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PHYTOCHEMICAL SCREENING AND ANTI-MICROBIAL ACTIVITY EVALUATION OF LEAF EXTRACT OF *OCIMUM SANCTUM, ALLIUM SATIVUM, ACACIA NILOTICA* AGAINST PATHOGENS

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ABSTRACT

Background: *Plant extracts are actively being used worldwide due to the presence of biologically active constituents helping in the preservation of food, and to aid against various diseases owing to their antimicrobial and antioxidant potential.*

Objective: *The main objective of this study was to conduct phytochemical screening and antimicrobial activity evaluation of Ocimum Sanctum, Allium Sativum and Acacia Nilotica.*

Methods: *The present research work was carried out to investigate the phytochemical constituents and antimicrobial activity of leaf extract of Ocimum Sanctum, Allium Sativum and Acacia Nilotica. The bioactive components were analysed in leaf extract of different solvent like Acetone, ethanol, petroleum ether, n-butanol and aqueous extract for Ocimum sanctum, Allium Sativum and Acacia Nilotica. Anti-microbial activities of plant extracts were evaluated against Klebsiella pneumoniae, Acinetobacter baumannii, Staphylococcus aureus, and Enterococcus Faecalis. Antimicrobial property was shown in terms of zone of inhibition by agar well diffusion methodologies.*

Results: *The phytochemical constituents identified were flavonoids, alkaloids, glycosides, phenols, saponins, steroids, and terpenoids. The number of flavonoids, alkaloids, and phenols were found higher in aqueous extract and ethanolic extract, and therefore, used for estimation of antimicrobial activity. Thee extract showed antimicrobial activity against Klebsiella pneumoniae, Enterococcus Faecalis, Staphylococcus aureus, and Acinetobacter Baumannii. Zone of inhibition was found higher against Staphylococcus aureus and Acinetobacter Baumannii in comparison to that against Klebsiella pneumoniae, and Acinetobacter Baumannii.*

Conclusion: *The majority of evaluated medicinal plants demonstrated remarkable activity against tested microbial strains, which can be attributed to the presence of secondary metabolites of different classes of compounds. The finding provided scientific evidence for the use of these traditionally used medicinal plants.*

Keywords: *Ocimum Sanctum, Allium Sativum, Acacia Nilotica, phytochemical screening, antimicrobial activity.*

Introduction

Plants are the organisms which are basic and broad spectrum of human lifestyle which demand different needs. As for the different purpose it is being one of the inseparable parts of life such as for medicine, food, cosmetics, industries, pharmaceutics etc. The importance of plants pioneered by the naturally present metabolites such as tannins, saponins, flavonoids, terpenoids, steroids, phenols, carbohydrates, alkaloids glycosides etc., which are known as phytochemicals**(Cowan, 1999)** . The different equations of presence of these phytochemicals made them essential for the daily uses and industrial processes like synthesis and production sector. In the health care and R&D department plants are commonly used for the extraction and isolation of secondary metabolites for its medicinal properties and drug productions such as antioxidants, antimicrobial, antiviral, antibacterial, antifungal, analgesics etc **(Abubakar & Haque, 2020).** Historically, medicinal plants have a great role in herbal drugs as it is taken as alternatives which have lesser side effect than the synthetic ones.

Medicinal plants generate a variety of bioactive compounds and are a significant source of medications. Over 120 active compounds have been extracted from various medicinal plants and are currently being used as herbal remedies **(Adeleye et al., 2022)**. According to Indian ethnobotanical literature, plants have the ability to cure a wide range of illnesses. *Ocimum sanctum L.*, also referred to as holy basil or Tulsi, is

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a perennial herbaceous plant that is a member of the Lemnaceae family and is used extensively in medicine and pharmaceuticals (**Fotsing Yannick Stéphane et al., 2022)**.

