

Phytochemical Analysis and Antimicrobial Activity of *Ocimum sanctum* (Tulsi) and *Hemidesmus indicus*(Anantamul)

Sucheta Karande, Palak Balak, Pooja Saroha and Saloni Patel

Department of Botany
Mehsana Urban Institute of science
Ganapat University

Corresponding author: snk01@ganpatuniversity.ac.in

ABSTRACT

Phytochemicals are most important source of antibiotics that are used to treat disease. Present study or investigation deals with phytochemical analysis of leaf of *Ocimum sanctum* and root of *Hemidesmus indicus*. The extractive value estimations of *Ocimum sanctum* and root of *Hemidesmus indicus* were resolved utilizing various solvents, for example water, methanol, ethanol, acetone and chloroform. Phytochemical analysis was done by available standard methods. Phytochemical screening indicates presence of flavonoids, tannin, sterol, carbohydrates etc. An experiment was performed to investigate the antibacterial movement of *Ocimum sanctum* and root of *Hemidesmus indicus* using methanol and chloroform concentrates. Agar well diffusion method was applied to access the antibacterial activity of *Ocimum sanctum* and root of *Hemidesmus indicus* against *Escherichia coli* (NCIM-2066), *Pseudomonas aeruginosa* (NCIM-5514), *Staphylococcus aureus* (NCIM-2079). *Ocimum sanctum* and *Hemidesmus indicus* revealed the presence of secondary metabolites or phytochemicals like flavonoids, tannin etc. which are known to exhibit medicinal as well as physiological activities.

Key Words: Phytochemical, Antibiotic, *Ocimum sanctum*, *Hemidesmus indicus*, antimicrobial, *E.coli*, *Pseudomonas vulgaris*, *Staphylococcus aureus*, secondary metabolites.

INTRODUCTION

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Valko M *et al.*, 2003). Phytochemical analysis of this medicinal herb can identify the nature of compounds present in the extract of *Ocimum sanctum*. It is also to identify the bioactive compound and its effect. They are commonly helpful as a model for the synthetic of new medicine (Kothari *et al.*, 2005). *Ocimum sanctum* is also known as Tulsi. The family of

the *Ocimum sanctum* is Lamiaceae. *Ocimum sanctum* is produced in India and Southeast Asia. India is the largest source of medicinal plants in the whole world (Panchal P. *et al.*, 2019). Herbs have been provided the therapeutic potential to the health of the individual. The demand for this plant is increasing day by day for medicinal purposes (Gupta SK *et al.*, 2002) *Hemidesmus indicus* (L.) R. Br. (Family: Apocynaceae), commonly known as Indian sarsaparilla or Anantamul, is a slender, laticiferous and twining shrub, occurs over the greater part of India (Anon., 1997).

It is widely recognized in folk medicine and as an ingredient in Ayurvedic and Unani preparations against the disease of biliousness, blood diseases, diarrhea, skin diseases, respiratory diseases, fever, bronchitis, eye diseases, burning sensation, rheumatism, and gastric disorders. The roots served as a remedy for leprosy, syphilis, leucoderma, asthma, dysentery, fever and, blood, kidney and urinary diseases and root extracts have been found to exhibit various pharmacological properties (Sharma B.K., 2000).

Antibacterial resistance has become a global problem. In developing countries, due to the cost of efficient, Antibacterial a large proportion of the population utilizes medicinal plants for the treatment of infectious diseases. According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine (Andy *et al.*, 2008). The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. The most important bioactive compounds of plants are alkaloids, Flavanoids, tannins and phenolic compounds. The phytochemical research based on ethnopharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants. The necessity to search for plant-based antimicrobials is increasing due to high cost, and increased resistance to conventional medicines. This study analyzed the phytochemical composition of *Moringa olifera*, and antimicrobial potential of its methanol extracts on *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. (Duraipandiyar, V. *et al.*, 2006). To evaluate the antibacterial activity of *Moringa oleifera* leaf extracts and *Terminalia arjuna* bark extract against three microorganisms, viz., *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa* were carried out.

