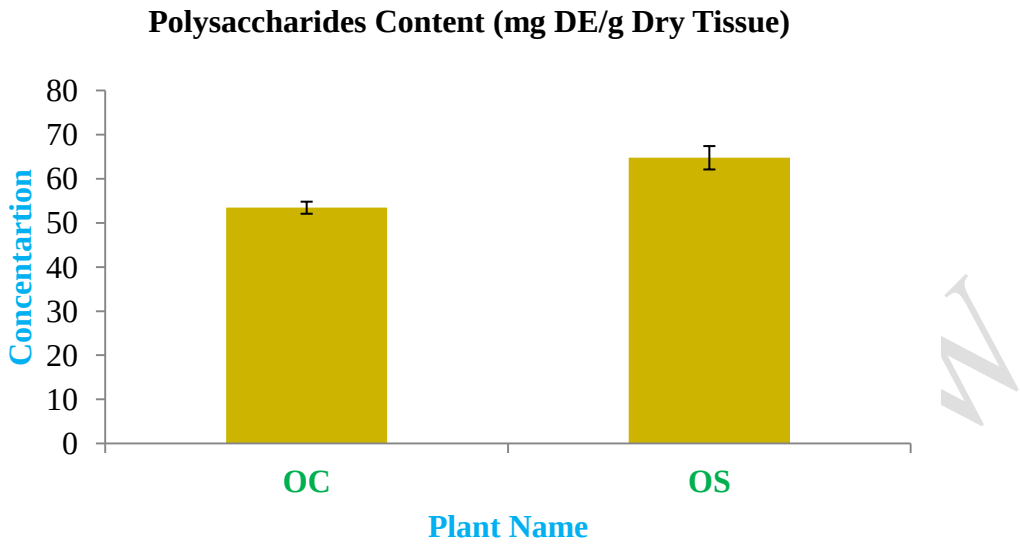

Figure 5: Total Polysaccharides Content (mg DE/g Dry Tissue)

Protein

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

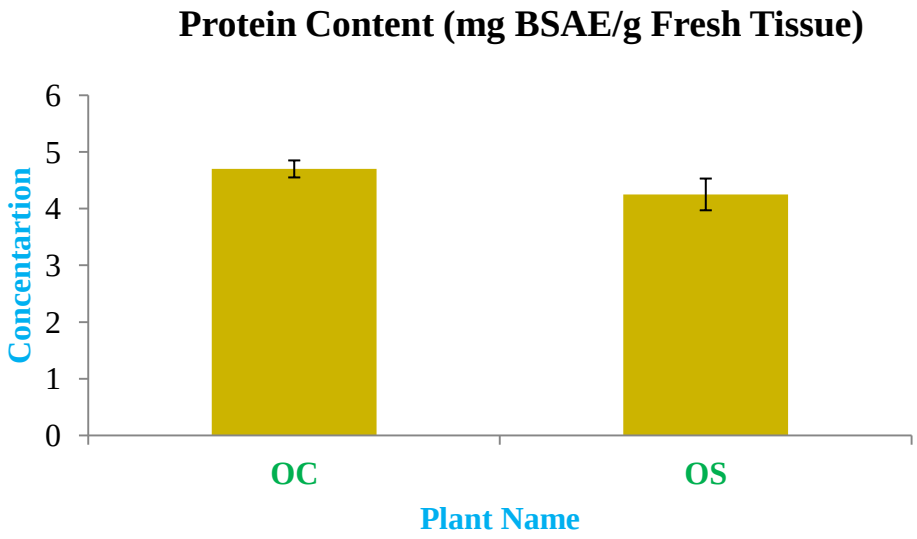

Figure 6: Total Protein content (mg BSAE/g Fresh Tissue)

HPLC-DAD Analysis

A simple, authentic, and productive HPLC online method has been used and validated for the identification and estimation of 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 profile analysis represented in figures.