Ocimum sanctum also exhibits antibacterial, antifungal, antifertility, hepatoprotective, antispasmodic, cardioprotective, antiemetic, antidiabetic, analgesic, adaptogenic, diaphoretic, and many more qualities. Thus, the current investigation was carried out to carry out the extraction and initial phytochemical of *o. sanctum* (**Bhattarai et al., 2024)**. Oleanolic acid, rosmarinic acid, ursolic acid, eugenol, linalool, carvacrol, *β*-elemene, β-caryophyllene, and germacrene are only a few of the numerous chemical constituents found in *Ocimum sanctum*. *Ocimum sanctum* is said to possess stimulating and diuretic properties. The leaves of medicinal herbs are also used to make volatile and fixed oils. Some monoterpenes, like linalool and borneol, create oxygen (*(PDF) Phytochemical Analysis and Medical Benefits of Ocimum Sanctum (Tulsi)*, n.d.). Monoterpenes are derived from volatile oils like camphene, myrcene, and sabinene. Phytochemical analysis of this medicinal herb can identify the nature of compounds present in the extract of *Ocimum sanctum****(Bhattarai et al., 2024)*** . It is also for identify the bioactive compound and their effect. They are commonly helpful as model for the synthetic of new medicine **(Riaz et al., 2023).**

One plant that has been used for millennia to treat infectious disorders is garlic (*Allium sativum L.)*, which has been the subject of extensive research throughout the years. Garlic, or Allium Sativum Kingdom: Plantae clade: Angiosperms is the scientific categorisation. Genus: *Allium sativum L* species; Order: *Asparagales*; Family: *Liliaceae* ***(Harris et al., 2001)***. It is a farmed food that is highly valued globally. One of the first plants to be cultivated, garlic originated in Central Asia. For thousands of years, various bacteria have recognised the potential medical benefits of garlic. Garlic, for instance, has well-established antifungal, antiviral, antibacterial, anthelmintic, antiseptic, and anti-inflammatory qualities (**Batiha et al., 2020)**. Additionally, gram positive *(S. aureus, S. pneumonia, streptococcus*, and *Bacillus anthrax*) and gram negative (*E. coli, Salmonella sp., Citrobacter Enterobacter, Pseudomonas Klebsiella)* bacteria that cause morbidity globally were both active against garlic extracts. Garlic's volatile oils contain flavonoids that include methyl allyl trisulphide, dialkyl disulphide, and dialkyl trisulphide (**Sasi et al., 2021)**. Garlic has been used for centuries to prevent and treat a variety of illnesses, including cancer, heart disease, atherosclerosis, and cholesterol metabolism. It has also been shown to have antihypertensive, antioxidant, and antibacterial properties. According to current studies, garlic can be utilised as a repellent against certain plant pests and illnesses in addition to being a useful medicinal herb (**Santhosha et al., 2013)**.

With its many uses, *Acacia Nilotica* tree is the world's most exclusive source of herbal and life-saving medications. The presence of beneficial natural organic groups such as proteins, carbohydrates, amino acids, alkaloids, flavonoids, tannins, phenols, Tannis, and saponins makes this plant noteworthy (*View of Medicinal Properties Of Different Parts Of Acacia Nilotica Linn (Babul), Its Phytoconstituents And Diverse Pharmacological Activities*, n.d.). Nearly every part of *Acacia nilotica*, including the bark, root, gum, leaves, and flowers, has been used to treat a variety of conditions, including diabetes, eczema, cough, diarrhoea, astringents, and multifunctional illnesses. Historically, plants have been a source of inspiration for innovative medicinal molecules, since plant derived medicines have made substantial contribution to human health and wellbeing (**SAEEDI et al., 2020)**. Medicinal plants have been recognised and used throughout human history. Plants have the ability to synthesize a vast array of chemical substances that are used to accomplish key biological processes and to defend against attack from predators. *Acacia nilotica* is therefore utilised to treat different illnesses. Polyphenols are derived from it**(Angelo, 2015)** . The plant has a profile of several different bioactive elements. Acute diarrhoea

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has several therapeutic benefits. Many people utilise the plant's bark to treat colds, bronchitis, diarrhoea, bleeding piles, and Leukoderma **(SAEEDI et al., 2020).**

The goal of the current investigation was to screen the various phytochemicals found in the and compare them to ethanol, aqueous, acetone, n-butanol. Polyphenolic chemicals and flavonoids were found in these plants, according to a phytochemical examination of the plant's aerial parts(**Agidew, 2022**) . the presence of Tannis, flavonoids, terpenoids, amino acids, saponins, steroids, phenols, carbohydrates, glycosides, alkaloids are the phytochemical component were checked in the phytochemical testing. The test acknowledges us about the different chemical components**(Panchal & Parvez, 2019a)** .