For phytochemical screening, some common and available standard tests were done. Antimicrobial bioassay was done through agar well diffusion method.

MATERIALS AND METHODS

Collection of the plant samples and Preparation of the plant extracts of *Ocimum sanctum* (Tulsi) and root of *Hemidesmus indicus* (Anantamul)

1. COLLECTION OF PLANT MATERIALS:

Leaves of Tulsi and root of Anantamul were secured from nearby markets from Pune. It was guaranteed that the plant was solid and uninfected. The leaves were washed under running faucet water to dispense with dust then the plant materials were kept in until all the water content dissipated and the plant turned out to be all around dried for pounding. Subsequent to drying the plant material were ground utilizing with mechanical blender to get fine powder and the powder was put away in impermeable plastic holder with appropriate marking for sometime later

2. IDENTIFICATION

Fig.1



Ocimum sanctum
(Whole plant)



Ocimum sanctum
(Whole powder extract)

Fig.2



Hemidesmus indicus
(Wholeplant)



Hemidesmus indicus
(Whole powderExtract)

3. PREPARATION OF LEAF AND BARK EXTRACTS:

The dry powder plant material of Tulsi leaf and Anantamul's root was extracted with water, methanol, ethanol, acetone, chloroform, ether utilizing a maceration procedure. 2 gm of powdered plant material said something a gauging bottle moved into a 250ml dry funnel shaped flask. Flagon was loaded up with various solvents (30ml) independently. flak were saved aside for 24 hrs at room temperature shaking much of the time. Blend was separated through whatman no. 1 channel paper. Filtrate moved into wet Petri plate. Gotten extricate were concentrated to dryness by saving filtrate for complete vanishing of dissolvable. Concentrate were exposed to subjective phytochemical examination.

4. DETERMINATION OF EXTRACTIVE VALUE OF TULASILEAF AND ANANTMUL'S ROOT(Khandelwal K. R. ,2002)

The extracts obtained were concentrated to dryness by maintaining a filtrate for complete solvent evaporation. The extractive value in percentage was calculated by using Aqueous, methanolic, ethanolic, acetone, chloroform and petroleum ether extracts

$$\text{Extractive value (\%)} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Table 1: Extractive value of *Ocimum sanctum* (Tulsi).

Solvents	Weight of Plant material (g)	Colors of extract	Extractive value (%)
Aqueous (Water)	2	Green	1
Methanol	2	Dark green	12.00
Ethanol	2	Dark green	6.50
Acetone	2	Green	3.00
Chloroform	2	Green	4.00
Petroleum Ether	2	Green	1.00
Ethyl acetate	2	Green	3.50

Figure 1: : Extractive value % of different extracts of *Ocimum sanctum* (Tulsi).

