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Research Article

**ESTIMATION OF PHYTOCHEMICAL SCREENING,
ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY ON
ETHANOL EXTRACT OF *SOLANUM LYCOPERSICUM*,
POUTERIA SAPOTA, *MUSA ACUMINATA*, AND *BETA
VULGARIS*****K.Ramanji Reddy*¹, Dr.Chandaka Madhu ², Dr.J.Balaji Chandra Mouli³, Dr.B.Jagan
Mohan Reddy³, Penumala Joshi ², Ballavarapu Sharanya ²**¹Scientist, Startech Labs Pvt.Ltd, Madinaguda, Hyderabad, TS-5000050²MLR Institute of Pharmacy, Dundigal (V), Quthbullapur (M), R.R.Dist-500043³Department of Chemistry, Adikavi Nannaya University, Rajamahendravaram-533296**Abstract:**

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. So the present study is to know that the efficiency of the herbal extracts on microbial activity (Pouteria Sapota, Solanum lycopersicum, Musa Acuminata, and betavulgaris). Estimation of antimicrobial activity and antifungal activity on E.coli, bacillus, salmonella, candida and aspergillus by using Soyabean casein digests agar media, Sabouraud dextrose agar media by the technique cup-plate method in which all the extracts have antibacterial activity where as absent in antifungal activity.

Keywords : *resistance, herbal extracts, Pouteria Sapota, Solanum lycopersicum, Musa Acuminata, betavulgaris, E.coli, bacillus, salmonella, candida, aspergillus, Soyabean casein digests agar media, Sabouraud dextrose agar media and cup-plate method*

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INTRODUCTION:

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (1). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality.

From 1980 to 1990, Montelli and Levy (2) documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics around all over world.

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization (3) medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (4).

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (5,6,7,8,9,10,11). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (12), as well as in tannin (13).

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to

evaluate the potential of plant extracts and phytochemicals on standard microorganism strains as well as multi-drug resistant bacteria, which were isolated from hospitals. Moreover, we investigated the synergistic effects of extracts with antimicrobial activity in association with antibiotics against drugs resistant bacteria

METHOD AND METHODOLOGY:

Instruments:

Autoclave, incubator, hot air oven, Weighing balance, micropipette, inoculation loop, laminar air flow.

Glass Ware:

Petri plates, volumetric flask, test tube, beakers, conical flask, glass rod.

Collection and Authentication of Plant Material

The plant material *Pouteria Sapota*, *Actinidia Deliciosa*, *Solanum lycopersicum*, *Musa Acuminata*, *Carica Papaya*, *Daucus Carot* and *Mangifera Indica* were collected in the month of MAY -2017 from local market, madinaguda in Hyderabad.

Preparation of Ethanolic Extract

Method: The Ethanolic extract of the plant was prepared using reflex condensation process. The fresh fruits about 200g was weighed and placed in a 500 ml round bottom flask with 200ml of ethanol and its refluxed for 8 hrs at 40°C. Then suspension was filtered through a fine muslin cloth. The solvent was evaporated by heating until $\frac{3}{4}$ is reduced. The remaining solvent is evaporated under room temperature. A semisolid residue was obtained.

Phytochemical Screening

TEST FOR GLYCOSIDES

A portion of the extract was hydrolysed with HCl and the hydrolysate was subjected to Legal's and Borntrager's test to detect the presence of different Glycosides.

Legal's Test

To the extract, 1ml of Pyridine and few drops of Sodiumnitropruside were added and it was made alkaline with NaOH. Appearance of pink to red colour shows the presence of glycosides.

Borntrager's Test

Extract was treated with Chloroform and then the chloroform layer was separated. To this equal quantity of dilute Ammonia solution was added. Ammonia layer acquires pink colour showing the presence of Glycosides.

TEST FOR SAPONINS

Froth Test

Place 2ml of extract in water in a test tube. Shake well, stable froth (Foam) is formed.

Hence saponins

TEST FOR ALKALOIDS

Dragendorff's Reagent

Drug extract when treated with Potassium Bismuth Iodide Solution gives reddish brown ppt.

Hence Alkaloids are present.

Mayer's Reagent

Drug extract when treated with Potassium Mercuric Iodide solution gives cream color ppt.