Figure 7: OC Chromatograms

Print of window 38: Current Chromatogram(s)

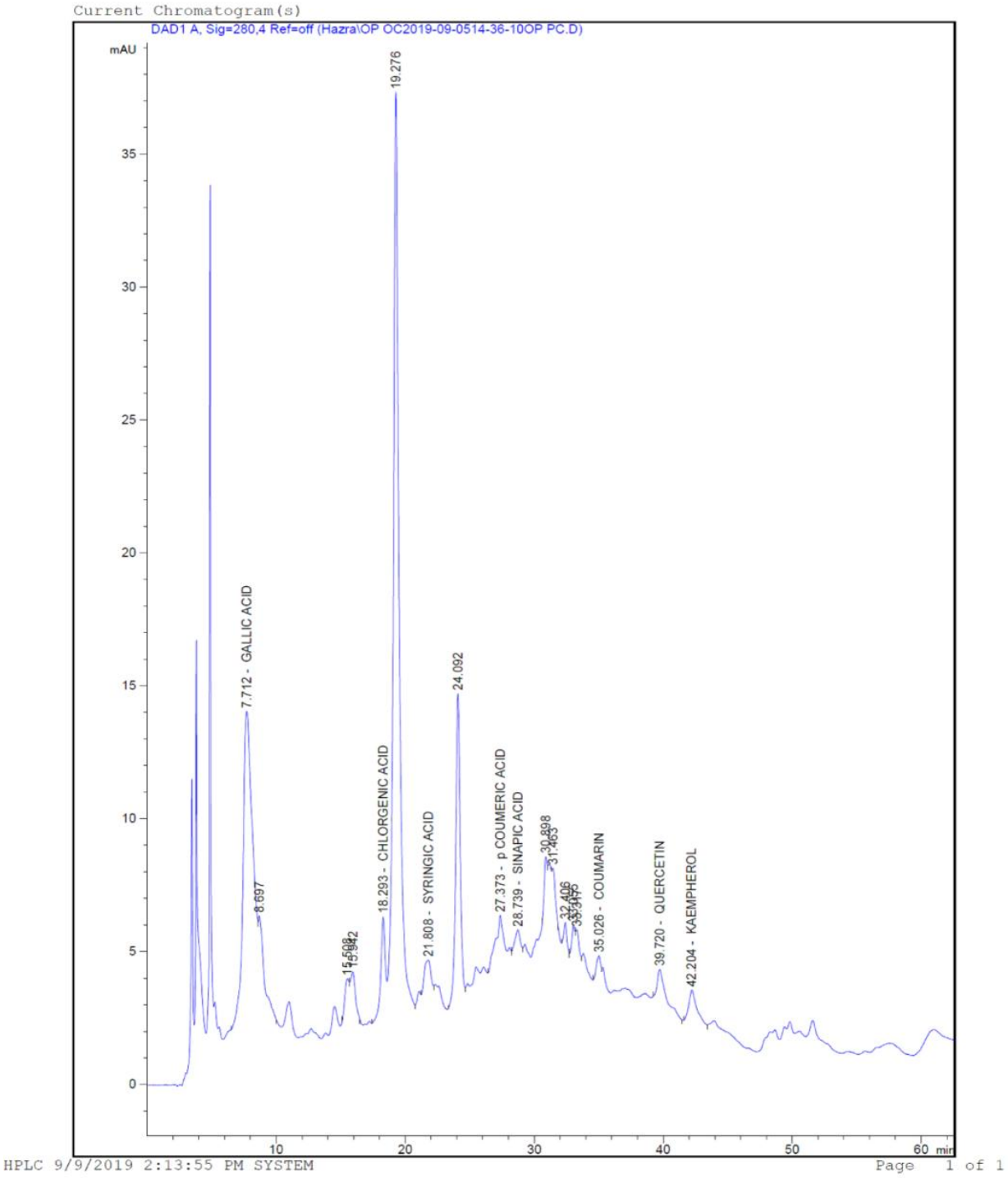

Figure 8: OS Chromatograms

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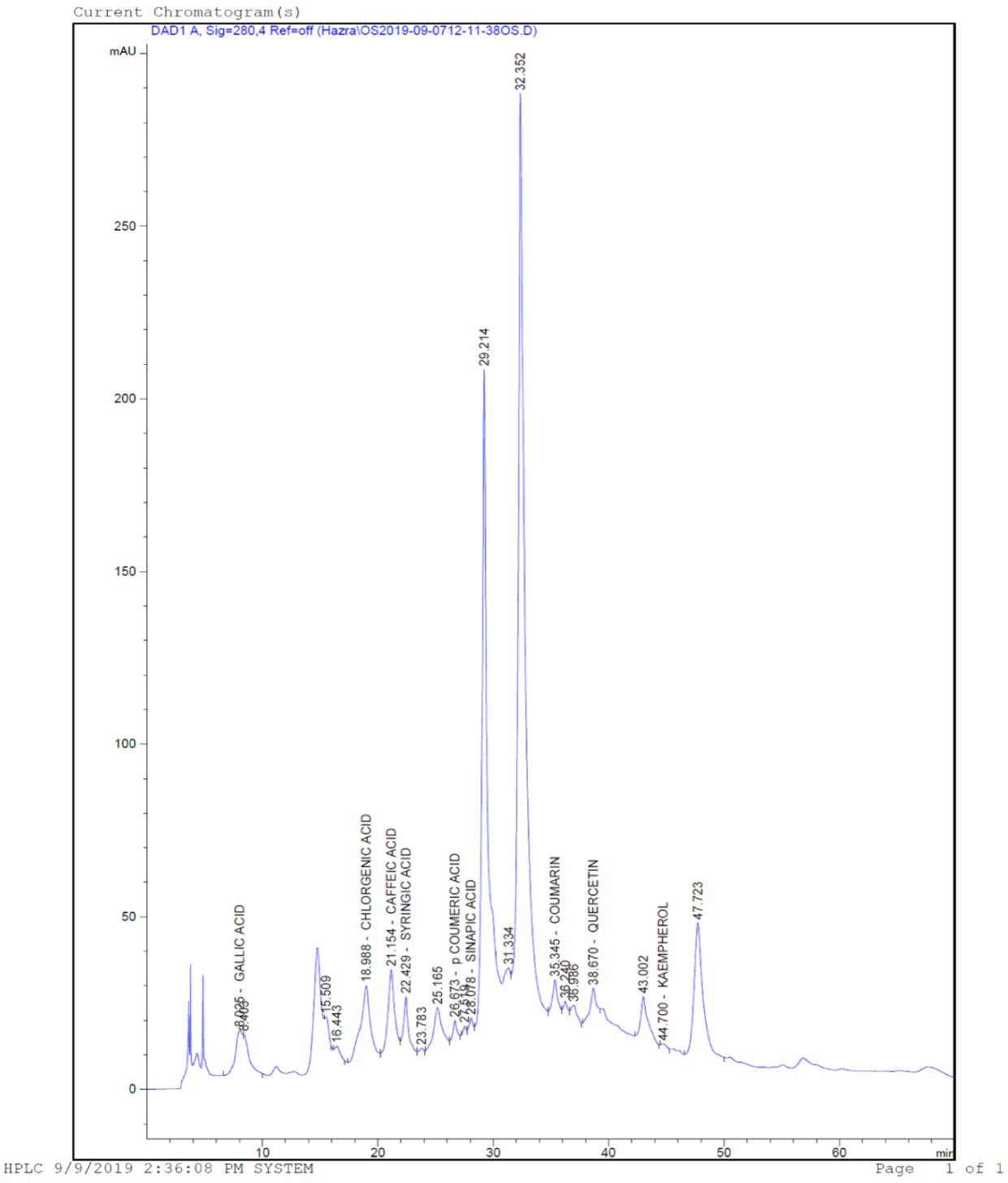

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Image 1: Inhibition of Free Radical Scavenging Assays

The inhibitory capacity of DPPH free radical scavenging test was showed to be 87.30±0.86% for leaf of OC and 89.06±0.56% for leaf of OS as compared to maximum inhibition percentage for standard reagent ascorbic acid which is 93.57% (Figure 9). Standard curve was prepared (R^2=0.996).

