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PHYTOCHEMICAL SCEENING, TPC, TFC AND ANTIBACTERIAL ACTIVITY AGAINST UTI PATHOGEN OF OCIMUM SANCTUM PLANT LEAF HYDRO ALCOHOLIC EXTRACT

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ABSTRACT

Hydroalcoholic extract of Ocimum sanctum plant leaf has been routinely assessed as a rich source of therapeutic compounds with tremendous applications in the pharmaceutical industry to identify new sources of phytochemicals antimicrobial agents. It is commonly used for treating different ailments in the Ayurveda sidda medicine system. Total phenolic compounds, Total Flavonoid Compounds, and antibacterial activity against two UTI (E.coli & Pseudomonas) pathogens were used in this study phytochemical test through traditional methods. Furthermore, several forms of Phyto-compounds and potent antibacterials have been found.

INTRODUCTION

In literature dating back several thousand years, medicinal plants' uses in traditional medicine have been identified [2]. Quite old and thoroughly used in traditional medicinal Tulsi plants. A widely revered culinary, medicinal aromatic herb is Tulsi in Hindi, or Tulsi in Sanskrit (holy basil in English). Ocimum sanctum (Tulsi) is a plant for preventing and treating diseases, ranging from traditional and standard medicinal products in all countries to herbal extracts. The Ocimum sanctum Tulsi family is grown in India and Southeast Asia, with India being the world's largest medicinal plant source. The market for this plant for medicinal purposes is growing day by day [6]. It is used to treat various diseases such as antimicrobial infection, anticancer, chronic fever, eye disease, antifungal, antispasmodic, etc. [13].

The nature of compounds present in the Hydroalcoholic extract of Ocimum sanctum can be established by phytochemical analysis of this medicinal herb. It is also for the identification and effect of biologically effective components. There are many large classes of compounds that play a role in the plant activity of phytomedicine. That includes alkaloids, coumarins, phenolic acid, tannins,

flavonoids, and phenolics. As a model for synthetics of new medicine, they are generally helpful [11]. In the treatment of bacterial infections, second thoughts on antibacterial medicines are used. Prolonged use of such antibacterial drugs will reduce the amount of intestinal flora, which can negatively affect health. Antibiotics, including hypersensitivity, immune suppression, and allergic reactions, are often associated with adverse effects. This problem forced researchers to look for new antimicrobial material. Therefore, an alternative antimicrobial drug from a medicinal plant to treat infectious diseases needs to be created [4-5]. Tulsi may be a Cox-2 (cyclooxygenase-2) inhibitor, an alcoholic tulsi extract that modulates immunity, like many painkillers, thus promoting immune system function [9, 12]. Tulsi is one of the successful immunomodulators. Therefore, the phytochemical evaluation of Ocimum sanctum leaf Hydroalcoholic extract and antimicrobial activity against two pathogen-E.coli & Pseudomonas was studied in current research.

MATERIAL AND METHODS

PREPARATION OF PLANT EXTRACT

40 GM of Soxhlet-extracted Ocimum sanctum leaf. With alcoholic hydro-solvent (D/W and methanol). The extraction was carried out for 72 hours at 65^0C. To form a paste, extracts were then evaporated at 40^0C and further transferred to sterile and refrigerated until used.

REQUIREMENTS

Acetic acid, Mercuric chloride, Potassium iodide, Mercury, Nitric acid, Napthol, Butanal, Hydrochloric acid, Tannic acid, Sodium chloloride, Sulphuric acid, Cobalt chloride Sodium hydroxide, Copper sulphate, Lead acetate,, Glacial acetic acid, Gelatine, Ferrous sulphate, Methanol, Acetone, etc.

TEST FOR COBAL CLOROID

3 ML measurement solutions combined with 2 ML. Boil cobalt chloride in a water bath at 50^0C for 5 to 10 minutes and apply a few NaOH solution drops to cool. If the solution tends to be declared greenish blue, it means the test is positive.

TEST FOR PROTEIN

Biurets Test: The test solution was treated with 4% NaOH solution, and 2-3 drops of 1% Cuso4 and violet/pink color formation was observed.