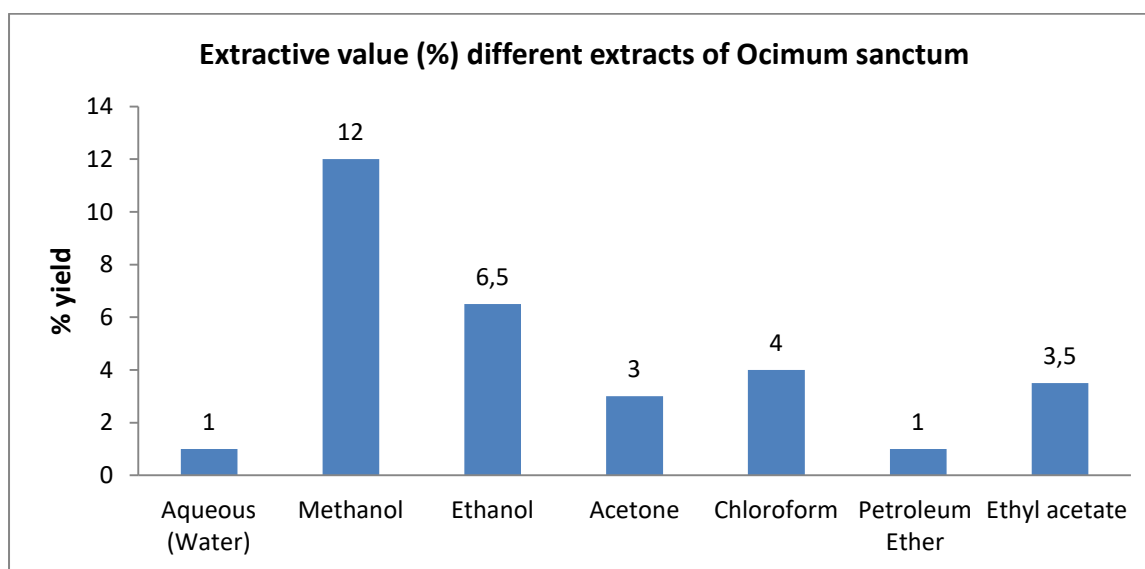
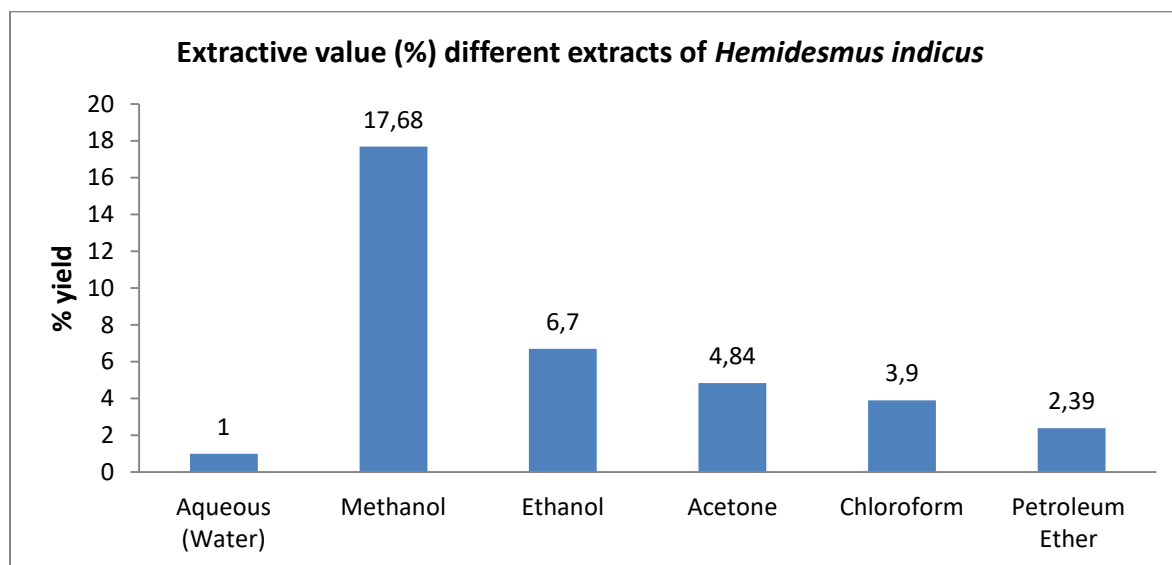


Table 2: Extractive value of *Hemidesmus indicus* (Annatmul)

Solvents	Weight of Plant material (g)	Colors of extract	Extractive value (%)
Aqueous (Water)	2	Brown	1
Methanol	2	Brown	17.68
Ethanol	2	Slightly Brown	6.70
Acetone	2	Brown	4.84
Chloroform	2	Turbid solution	3.90
Petroleum Ether	2	Turbid solution	2.39
Ethyl acetate	2	Turbid solution	3.31

Figure 2: : Extractive value % of different extracts of *Hemidesmus indicus* (Annatmul)



5. Preliminary Phytochemical Analysis:

Phytochemical analysis (Rangari VD. 2002, Harbone JB1998) was done by the accompanying techniques to test for the nearness of the Phytosterol, Terpenoids, Total alkaloids, Carbohydrates, Flavonoids, Tannins, Proteins, Glycoside, Starch, Amino acids.