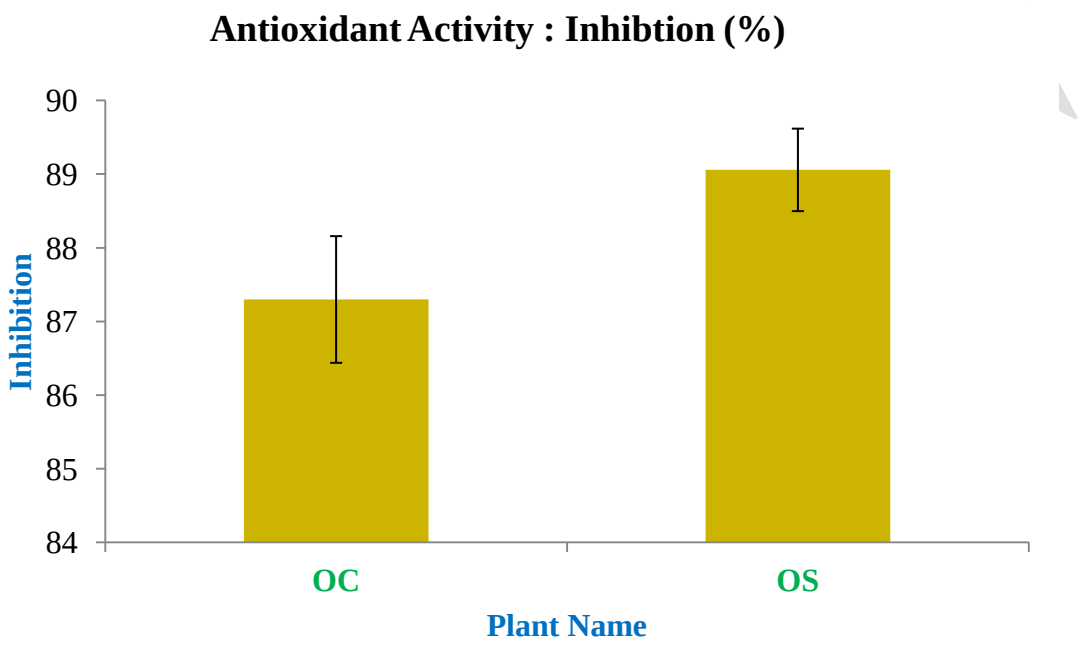


Figure 9: % Inhibition for DPPH Free Radical Scavenging Assay

The inhibitory percentage of the H_2O_2 free radical scavenging test for leaf of OC was observed to be 77.63±0.67% and for leaf of OS it is 82.15±0.68%, as compared to inhibition percentage for standard reagent Gallic acid which is 92.96% (Figure 10). Standard curve was prepared (R^2=0.994). In both scavenging assay OS leaf show higher amount of anti-oxidant property than OC leaf extract.