TEST FOR STEROIDS

SALKOWSKI REACTION

To 2 ML of extract added 2 ML chloroform and 2 ML conc. H2SO4 shake well. If the layered chloroform appears red and the layered sulfuric acid displays greenish-yellow fluorescence, steroids are indicated.

TEST FOR ANTHRAQUINONE GLYCOSIDES

BORNTRAGER'S TEST FOR ANTHRAQUINONE GLYCOSIDE

To 2 ML extract added dilute H2SO4 boiled and filter two cooled filtrates added equal volume benzene or chloroform shake well then separately added ammonia. If turn pink and red color on ammonia layer that hint presence of anthraquinone glycosides.

TEST FOR FLAVONOIDES

Add the lead acetate solution to the small amount of waste. It forms a yellow colored PPT. After adding acid, increasing quantities of NaOH to the residue shows that the yellow color decolorizes. Presence of flavonoids as seen.

ALKAOIDES TEST

Mayer's Test: If seen, PPT confirms the test as positive, 2 ML of extract filtrate solution and added a few drops of Mayer reagents.

Wagner's Test: 2 ML Extract filtrate solution and applied a few drops of Wagner's reagents, verifying the test if seen radish brown PPT.

TEST FOR TANNIN COMPOUND

Ferric Chloride Test: 2 to 3 ML test solution with 5% Fecl3 solution applied. The presence of tannins was announced by the creation of a deep blue and black color.

TEST FOR PHENOLIK COMPOUND

Iodine Test: 3 ML test solution and the iodine solution was applied to the test tube. Phenol is declared to be present after 1 to 2 minutes of red color formation.

TEST FOR ORGANIC ACID

Confirmatory test for oxalic acid.

Lead Acetate Test: 3 ML test solution and added five percent lead acetate to some drops. The white PPT formation suggested the presence of oxalic acid.

TEST FOR CORBONET

With Mercuric Chloride: 2 ML test solution in the test tube and a few drops of Hgcl2 solution were applied. Brownish red PPT formation, white PPT unfolding with carbonate presence.

TOTAL PHENOLIC CONTENT ASSAY

Total phenolic content ability was calculated using a slightly changed process. The isolated crud's total phenolic content (leaves powder) was measured using the method mentioned [14]. 1 ML of the sample was combined with 1 ML of Folin and the phenol reagent Ciocalteu. After 3 Minutes, 1 ML of saturated Na CO (~35%) was applied to 2 to 3 ML of the mixture, and up to 10 ML of distilled water was added. The reaction was sustained in the dark for 90 Minutes, observed at 760 nM absorption under the UV-Vis spectrophotometer. As normal, tannic acid with varying concentrations from 20 ppm to 100 ppm was used. With different catechol concentrations (0.01- 0.1 mM) as normal, a calibration curve was constructed. The findings were expressed as mg of catechol equivalent/g of extract, and the same method was performed with Plant Leaves Extract.

TOTAL FLAVONOID CONTENT ASSAY

This method was determined to produce flavonoids in isolated crud leaf powder (Piper betel) [8]. Take a sterile test tube and apply 1.25 ML of distilled water to 0.5 ML of the sample (extract). Then 0.075 ML of 5% sodium nitrite solution was added and permitted to stand for 5 Minutes. 0.15 ML of 10% aluminum chloride was added, 0.5 ML of 1.0 M sodium hydroxide was added after 6

minutes, and the mixture was diluted with another 0.275 ML of distilled water. The mixture's absorbance at 510 nm was measured immediately. The flavonoid content was expressed as / g sample mg catechin equivalents. The same procedure was performed with Plant Leaf Extract and the measured formula [7].

TFC = (R×D.F× V×100) /W

When R-outcome obtained from the standard curve, D.F-dilution factor, V-stock solution length, 100 by 100 gm dried plant, W-weight of the experimental plants.

ANTIBACTERIAL ACTIVITY TEST

SELECTION AND SOURCE OF MICROORGANISM

Human patients collected urine samples from the Peoples Hospital and Research Centre Bhopal in pathology and then isolate and found two significant pathogens (E.coli & Pseudomonas) in urine samples at the Bhopal CMBT research centre. Who is mainly responsible for urinary tract infection (UTI).