Hence alkaloids are Present.

TEST FOR TANNINS

To the extract few ml of Chromic acid was added. No ppt was found.

Hence Tannins are present.

TEST FOR FLAVONOIDS

Shinoda Test

To the extract add few Magnesium turnings and con HCl dropwise. Pink scarlet, crimson red or occasionally green to blue colour appears after few mins.

Hence Flavonoids are present.

Zinc Hydrochloride Test

To the extract add a mixture of Zn dust and con HCl. Gives red colour after few min.

Hence Flavonoids are present.

TEST FOR MUCILAGE

To the extract Ruthenium red solution is added, pink colour is obtained.

Hence mucilage is present.

TEST FOR CARBOHYDRATES

Molisch Test

To the extract add few drops of alcoholic α -naphthol, then add few drops of con. H_2SO_4 through sides of the test tube, violet colour ring is appeared at the junction.

Hence Carbohydrates are present.

TEST FOR PROTEINS

Xanthoproteic Test

To 5ml of extract add 1ml of con Nitric acid and boil. Yellow ppt was obtained. After cooling add 40% NaOH solution. Orange colour was obtained.

Hence proteins are present.

TEST FOR PHYTOSTEROLS

Salkowski Test

To the extract add few drops of con H_2SO_4 , re colour at the lower layer indicate the prescence of sterol and yellow colour prescence indicate

Hence Phytosterols are present.

ESTIMATION OF ANTIMICROBIAL ACTIVITY AND ANTIFUNGAL ACTIVITY:

Media:

Soyabean casein digests agar media

Sabouraud dextrose agar

Method of Preparation:

Preparation of Media for Bacterial:

40 gms of soyabean casein digest media was added to 1000 ml of purified water in a conical flask. Then the media was subjected to sterilization in autoclave for $121^\circ c$ for 15 min.

Preparation of Media for Total Fungal Count:

35 gms of sabouraud dextrose agar media was added to 1000 ml of purified water in a conical flask. Then the media was subjected to sterilization in autoclave for $121^\circ c$ for 15 min.

METHODS

A. Estimation of TBC:

Take 13 Petri plates in which 4 Petri plates was act as test for each extarct and 1 petri plate acts as blank

Add 1ml of test sample media into the petri plate (which was prepared for total bacteria count) and then it was incubated at $22^\circ c$ for 5 days. After incubation the zone of inhibition was studied in the petri plates.

B. Estimation of TFC

Take 9 Petri plates in which 4 Petri plates was act as test and 1 petri plate acts as blank

Add 1ml of test sample media into the petri plate (which was prepared for total fungal count) and then it was incubated at $32^\circ c$ for 7days. After incubation the zone of inhibition was studied in the petri plates

S.no	Media type	technique	Sample Induced (test)	Pathogen	No.of plates		Temperature	Incubation period
					Blank	test		
1	SCDA	Cup-plate	1ml each	Ecoli	1	4	$22^\circ C$	5 days
2	SCDA	Cup-plate	1ml each	Bacillus	1	4	$22^\circ C$	5 days
3	SCDA	Cup-plate	1ml each	Salmonella	1	4	$22^\circ C$	5 days
4	SDA	Cup-plate	1ml each	Candida	1	4	$32^\circ C$	7 days
5	SDA	Cup-plate	1ml each	aspergillus	1	4	$32^\circ C$	7 days

RESULTS AND DISCUSSION:**Table1: Percentage Yield of the Extract**

S.No	Name of The Plant	Percentage Yield (%)
1	<i>Pouteria Sapota</i>	12.3 %
2	BETA VULGARIS	9.8 %
3	<i>Solanum lycopersicum</i>	11.7 %
4	<i>Musa Acuminata</i>	13.1 %