Globally, antimicrobial resistance (AMR) has become a serious public health concern. Controlling the emergence and spread of antimicrobial resistance (AMR) requires accurate and timely identification of drug resistance, followed by appropriate antimicrobial treatment and antimicrobial control (**Prestinaci et al., 2015)**. The further investigation turns towards the anti-microbial susceptibility test of the plant extract, where we took ethanolic and aqueous extract as sample. The microbes used under it was two species of gram positive and gram-negative bacteria which were *staphylococcus Aureus*, *Enterococcus Faecalis*, *Klebsiella Pneumonia* and *Acinetobacter Baumannii* respectively. The Agar well diffusion method is used for the antimicrobial testing (**Khan et al., 2009)**.

Material and Methods

Material Collection: The leaves of *Ocimum sanctum* and *Acacia nilotica* from botanical garden and nursing ground of Nims university campus, Jaipur, Rajasthan, and leaves of *Allium sativum* were collected from the Achrol market, Jaipur, Rajasthan. Then after collection they were washed thoroughly. We let it dry till it become brittle and grinded it into a fine powder form.

Crude extract preparation: The leaves of *Ocimum sanctum*, *Allium sativum*, *Acacia nilotica* dried for 3 to 5days as it became brittle for grinding. We grinded it in the fine powder then prepared it in different solvents with the ratio 1:10 i.e. 1g powder of laves and 10 ml solvent. the preparation was of totally 12 extracts, where 3 solutes i.e. leaf powders of Tulsi, garlic and babul are dissolved in 4 different solvents viz. ethanol, distilled water, acetone and n-butanol respectively. All were incubated for 24 hours. Then next day they were filtered with the Whatman filter paper and funnel and filtrate were kept in the different vials with name of type of solvent and plant marked respectively.

Table 1- preparation of different solvents from the powder of leaves of Tulsi, garlic & babul

	ETHANOL	DISTILLED WATER	ACETONE	N-BOTANOL
TULSI	S1=T+ETHANOL	S2=T+DW	S3=T+ACETONE	S4=T+N-BUT.
GARLIC	S5=G+ETHANOL	S6=G+DW	S7=G+ACETONE	S8=G+N-BUT.
BABUL	S9=B+ETHANOL	S10=B+DW	S11=B+ACETONE	S12=B+N-BUT.

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Fig1- preparation of different extracts of leaves of Tulsi, garlic and babul

Phytochemical screening-

1.Test for tannin (ferric-chloride test): The presence of tannins is shown by the emergence of a brownish green or black hue when 1 millilitre of the sample was mixed with 20 microlitres of 0.1% ferric chloride.

2.Test for flavonoids (lead -acetate test): One millilitre of the plant extract was mixed with a few drops of a 10% lead acetate solution. When flavonoids are present, a yellow precipitate forms.

3.Test for terpenoids (Salkowski's test): To find out if the extract included terpene chemicals, Salkowski's assay was used. After shaking 1 mL of the extract with 2 mL of chloroform and 2 mL of concentrated H2SO4, the test tube was heated in a water bath. A shade of reddish-brown denotes that the extract contains terpenoids **(Usman et al., 2009)**.

4.Test for amino acids (biuret test): A few drops of the biuret's reagent were added along with 5 mg of extract. After giving the resulting mixture a good shake, it was left to warm for one to five minutes. The presence of proteins was indicated by a red or violet appearance.

5.Test for saponins (foam test): Two millilitres of distilled water were added to one millilitre of the sample, agitated quickly, and checked for the formation of foam, which is a sign that saponins are present.

6.Test for steroids (Liberman-Buchard reaction): One millilitre of chloroform was combined with five milligrams of extract, and a few drops of strong sulphuric and acetic acids were then added. The presence of steroids was indicated by the greenish hue.

7. Test for phenols (ferric-chloride test): After adding two thirds of drops of a 10% FeCl3 solution, the aqueous extract was shaken. The presence of phenolic chemicals in the extract is indicated by the production of a blue or dark green colour or precipitate **(Panchal & Parvez, 2019b).**

8. Test for carbohydrates (Molisch's test): Firstly, a test tube containing 5 mg of extract was filled with 1 ml of Molisch's reagent. The mixture was thoroughly shaken. Next, two millilitres of sulphuric acid concentrate were cautiously poured along the test tube's side. The presence of carbohydrates was revealed by the appearance of a violet ring at the contact **(*Untitled Document*, n.d.).**

9. Test for glycosides (killer-Kilani test): 5 mg of the extract was placed in test tubes, and 1 ml of glacial acetic acid was then added to validate the glycoside content of the extract. A few drops of a ferric chloride solution (2%), were added. After that, 1 millilitre of sulphuric acid was added to the mixture. There will be a brown ring at the margin if cardiac glycosides are present.