1. Test for Phytosterol:

Leibermann- Burchard reaction – To 3 ml extract, 10 ml chloroform was added followed by 2 ml of acetic anhydride. Then 2 drops of conc. Sulphuric acid was added from the side of the test tube. The blue green colour indicated the presence of steroids

2. Test for Terpenoids:

Salkowski test: The extract was mixed with 2 ml of chloroform and concentrated H₂SO₄ (3 ml) is carefully added to form a layer. A reddish brown colouration of the interface is formed to show positive result of the presence of terpenoids. Salkowski test gave a positive result hence confirms the presence of Terpenoids

3. Test for Alkaloids:

Mayer's reagent & Wagner's reagent confirmed the presence of Alkaloids in the extract. The Methanolic plant extract was warmed with 2% H₂SO₄ for two minutes. It is filtered and few drops of reagents were added separately.

a. Mayer's reagent- A creamy- white colored precipitation appeared giving a positive result.

b. Wagner's reagent-A reddish-brown precipitate appeared which also confirms the presence of alkaloids in the extract.

4. Test for Carbohydrate:

Fehling's and Anthon's test confirmed the presence of carbohydrate. Fehling's Test: Fehling A and Fehling B reagents were mixed and few drops of extract was added and boiled. A brick red coloured precipitate of cuprous oxide forms, confirming the presence of carbohydrates

5. Test for Flavonoids:

Ammonium Test: Plant extract heated with ethyl acetate for 3 min + mixture was filtered + 1ml ammonia solution was added. Layer's were allowed to separate. Yellow coloration at ammonia layer indicate the presence of flavonoid

6. Test for Tannins:

To 1.2 ml of extract of drug, added few drops of 5% FeCl₃ solution. A greenish colour indicates the presence of Galactotennins while brown colour indicates Tannins

7. Test for Protein –

Millon's test - Dissolved small quantity of aqueous extract of drug in 1 ml of distilled water and 5-6 drops of millon's reagent. A white precipitate is formed which turns red on heating.

8. Test for Glycosides:

Keller-Kiliani Test and Concentrate H₂SO₄ Test confirmed the presence of Glycosides in the methanolic plant extract. Keller-Kiliani Test: In 2 ml plant extract, glacial acetic acid, one drop of 5% FeCl₃ and conc. H₂SO₄ were added. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green, confirming the presence of glycosides. Concentrate H₂SO₄ Test: In 5 ml plant extract, 2 ml glacial acetic acid, one drop of 5% FeCl₃ and conc. H₂SO₄ were added. Brown ring appears, indicating the presence of glycosides.

9. Test for Starch:

Dissolved 0.015 g of Iodine and 0.075 g of KI in 5 ml of distilled water and added 2-3 ml of an aqueous extract of drug. A blue colony is produced.

10. Test for amino acids :

Ninhydrine test - About 3ml of plant extract solution was heated followed by addition of 3 drops of 5% Ninhydrine solution. The test tubes with this solution were kept in boiling water bath for 10 minutes. The purple colour was observed. It indicated the presence of amino acids.