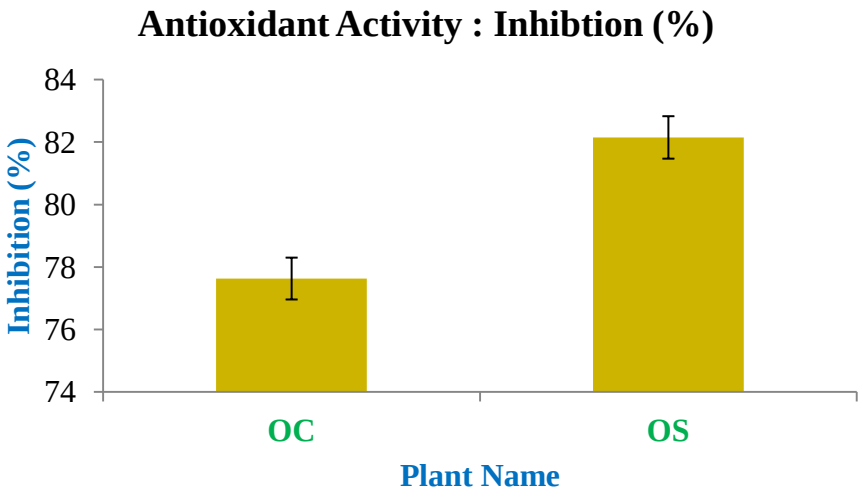


Figure 10: % Inhibition for H_2O_2 Radical Scavenging Assay

Antimicrobial Activity

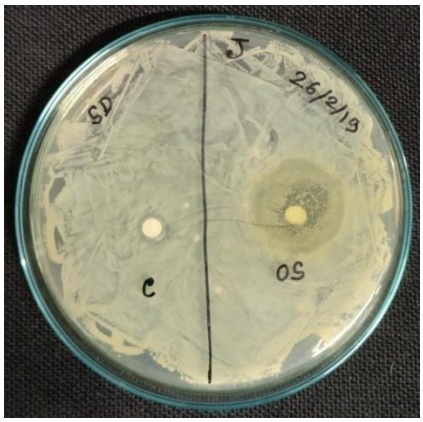
The obtained results of the investigation showed that the 70% ethanol extracts prepared from the leaves of showed inhibitory activity against these two bacteria, shown in Table 3. OC shows zone of inhibition against two strains EC and SA (Figure 11 & 12). It gives highest zone of inhibition against EC (Figure 13 & 14). OS shows comparatively less zone of inhibition against these two strains.

Antimicrobial Activity of OC:

Figure 11: SA

Figure 12: EC

Antimicrobial Activity of OS:



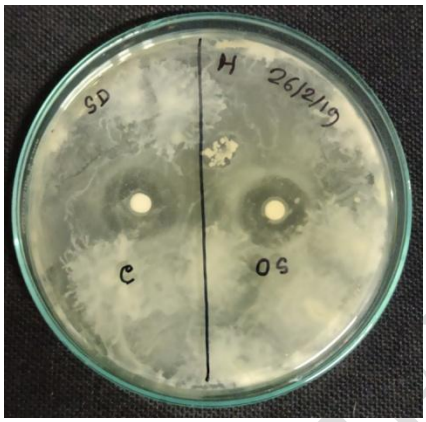


Figure 13: SA **Figure 14: EC**

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 concentrations of leaves extract of OC and OS were selected for the assay between 10 mg/ml and 0.0195 mg/ml. The results of the study showed the maximum inhibition percentage of α-amylase inhibitory experiment was found 72.81±0.96% for OC 70% ethanol extract. The minimum is 63.53±1.62% for OS 70% ethanol extract (Figure 15), and the inhibitory percentage for standard acarbose was found to be 98.69% at 10 mg/ml of concentration. IC_{50} analysis showed that acarbose is needed 3.55 mg/ml, whereas 70% ethanol leaves extracts need higher amount to do IC_{50} (Table 4). Standard curve was prepared (R^2= 0.972).