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10. Test for alkaloids (Wagner's test)

In this test, 0.5 of the Wagner reagents was added to a solution that had been thoroughly shaken after 5 mg of extract had been taken in a test tube. Reddish-brown appearance indicates the presence of alkaloids. The hue is reddish brown due to the formation of an insoluble complex by iodine **(*Document*, n.d.).**

Table 2- representing all the conducted phytochemical tests according to different secondary metabolites of plants with there positive& negative results

	Name of test	reaction	Result (+ve)	Result (-ve)
Tannins	Ferric-chloride test	FeCl3+filterate	Blue-green colour	Clear/light Yellow colour
Flavonoids	Lead-acetate test	Filtrate+ Pb(C2H3O2)2	Yellow precipitate	No precipitation
Terpenoids	Salkowski's test	Filterate+chloroform+H2SO4	Red-brown ring	No colour change
Amino acids	Biuret test	CuSO4+filterate+ethanol+excess KOH	Violate/purple colour	No colour change
Saponins	Foam test	Filtrate+ ethanol+ distil water	Persist foam or lather formation	No foam or lather formation
Steroids	Liebermann-Burchard reaction	Filtrate+(CH3CO)2O+H2SO4	Yellow/blue /purple colour change	No colour change
Phenol	Ferric-chloride test	FeCl3+filterate	Blue/green/red colour	No colour change
Carbohydrate	Molisch's test	2-3 drops α-naphthol solution+ filtrate	Purple/violate ring formation	No colour change
Glycosides	Keller-Kilani test	Filtrate+ glacial acetic acid+FeCl3	Blue-green or brick red colour	No colour change
Alkaloids	Wagner's test	Wagner's solution +filtrate	Yellow/brown /purple colour change	No colour change

Table 3-Phytochemical analysis of *Ocimum sanctum*

	ETHANOL	DISTILLED WATER	ACETONE	N-BUTANOL
TANNINS	+	-	+	-
FLAVONOIDS	+	+	+	+
TERPENOIDS	+	-	-	-
AMINO ACIDS	-	-	-	-
SAPONINS	+	-	+	-
STEROIDS	-	+	-	-
PHENOL	+	-	+	+
CARBOHYDRATE	+	+	-	-
GLYCOSIDE	+	+	-	-
ALKALOIDS	-	-	+	-

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Table 4- phytochemical analysis of *Allium sativum*

	ETHANOL	DISTILLED WATER	ACETONE	N-BUTANOL
TANNIN	+	+	+	-
FLAVONOID	+	+	+	+
TERPENOIDS	-	-	-	-
AMINO ACIDS	+	-	+	+
SAPONINS	+	-	+	-
STEROIDS	+	+	-	-
PHENOL	+	-	+	-
CARBOHYDRATE	+	+	+	-
GLYCOSIDES	+	+	+	-
ALKALOIDS	+	+	+	-

Table 5- Phytochemical analysis of *Acacia nilotica*

	ETHANOL	DISTILLED WATER	ACETONE	N-BUTANOL
TANNIN	-	-	-	-
FLAVONOID	+	-	-	+
TERPENOIDS	-	-	-	-
AMINO ACIDS	-	-	-	-
SAPONINS	+	+	-	+
STEROIDS	-	-	-	-
PHENOL	+	-	-	-
CARBOHYDRATE	+	+	-	+
GLYCOSIDES	+	+	+	+
ALKALOIDS	-	+	-	-

Anti-Microbial Property

The antimicrobial susceptibility test was done using four different microbes, in which two were gram positive and two were gram negative. The gram-negative bacteria were - *Klebsiella Pneumonia* and *Acinetobacter Baumannii* whereas *Staphylococcus Aureus* and *Enterococcus Faecalis* were gram negative bacteria **(Alhumaid et al., 2021).**

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- **Required material** – bacterial strains mother plate, blood agar plates, muller-Hinton agar plates, sterile swabs, inoculation loop, sterile saline water, burner, plant extracts, ethanol, forceps, agar well puncher, antibiotics (penicillin and tetracycline).