Table 3:- Qualitative phytochemical analysis of *Ocimum sanctum* leaf extract

Sr No	PHYTO-CONSTITUENT	PHYTOCHEMICAL TEST	RESULTS				
			WATER	MEOH	ETOH	CHLOROFORM	ACETONE
1	Phytosterol	Liebermann-Burchard's test	-	+	-	+	+
2	Terpenoid	Salkowski reaction	+	-	+	-	-
3	Alkaloid	Mayer's Test	-	-	-	-	-
		Wagner's Test	-	+	+	+	+
4	Carbohydrates	Anthrone's Test	-	+	+	+	+
		Feling Test	-	+	+	-	-
5	Flavonoids	Lead acetate test	-	+	+	-	-
6	Tannins	5%FeCl ₃ Test	+	+	+	-	+
7	Protein	Ninhydrin Test	-	-	-	-	-
8	Glycosides	Keller-Killiani test	-	+	+	-	+
9	Starch	Iodine	-	-	-	-	-
10.	Amino Acid	Ninhydrin Test	+	-	-	-	-

Note:-The presence of phytochemical is indicated by “+” and absence is indicated by “-” sign.

Table 4:- Qualitative phytochemical analysis of *Hemidesmus indicus* leaf extract

Sr No	PHYTO-CONSTITUENT	PHYTOCHEMICAL TEST	RESULTS				
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			WATER	MEOH	ETOH	CHLOROFORM	ACETONE
1	Phytosterol	Liebermann-Burchard's test	-	+	-	+	+
2	Terpenoid	Salkowski reaction	+	-	+	-	-
3	Alkaloid	Mayer's Test	-	-	-	-	-
		Wagner's Test	+	+	+	+	+
4	Carbohydrates	Anthrone's Test	-	+	+	+	+
		Feling Test	-	+	+	-	-
5	Flavonoids	Lead acetate test	-	+	+	-	-
6	Tannins	5%FeCl ₃ Test	-	+	-	+	+
7	Protein	Ninhydrin Test	+	-	+	-	-
8	Glycosides	Keller-Killiani test	-	-	-	-	-
9	Starch	Iodine	+	+	+	+	+
10.	Amino Acid	Ninhydrin Test	-	+	+	+	+

Note:-The presence of phytochemical is indicated by “+” and absence is indicated by “-” sign.

6. Determination of the Antibacterial Activity: Selection of the Microorganisms:

The bacterial strains utilized for this investigation were *Escherichia Coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. All the bacterial strains were developed and kept up in supplement agar. These living beings utilized were gotten from the Microbiology Department of MUIS of Ganpat University.

Well Diffusion Method:

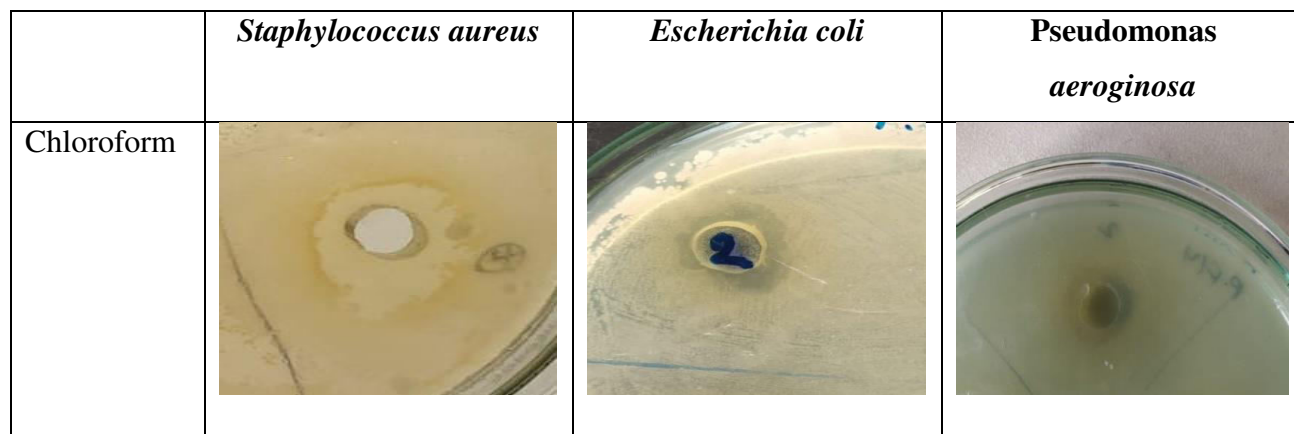
The antibacterial movement of the leaf removes was resolved utilizing agar well dispersion strategy. The antibacterial movement of methanolic and chloroform concentrate of *Ocimum sanctum*(Tulsi)and root of *Hemidesmus indica*(Anantamul) was tried on microbes viz. *Escherichia coli*, *Pseudomonas*