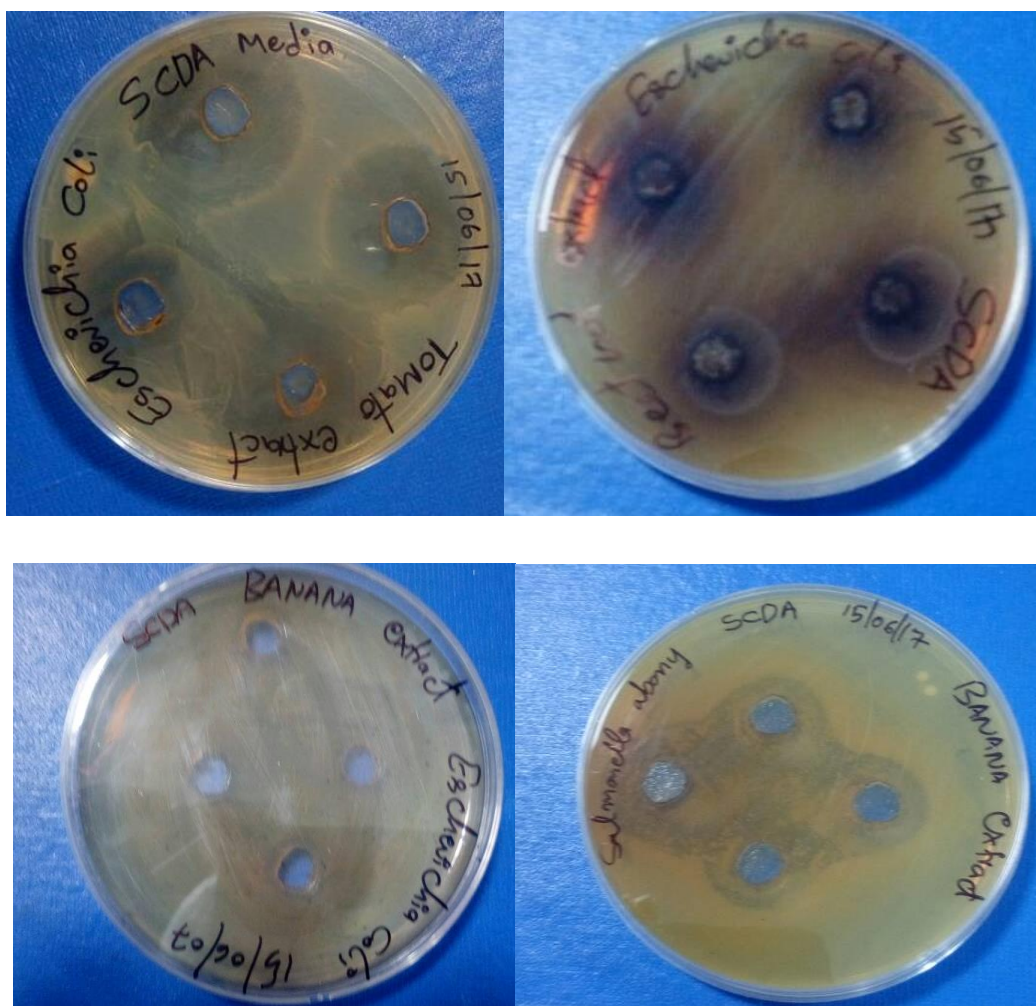
Table 2: Phytochemical Screening:

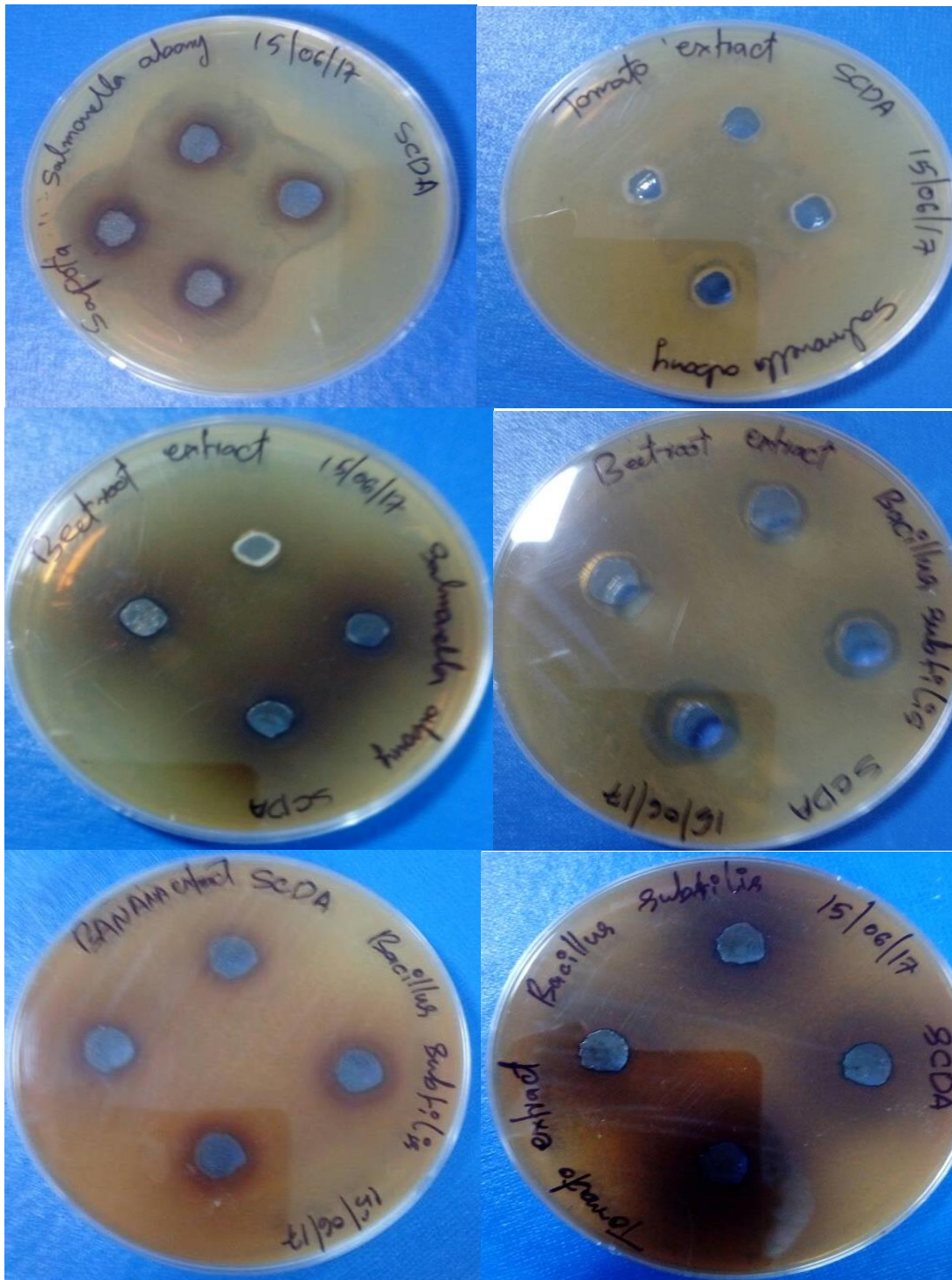
s.no	Name of the plant	Alk	Carb	Gly	Tan	Phytos	Flav	sapo	Pro	muci
1	<i>Pouteria Sapota</i>	+	+	-	+	+	+	+	-	+
2	BETA VULGARIS	-	+	-	+	+	+	+	+	+
3	<i>Solanum lycopersicum</i>	-	+	+	+	+	+	+	-	+
4	<i>Musa Acuminata</i>	+	+	+	+	+	+	+	-	+

The above table indicates the presence (+) or absence (-) of phytochemicals in ethanolic extract(Alk:Alkaloids , Carb:Carbohydrates , Gly:Glycosides, Tan:Tannins, Phtos:Phytosterol,Flav:Flavanoids , Sapo:Saponins , Pro:Proteins , Muci:Mucilages)

3. ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY

Images:





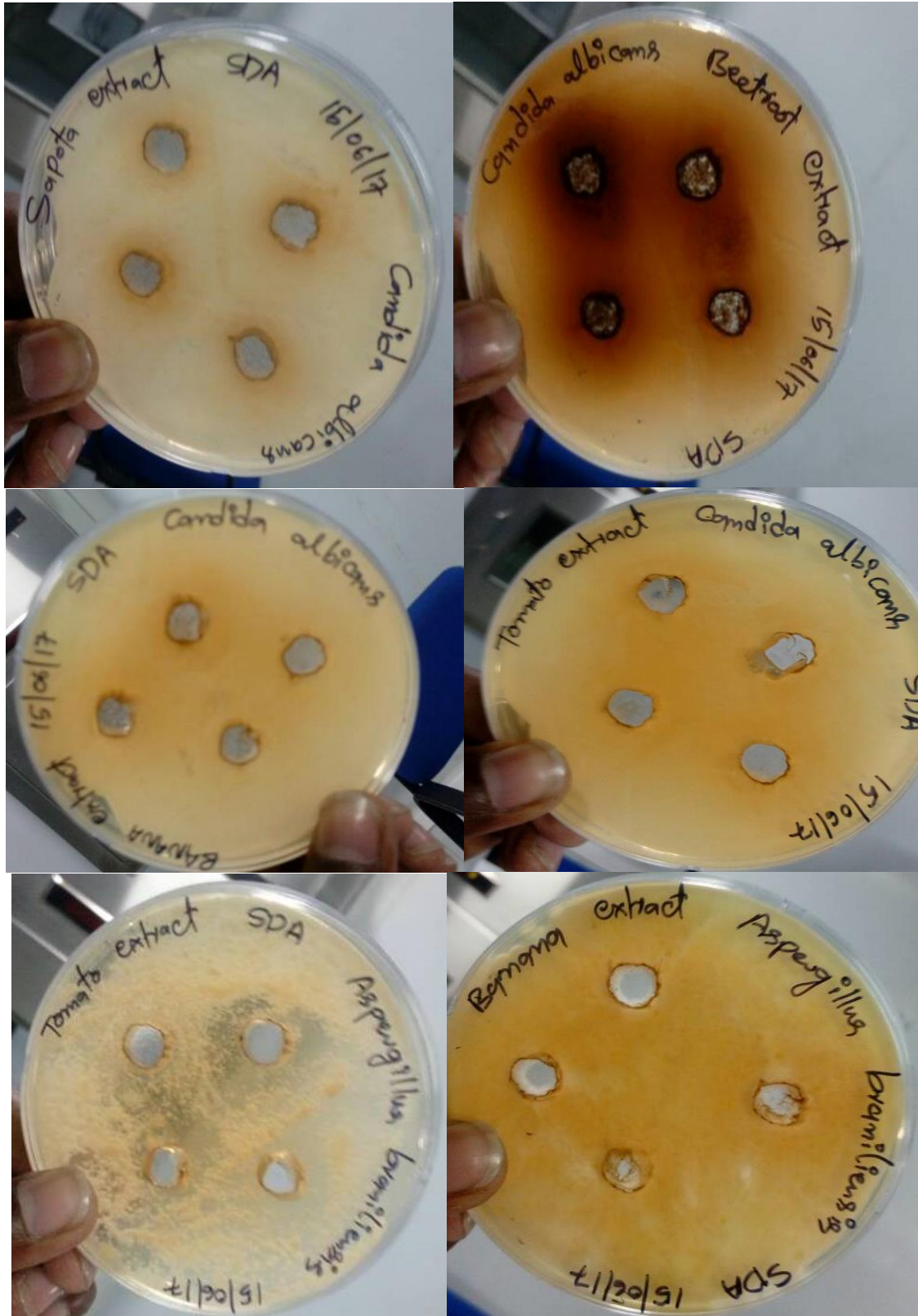




Fig 1: Antimicrobial and Antifungal Activity

Table 3: Estimation of antimicrobial activity for ecoli

s.no	Name of extract	Zone of inhibition (mm)
1	<i>Pouteria Sapota</i>	No zone seen
2	BETA VULGARIS	30.00
3	<i>Solanum lycopersicum</i>	30.27
4	<i>Musa Acuminata</i>	31.64