Procedure

The antibiotics were planted with the help of forceps by Kirby- Bauer disc diffusion method and the extracts were planted with the well diffusion method by making wells in the M-H agar plate. Pouring extracts in wells and a positive control of antibiotics (tetracycline and penicillin) were added on another plate.

Table 6- MIC of antibiotics on different pathogens

Control name	**penicillin**	***tetracycline***
Microbes		
Klebsiella pneumonia	0mm	*15mm*
Acinetobacter Baumannii	0mm	*13mm*
Staphylococcus Aureus	9mm	*24mm*
Enterococcus faecalis	0mm	*0mm*

Table7- MIC of garlic leaf extract on different pathogens

GARLIC EXTRACTS	**ETHANOLIC**	**DISTILL WATER**
Microbes		
Klebsiella Pneumonia	10mm	8mm
Acinetobacter Baumannii	18mm	15mm
Staphylococcus Aureus	15mm	10mm
Enterococcus Faecalis	14mm	5mm

Table8- MIC of Tulsi leaf extract on different pathogens

TULSI EXTRACTS → / *Microbes* ↓	**ETHANOLIC**	**DISTILL WATER**
Klebsiella Pneumonia	14mm	3mm
Acinetobacter Baumannii	3mm	2mm
Staphylococcus Aureus	7mm	6mm
Enterococcus Faecalis	19mm	7mm

Table 9- MIC of babul leaf extract of different pathogens

BABUL EXTRACTS	**ETHANOLIC**	**DISTILL WATER**
Microbes		
Klebsiella Pneumonia	17mm	1mm
Acinetobacter Baumannii	10mm	0mm
Staphylococcus Aureus	13mm	7mm
Enterococcus Faecalis	9mm	4mm

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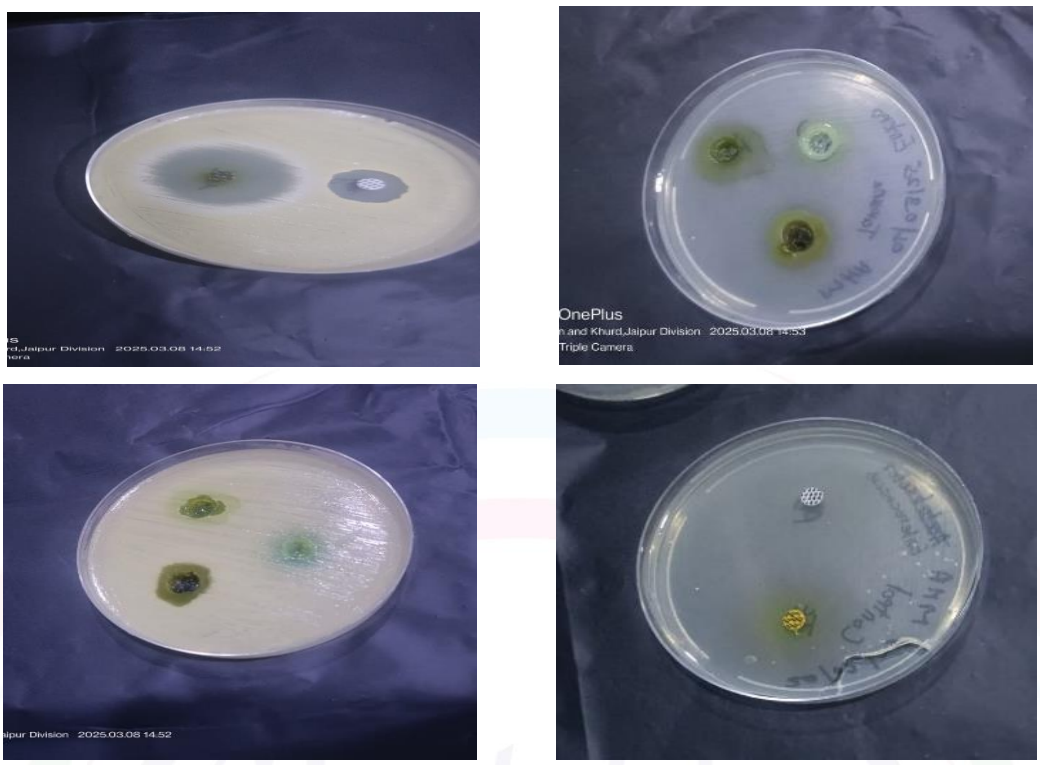