aeruginosa, *Staphylococcus aureus* by standard agar well dissemination technique. 1 ml Organisms stock culture were vaccinated on supplement agar medium and filled sanitized Petri dishes. Wells of 5 mm distance across were made on the supplement agar utilizing a sterile plug borer. The cut agar plates were deliberately evacuated by the utilization of cleaned forceps. To each well, 20 µL of plant separates were stacked with the assistance of micropipette under aseptic conditions. The plates were containing development of the test living being and concentrates were hatched in well and plates were kept in hatchery at 37 °C for 24 h. The plates were analyzed for proof of zones of hindrance, which show up as a reasonable territory around the wells. The breadth of such zones of restraint was estimated . Control tests involving inoculums with gentamicin anti-toxin circles were arrangement and the plates were brooded at 37 °C for 24 h. The zones of restraint were then recorded and thought about.

Table 5: Antimicrobial activity of Chloroform, Methanol extract of *Ocimum sanctum* against pathogens

Plants	Extracts	Zone of Inhibition (mm)		
		<i>Staphylococcus aureus</i>	<i>Escherichia Coli</i>	<i>Pseudomonas aeruginosa</i>
<i>Ocimum sanctum</i>	Methanol	11	8	0
	Chloroform	6	8	7

Figure 3: Antibacterial activity of *Ocimum sanctum*



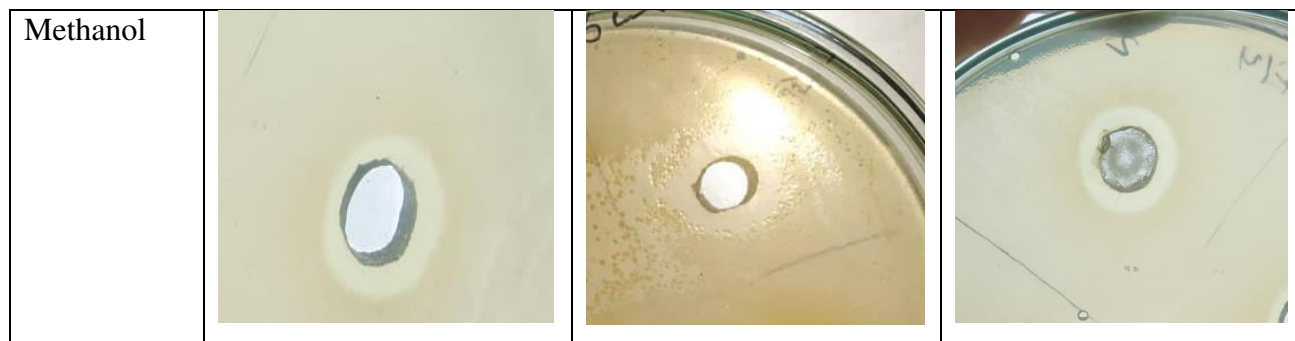
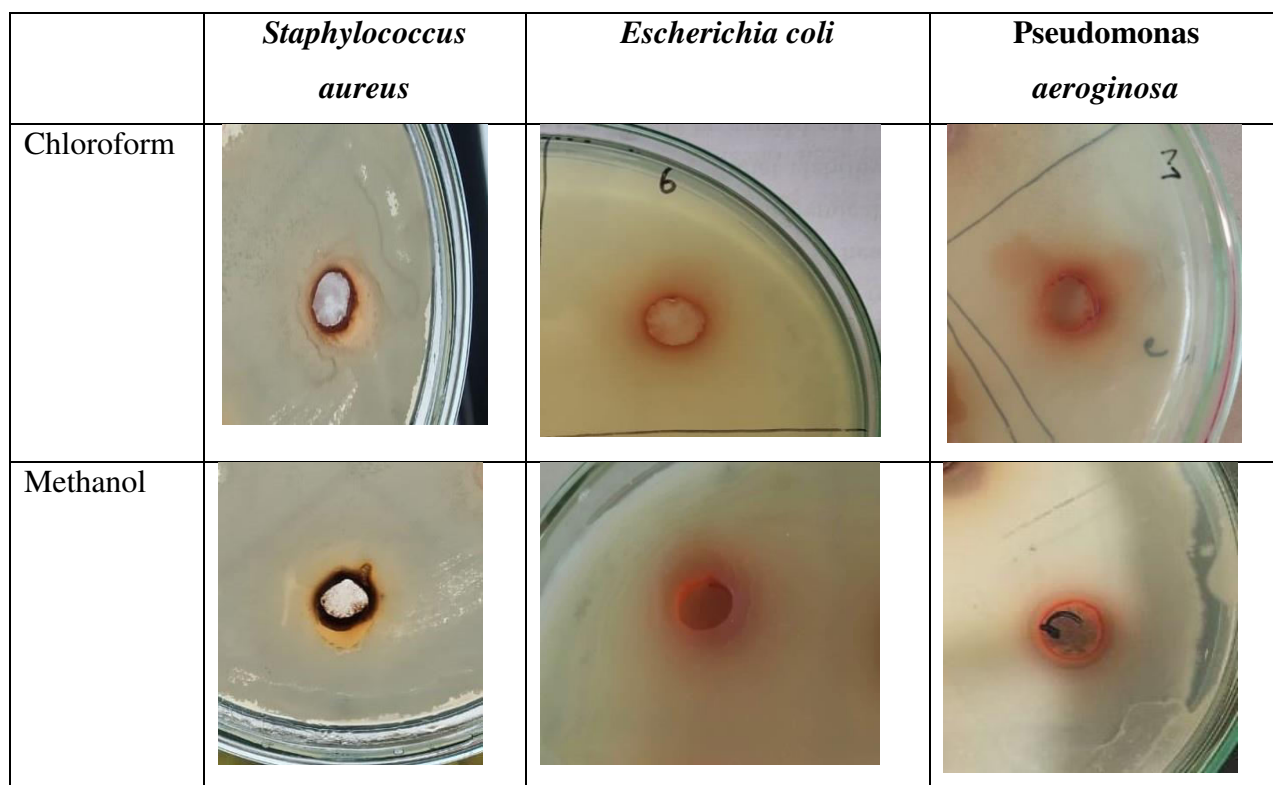


Table 6: Antimicrobial activity of Chloroform, Methanol extract of *Hemidesmus indicus* against pathogens

Plants	Extracts	Zone of Inhibition (mm)		
		<i>Staphylococcus aureus</i>	<i>Escherichia Coli</i>	<i>Pseudomonas aeruginosa</i>
<i>Hemidesmus indicus</i>	Methanol	7	0	6
	Chloroform	5	6	4

Figure 4: Antibacterial activity of *Hemidesmus indicus*



RESULT AND DISCUSSION

The extractive worth and shade of concentrates of *Ocimum sanctum* and *Hemidesmus indicus* was explored and spoken to in Table No. 1 and 2 From the current examination it was discovered that, the extractive estimation of *Ocimum sanctum* in methanol was 12% and *Hemidesmus indicus* methanolic remove was most extreme 17.68% when contrasted with other dissolvable concentrates. The watery concentrate of Tulsi and Anantamul demonstrated lower extractive 1% than other dissolvable concentrates.