DISC DIFFUSION METHOD

To test the antimicrobial activity of the extract against the test microorganism, the disc diffusion method [1] was used.

- The known concentration (µg/ml) solutions of the test samples are obtained by dissolving the samples' measured quantity into the determined volume of solvents.
- Dried and sterilized filter paper discs (6 MM in diameter) were then impregnated with a known quantity of micropipette-based test substances. The residual solvent was completely evaporated.
- Discs containing the test material were placed uniformly seeded with the test microorganisms on nutrient agar medium.
- As positive and negative power, regular discs of Ocimum sanctum extract (30µg/disc) and blank discs (impregnated with solvent followed by evaporation) were used.
- These plates were then held for 24 hours at a low temperature (4^0C) to allow full diffusion. There was a gradual shift in the concentration of test materials in the media covering the disks.
- To allow optimum growth of the species, the plates were then incubated for 24 hours at 37^0C.
- The antimicrobial activity test material inhibited the microorganisms' growth and visualized a robust and distinct inhibition zone surrounding the medium.
- The test agents' antimicrobial activity was measured by calculating the inhibition zone diameter expressed in MM (Millimeter).

RESULTS AND DISCUSSION

In the present research, the phytochemical analysis, Total Phenolic Content (TPC) assay and Total Flavonoid Content (TFC) assay, and Antibacterial Activity against E.coli and Pseudomonas bacteria were evaluated using Ocimum sanctum hydro alcoholic extracts.

PHYTOCHEMICAL SCREENING

We found current bioactive compounds in the phytochemical screening test shown in Table 1.

Table 1: Phytochemical Screening Test Results

Phyto Constituents Test	**Performed Test**	**Hydro Alcoholic Extract**
TEST FOR COBAL CLOROID	COBAL CLOROID	+
TEST FOR PROTEIN	Biurets test	+
TEST FOR STEROIDS	Salkowski reaction	+
TEST FOR ANTHRAQUINONE GLYCOSIDES TEST FOR FLAVONOIDES	Borntrager's test for Anthraquinone glycoside	+
ALKAOIDES TEST	Mayer's test Wagner's test	+ +
TEST FOR TANNIN COMPOUND	Ferric chloride test	+
TEST FOR PHENOLIK COMPOUND	Iodine test	+
TEST FOR ORGANIC ACID	Confirmatory test Lead acetate test	+ +
TEST FOR CORBONET	With mercuric chloride	+

TOTAL PHENOLIC CONTENT ASSAY

Total phenolic content (TPC) determination of the total phenolic content was calculated based on a colorimetric reduction based approach using the Folin-Ciocalteu reagent. By extrapolation from the calibration curve (Y = 0.0085x + 0.7702; R2 = 0.9869) prepared from the Tannic acid concentration, the TPC of the plant extracts was resolved and expressed in mg of Tannic acid equivalence (TAE) per gram. The findings are shown below, as shown in Figure 1 and Table 2.