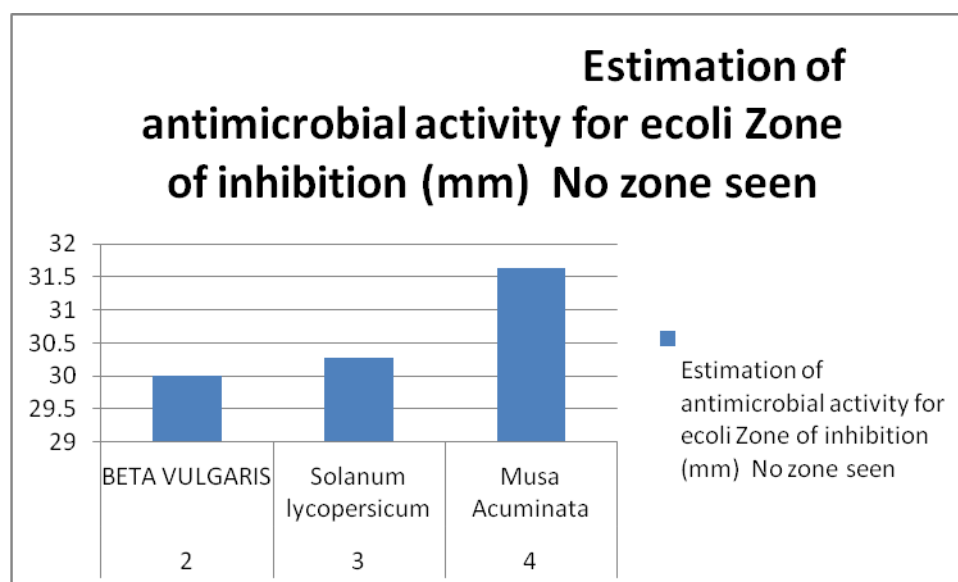


Fig 2: Estimation of antimicrobial activity for E- coli of inhibition (mm) No zone screen

Table 4: Estimation of antimicrobial activity for salmonella

s.no	Name of extract	Zone of inhibition (mm)
1	<i>Pouteria Sapota</i>	27.11
2	BETA VULGARIS	12.06
3	<i>Solanum lycopersicum</i>	10.23
4	<i>Musa Acuminata</i>	22.59

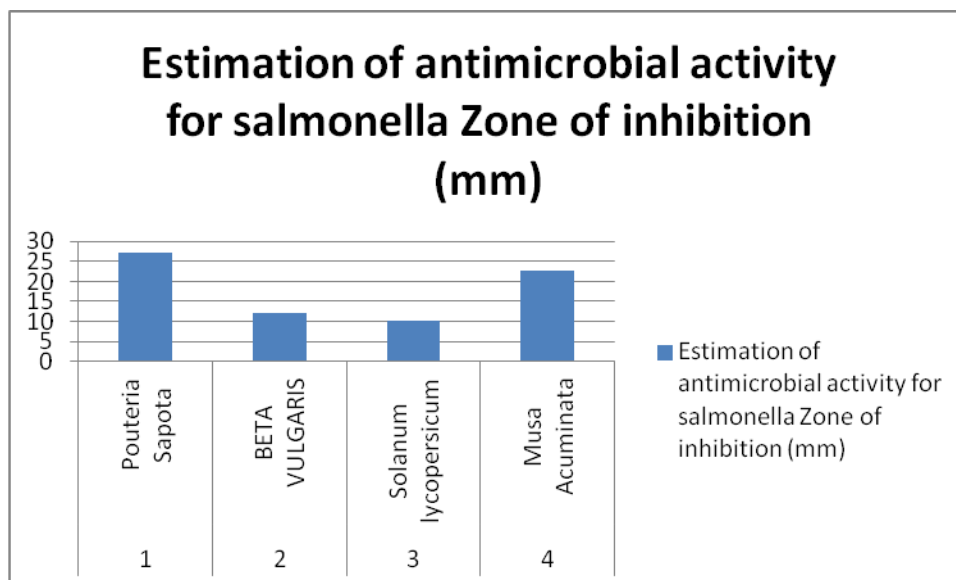


Fig 3: Estimation of antimicrobial activity for salmonella zone inhibition (mm)

Table 5: Estimation of antimicrobial activity for bacillus

s.no	Name of extract	Zone of inhibition (mm)
1	<i>Pouteria Sapota</i>	12.53
2	<i>BETA VULGARIS</i>	18.08
3	<i>Solanum lycopersicum</i>	15.54
4	<i>Musa Acuminata</i>	14.12