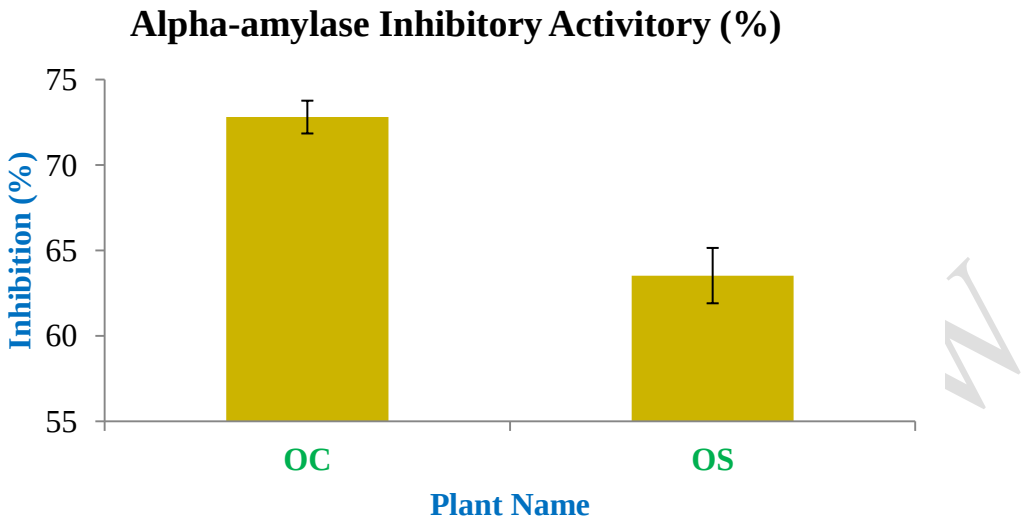


Figure 15: *In Vitro* Anti-diabetic Activity by Alpha-amylase Inhibition (%)

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

The obtained results of the current investigations qualitative studies observed that the experimental herbaceous medicinal plants contains huge amount of bioactive compounds which is necessary for preparing herbal formulations (Sahoo et al., 2018; Malliga et al., 2014).

UV-Vis absorption spectrum profiling of the extracts showed variations in wavelengths ranges. This variation happens due to the surface plasmon resonance of the particles that is present in the sample extracts (Ghosh P & Kulavi S et al., 2019; Ghosh P & Saha M et al., 2020).

Phytochemical composition analysis evaluates the concentration of the specific bioactive constituents in the plants extracts. Polyphenolic components are reactive species towards

oxidation, and it maintains physiological activities. The oxidation reactions and free radicals production leads to few oxidative stress-related physical ailments like diabetes, high blood pressure, arthritis and cancer. Polyphenols acts in the process that can have substantial pharmacological uses in the industrial sector (Chandha et al., 2009; Fukumoto et al., 2000).

Flavonoids are the kind of secondary metabolites with high pharmacological and chelating activities. Antioxidant effects of flavonoids class basically depends on structure and hydroxyl group reduction. Flavonoids antioxidant properties are happens due to many reaction processes and it acts in regulation of free radicals, metal ions chelating, and regulatory activity of enzymes. Earlier investigations showed the protective capacity of flavonoids classes against several microbial pathogenesis (Ghosh P & Biswas S et al., 2018; Chandha et al., 2009; Cowan MM, 1999).

Tannins are primarily found in barks of plants. The maximum quantity of tannins presence showed different properties. Tannins are basically astringent, bitter polymeric substances that can be used for tanning the leathers. Tannins categories of compounds precipitate proteins and organic substances like amino acids, alkaloids and nitrogenous components. Tannin-protein complex can be able to provide persistent pharmacological properties (Cowan MM, 1999; Chandha et al., 2009).

Alkaloids are necessary secondary metabolites, and it has huge applications in phytomedicine. It is observed that alkaloids have extensive uses in antimicrobial treatment purposes (Fazel et al., 2008; Biju et al., 2014).

Polysaccharides are 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. As polysaccharides is inexpensive, non-toxicity showing, and it has the capacity of reactive changes and biologically degradable and for these reasons it is highly applied in preparing many industrial drugs (Harshal et al. 2011). Polysaccharides can be easily oxidized to produce instant energy and its polymers also can act as storage molecules (Begum et al., 2018; Horo et al., 2015).

Protein is highly necessary nutrient components and it is also a primary metabolite, which is needed for human body functions (Ghosh P & Chatterjee S, 2020).

In the current research study, a simple, authentic and reproducible online HPLC-DAD technique has been applied for the identification and estimation of phenolic acids. Phenolics acids and flavonoids class of compounds are highly available plant derived natural products found which have antioxidant and antimicrobial properties. Phenolic acids are well known for its 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 signifies hydroxybenzoic acid and cinnamic acid derivatives of phenolic substances. These phenolics help in curing the oxidative stresses related diseases like microbial pathogenesis and diabetes (Hazra et al., 2018). Previous study showed that caffeic acid is the primary factor for antioxidant activities. Another phenolics categories compound sinapic acid also known for its antioxidant and antimicrobial activities (Ghosh P & Das C et al., 2020).