Figure 1: Standard Curve of Tannic Acid Ocimum Sanctum Leaf Extract

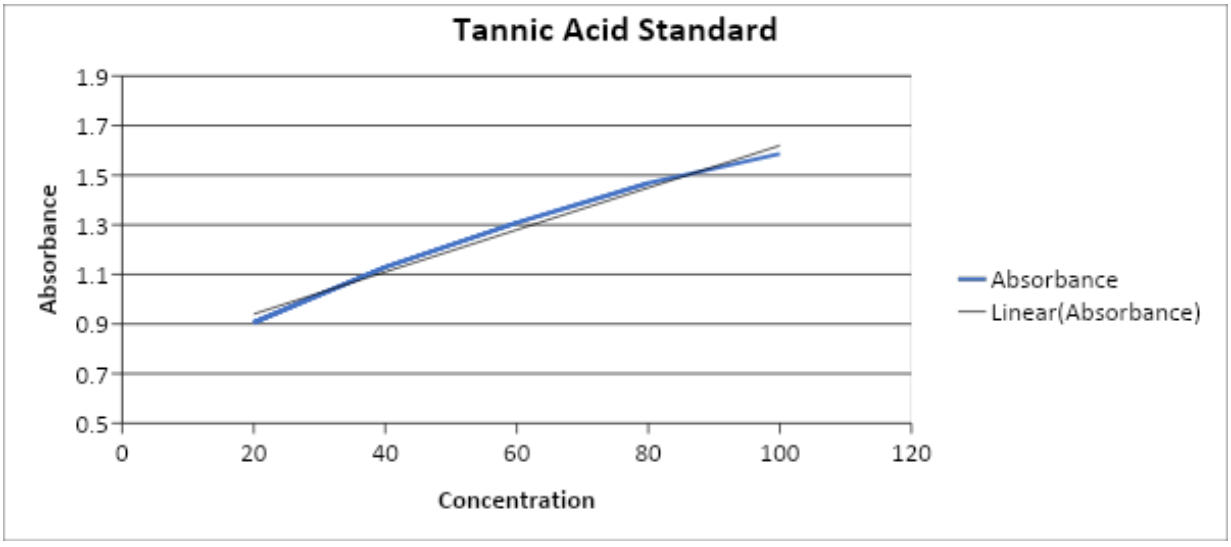


Table 2: Total Phenolic Content of Ocimum Sanctum

QUANTITATIVE ANALYSIS	**O.sanctum Extract**
TOTAL PHENOLS (µg of TAE/serving)	703.119±0.0015

TOTAL FLAVONOID CONTENT ASSAY

The total flavonoid content of the extract was measured according to the aluminum chloride process. For the calculation of the total flavonoid content of the extracts, the aluminum chloride method is used. By extrapolation from the calibration curve (Y = 0.0266x+0.2136; R2 = 0.9968) prepared from the quercetin concentration, the TFC of plant extracts was resolved and expressed in µg of the quercetin equivalence (QE) per ML. The findings are shown below, as shown in Figure 2 and Table 3.

Figure 2: Standard Curve of Quercetin Ocimum Sanctum Plant Leaf Extract

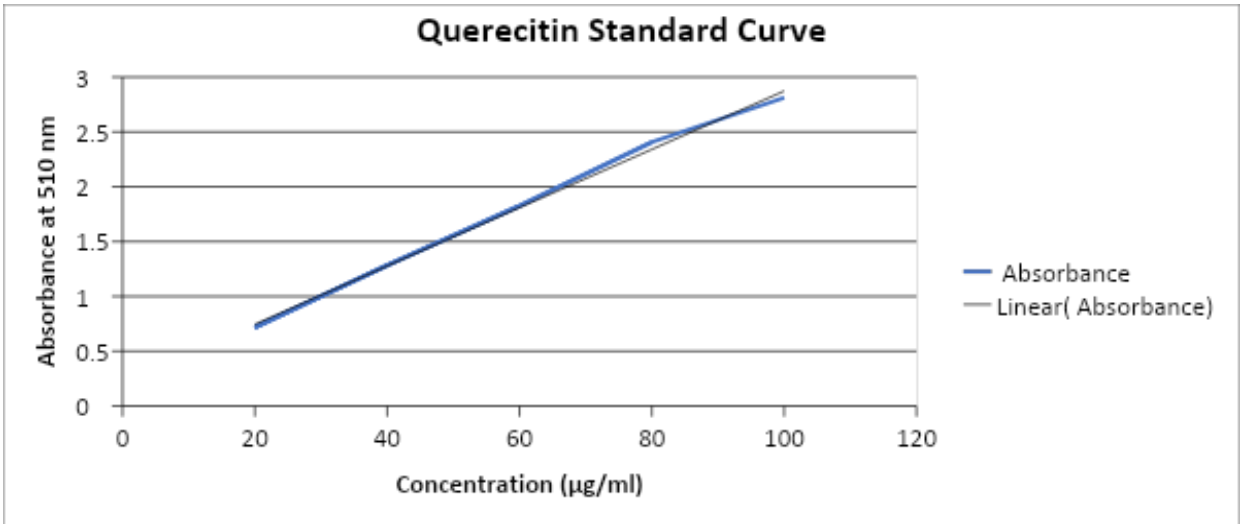


Table 3: Total Flavonoid In Ocimum Sanctum Plant Leaf Extract

QUANTITATIVE ANALYSIS	***O.sanctum* Extract**
TOTAL FLAVONOIDS (mg of QCE /serving)	100±0.0056