Fig 2 – representing the antimicrobial susceptibility test with different pathogens

RESULT & DISSCUSSION

The best results (Table 10) were shown in the ethanolic and extract of the leaf of *Ocimum sanctum*, *Allium sativum*, *Acacia nilotica*. The *Ocimum sanctum* and *Allium sativum* have the best results with ethanolic and acetonic extract whereas in *Acacia nilotica* ethanolic and distilled water extract gave the best results. If we component wise for tannin Tulsi and garlic shown better results rather than babul leaves and same goes for flavonoid and phenol. In terms of terpenoid none of the leaf extraction shown a good resultant as compare to ethanolic extract of Tulsi. For amino acid garlic shown a prominent result whereas for saponins were widely present in almost all extracts. Steroids were slightly present in tulsi and garlic as compare to babul. Carbohydrate and glycosides were present in garlic and babul. Alkaloids on the place were abundantly present in garlic. This study shows the variance of presence of chemicals in plant with compare to three different types of species of leaf of plants.

Table10: Results for phytochemical screening of leaf extract of Tulsi, garlic and babul

	TULSI EXTRACT					GARLIC EXTRACT					BABUL EXTRACT			
	Ethanol	**Aqueous**	**Acetone**	**n-butanol**		**Ethanol**	**Aqueous**	**Acetone**	**n-butanol**		**Ethanol**	**Aqueous**	**Acetone**	**n-butanol**
Tannin	+ve	-ve	+ve	-ve		+ve	+ve	+ve	-ve		-ve	-ve	-ve	-ve
Flavnoid	+ve	+ve	+ve	+ve		+ve	+ve	+ve	+ve		+ve	-ve	-ve	+ve
Terpenoids	+ve	-ve	-ve	-ve		-ve	-ve	-ve	-ve		-ve	-ve	-ve	-ve
Amino Acids	-ve	-ve	-ve	-ve		+ve	-ve	+ve	+ve		-ve	-ve	-ve	-ve
Saponins	+ve	-ve	+ve	-ve		+ve	-ve	+ve	-ve		+ve	+ve	-ve	+ve
Steroids	-ve	+ve	-ve	-ve		+ve	+ve	-ve	-ve		-ve	-ve	-ve	-ve
Phenol	+ve	-ve	+ve	+ve		+ve	-ve	+ve	-ve		+ve	-ve	-ve	-ve
Carbohydrates	+ve	+ve	-ve	-ve		+ve	+ve	+ve	-ve		+ve	+ve	-ve	+ve
Glycosides	+ve	+ve	-ve	-ve		+ve	+ve	+ve	-ve		+ve	+ve	+ve	+ve
Alkaloids	-ve	-ve	+ve	-ve		+ve	+ve	+ve	-ve		-ve	+ve	-ve	-ve

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Table 11: Results for anti-microbial susceptibility test

	TULSI		GARLIC		BABUL	
	ETH	DW	ETH	DW	ETH	DW
Klebsiella Pneumonia	14mm	3mm	10mm	8mm	17mm	1mm
Acinetobacter Baumannii	3mm	2mm	18mm	15mm	10mm	0mm
Staphylococcus Aureus	7mm	6mm	15mm	10mm	13mm	7mm
Enterobacter Faecalis	19mm	7mm	14mm	5mm	9mm	4mm

In the antimicrobial susceptibility test the ethanolic extracts shown more zone of inhibition rather than distil water extract (Table 11). The growth of *Klebsiella Pneumonia* was more actively seen on the M-H agar plate and the zone of inhibition was almost good in all the plant extract. The inhibition of *Acinetobacter Baumannii* was more efficient in allium sativum as compare to other plant extracts and same goes with the *Staphylococcus Aureus*. Ocimum sanctum and allium sativum shown good results with *Enterococcus Faecalis*. The main idea of anti-microbial susceptibility test was knowing how much the chosen plant species were effective against the different strains of bacteria.

CONCLUSION

The phytochemical tests show best results in ethanolic plant extracts whereas the antimicrobial susceptibility test given the best zone of inhibition in *Allium sativum* and *Ocimum sanctum*. These results can be use for the future aspects of the testing and pathogenic analysis of different plants and their different parts.

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