Phytochemical substances have huge importance in antioxidant activity with an effect in inhibiting the free radicals. DPPH is a widely applied stable radical to examine free radical regulation ability of the plant-derived antioxidants. DPPH free radical inhibitory assay associated with decreasing the DPPH in methanolic decoction in presence of hydrogen-donating natural antioxidant as it forms the non-radical structure of DPPH-H. DPPH is unaffected with some parallel reactions with metal ion chelation and enzymatic inhibitory activities. This technique is a fast, reproducible and consistent biochemical process. This technique also does not interfere with water or other alcohol presence (Ghosh P & Biswas S et al., 2018; Chandha et al., 2009; Fukumoto et al., 2000; Hazra et al., 2018).

H_2O_2 radical scavenging test is utilized for the reduction of free radicals which is present in phosphate buffer solution (pH 6.8) with the hydrogen donating antioxidants as it form the non radical shape of standard free radical. H_2O_2 sometimes becomes toxic to the human cells as it is quickly breaks down into oxygen and water and produces hydroxyl radical which can leads to lipid peroxidation by getting hydrogen atoms from unsaturated fatty acids and can cause DNA strands rupture. This phenomenon can be the prime reason for cancer, mutation and cell death. The study results showed that the two medicinal herbs derived antioxidants can be able to neutralize the hydroxyl radical-induced deoxyribose cleavage in a concentration-dependent way (Ghosh P & Biswas S et al., 2018; Sahoo et al., 2018; Ruch et al., 1989; Patel et al., 2010).

The results of the current study also supports that traditional medicinal plants are the prime source of clinically applied plant derived polyphenol or other bioactive substances which are highly act as antioxidants (Jagadeesan et al., 2011).

The results of the antimicrobial activity research investigation indicated that both the plants leaves extracts had shown antimicrobial property in 70% ethanolic extracts. The major bioactive substances present in the extracts such as polyphenols, flavonoids, tannins and alkaloids as reported previously is basically responsible for the antimicrobial activity. Previous study concluded that plants which are found with caffeic acid presence had shown 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 plants leaves extracts was done by α-amylase enzyme inhibition method. This Inhibitory activity happens with 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 basically generated after the treatment of a specific solution by α-amylase and plant extracts. Bioactive molecules are mainly act 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).

CONCLUSIONS

Herbal medicines are considered huge applications for the management of various diseases due to their effectiveness, availability, low cost, and fewer side effects. Identification of wild and ethnobotanically utilized medicinal herbs is the target for the industrial products. In this study 70% ethanol extracts of OS and OC were observed with enriched important metabolites, and it has sufficient amount of phytomolecules which showed antioxidant and other bioactivities.

Based on the study it is showed that the higher content of phytomolecules like phenolics, flavonoids, tannins and alkaloids are present in these medicinal plants. Nutritional components such as carbohydrate and proteins also present in these two medicinal herbs in good amount.

Antioxidant properties for DPPH and H_2O_2 radical scavenging assay in the extracts were showed in the experiment. The study concludes that folkloric plants are key contributors of naturally occurring antioxidants. Among these two medicinal herbs, OS has highest quantity of polyphenols and flavonoids contents and the highest antioxidant properties, too.

The antimicrobial activity was found to be moderate in case of both medicinal herbs. The therapeutic standpoint had focused on decreasing the hyperglycemia, which can be achieved through the inhibition of α-amylase. The current study concluded that 70% ethanol extracts showed the enzymatic inhibitions in the anti-diabetic activity. OC showed better inhibition capacity than OS.

In conclusions, the present study indicates that OC possesses a considerable amount of phytochemicals which is comparable with the standard medicinal herb OS. As OS have huge herbal applications in worldwide. The present investigation clearly spotted out that the OC extract possesses significant amount of bioactive compounds in comparison with OS extracts. Additionally, the OC extracts were able to inhibit alpha amylase enzyme effectively when compared with OS extracts.

Our findings provide a basic relationship between bioactive compounds and free radical scavenging properties as well. In future, *Oldenlandia corymbosa* can be utilized to separate, detect and identify active components in the 70% ethanol extract 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.

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