ANTIBACTERIAL ACTIVITY AGAINST OF E. COLI AND PSEUDOMONAS PATHOGENS

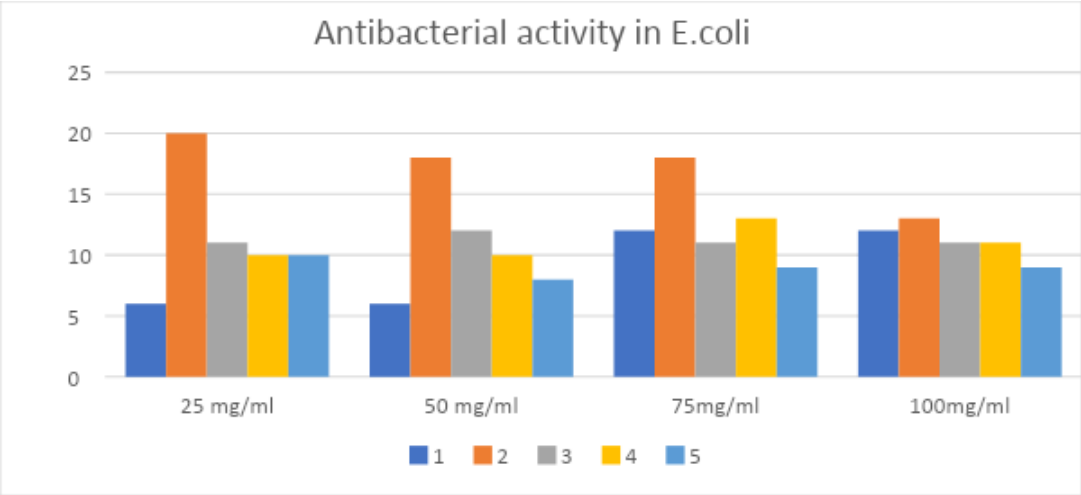
For their antibacterial activities, the Ocimum sanctum plant leaf extract has been tested. An interesting antibacterial profile against UTI pathogens E.coli & Pseudomonas has been observed. The operations of plant extracts are mentioned in terms of the inhibition zone (MM). The effects of all antibacterial activities are shown below. Table for the antibacterial activity of E.coli shown in Table 4 and Table 5 and Figure 3 and Figure 4.

Table 4: Antibacterial Activity Measured By Zonal Scale

S.NO	Sample ID	Zone of inhibition in mm in different concentration			
		25 mg/ml	50 mg/ml	75mg/ml	100mg/ml
1	E.Coli1	6	6	12	12
2	E.Coli2	20	18	18	13
3	E.Coli3	11	12	11	11
4	E.Coli4	10	10	13	11
5	E.Coli5	10	8	9	9

ANTIBACTERIAL ACTIVITY IN E. COLI

Figure 3: Antibacterial Activity *Ocimum Sanctum* Plant Leaf Extract Against UTI Pathogen E.Coli



ANTIBACTERIAL ACTIVITY IN PSEUDOMONAS

Table 5: Antibacterial Activity Measured By Zonal Scale of Pseudomonas

S.NO	Sample ID	Zone of inhibition in mm in different concentration			
		25 mg/ml	50 mg/ml	75mg/ml	100mg/ml
1	P1	11	12	11	14
2	P2	6	12	12	13
3	P3	10	11	13	11
4	P4	10	10	10	10
5	P5	6	6	12	12

Figure 4: Antibacterial Activity Ocimum Sanctum Plant Leaf Extract Against UTI Pathogen Pseudomonas