From the subjective examination of *Ocimum sanctum* extricates the nearness or nonappearance of alkaloids (Mayor's test), protein, starch and in *Hemidesmus indicus* extricates the nearness or nonappearance of alkaloids (Mayor's test) and Glycosides were explored.

The different concentrates of *Ocimum sanctum* and *Hemidesmus indicus* in particular Methanol and chloroform concentrates of its leaves, root were tried against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas vulgaris* for their antibacterial action.

Restorative plants are vital to the wellbeing of individual and networks (Pascaline J, 2011) Phytochemical examination led on the plant extricates uncovered the nearness of constituents which are referred to display therapeutic just as physiological activities (Sofowora A, 1993). The phytochemical examination of *Ocimum sanctum* plant removes utilizing water, methanol, chloroform, acetone, ethanol is appeared in Table no:3 From the phytochemical investigation it was discovered that protein and starch are missing in *Ocimum sanctum*. Terpenoids were available only in water and ethanol. Alkaloids show negative outcome in Mayer's test (absolutely missing) however show positive outcome in Wagner test (present) except in water. Starch was not found. Flavanoids were available in methanol and ethanol extract. Tannin was found in methanol, ethanol, acetone separate except, chloroform. Protein was missing. Glycosides found in methanol, ethanol and acetone extract. Starch was thoroughly missing. Amino acids were just found in watery concentrate. (Priya Panchal, 2019) The phytochemical examination of *Hemidesmus indicus* plant removes utilizing water, methanol, chloroform, acetone, ethanol is appeared in Table no:4 From the phytochemical investigation it was discovered that Glycosides are missing. Terpenoids were available in ethanol and watery separate. Alkaloids show negative outcome In Mayer's test (absolutely missing) yet show positive outcome in Wagner test

(present). Starch was found in *Hemidesmus indicus* root. Regular cancer prevention agents originate from plant as phenolic mixes, for example, flavonoids (Ali SSet.al'2008), (Nema R. et.al.2012). Tannin was found in all solvents methanol, chloroform and acetone. Protein was found in watery and ethanol extract. Amino acids were found all extract except in fluid (watery) (Gita Mishra 2016).

The antibacterial action of chloroform, and methanol extricates was explored utilizing agar well dispersion strategy, against the chose human pathogens, for example, *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*. All the inspected separate indicated fluctuating degrees of antibacterial exercises against the pathogens. Table-5 shows the antibacterial action of methanol concentrate of *Ocimum sanctum* indicated greatest zone of hindrance (11 mm) against *Staphylococcus aureus*. The antibacterial movement of methanol concentrate of *Ocimum sanctum* demonstrated no zone of restraint against *Pseudomonas aeruginosa*. The antibacterial action of chloroform concentrate of *Ocimum sanctum* indicated zone of hindrance (6mm, 8mm, 7mm) against the microbes on *Staphylococcus aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa* respectively (Napolean, P, 2009) Table-6 shows the antibacterial action of chloroform concentrate of *Hemidesmus indicus* indicated zone of restraint (5mm, 6mm and 4 mm) against all microbes *Staphylococcus aureus*, *Escherichia Coli* and *Pseudomonas aeruginosa* respectively. The antibacterial action of methanol concentrate of *Hemidesmus indicus* demonstrated no zone of hindrance against *Escherichia Coli* and zone of restraint (7mm and 6mm) against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. (Nagat Met.al. 2016)

CONCLUSION:-

The results of the present study revealed that *Ocimum sanctum* and *Hemidesmus indicus* are a rich source of phytochemical constituents. It can be concluded that the selected medicinal plants are the source of secondary metabolites like alkaloids, phytosterols, glycosides, phenols, Flavonoids and diterpens. Due to the presence of these secondary metabolites the selected medicinal plants have high healing potential. The antimicrobial efficacy of *Ocimum sanctum* leaves and *Hemidesmus indicus* roots indicates that the plants possess potent antimicrobial properties as well.

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