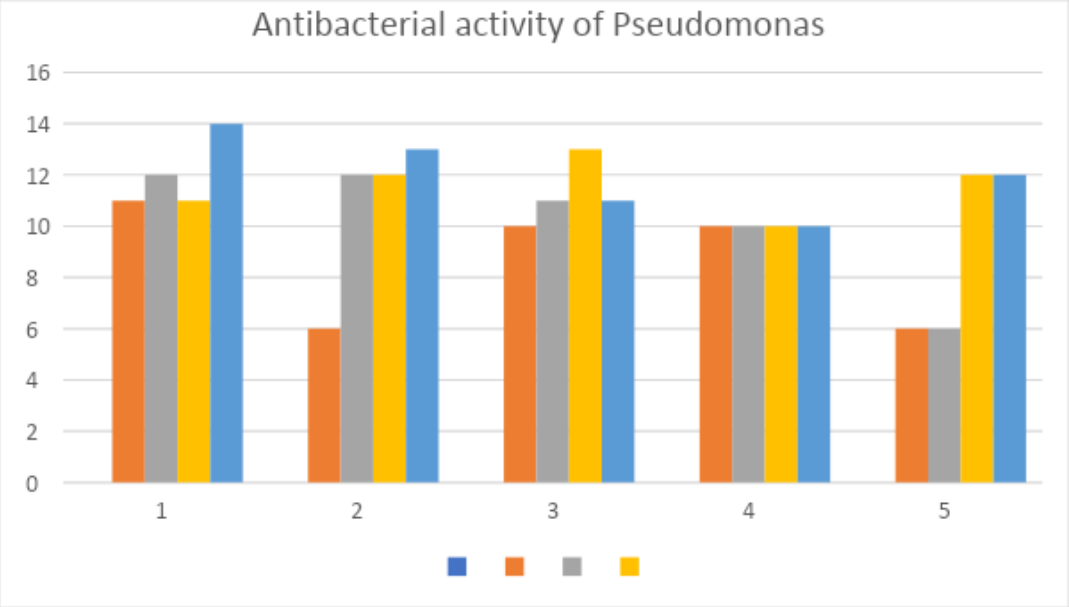
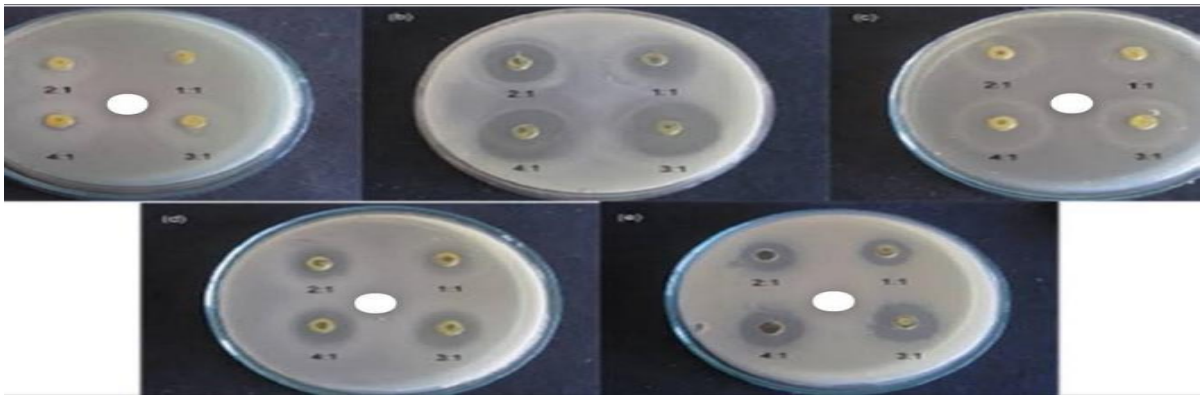


Figure 5: Antibacterial Activity In E.Coli On Petri Plate

Figure 6: Antibacterial Activity In Pseudomonas On Petri Plate



CONCLUSIONS

We find in this analysis that most biologically active phytochemicals are contained in plant extracts. Ocimum sanctum plant leaf Hydro alcoholic extract, such as Protein, Steroids, Flavonoids, Akaloids, Glycosides, Crbonet

etc. In this analysis, bacterial activity showed potent antibacterial activity against these pathogens with UTI pathogens (E.coli and Pseudomonas) plant extract. A significant portion of the world's population depends on the conventional medicine system. This research will use the more herbal medical value in the pharmaceutical industry for various therapeutic applications in the future.

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