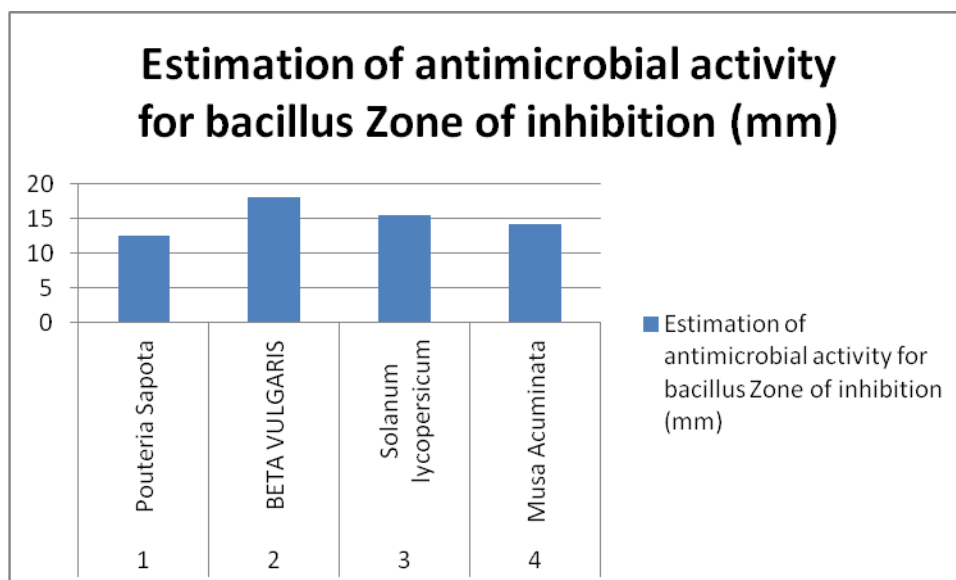


Fig 4: Estimation of antimicrobial activity for bacillus zone of inhibition (mm)

Table 6: Estimation of antifungal activity

s.no	Name of extract	Zone of inhibition (mm)
1	<i>Pouteria Sapota</i>	No activity was seen
2	BETA VULGARIS	
3	<i>Solanum lycopersicum</i>	
4	<i>Musa Acuminata</i>	

Discussion : From the table -1 we have come to know the percentage yield of the ethanolic herbal extract were obtained in which the *Musa Acuminata* is having highest yield is about 13.1 % and the lowest is *beta vulgaris* is about 9.8%

The percentage yield of the herbal extract was arranged in descending order:

Musa Acuminata > *Pouteria Sapota* > *Solanum lycopersicum* > *beta vulgaris*

From the table -2 shows all the ethanolic herbal extracts contain the above table indicates the presence and absence of phytochemicals in ethanolic extract was studied:

(Alk:Alkaloids, Carb:Carbohydrates, Gly:Glycosides, Tan:Tannins, Phtos:Phytosterol, Flav:Flavanoids, Sapo:Saponins, Pro:Proteins, Muci:Mucilages)

Pouteria Sapota has Alkaloids, Carbohydrates, Tannins, Phytosterol, Flavanoids, Saponins, Mucilages but absence of Glycosides and Proteins

beta vulgaris have, Carbohydrates, Tannins, Phytosterol, Flavanoids, Saponins, Proteins, Mucilages but absence of Glycosides and Alkaloids.

Solanum lycopersicum: Carbohydrates, Glycosides, Tannins, Phytosterol, Flavanoids, Saponins, Mucilages but absence of Proteins and Alkaloids

Musa Acuminata have Alkaloids, Carbohydrates, Glycosides, Tannins, Phytosterol, Flavanoids, Saponins, Mucilages but absence of Proteins

From the table no 3: the antibacterial activity *beta vulgaris*, *Solanum lycopersicum* and *Musa Acuminata* was seen for *ecoli*. where as all the three extract have a significant result i.e., 31.64, 30.27, 30.

In which *Musa Acuminata* have a higher antibacterial activity for *ecoli*.

From the table no 4: the antibacterial activity *Pouteria Sapota*, *beta vulgaris*, *Solanum lycopersicum* and *Musa Acuminata* was seen for *salmonella*. where as all the four extract have a significant result i.e., 27.11, 10.23, 12.60, 22.59.

In which *Pouteria Sapota* have a higher antibacterial activity for *salmonella*

From the table no:5 the antibacterial activity *Pouteria Sapota*, *beta vulgaris*, *Solanum lycopersicum* and *Musa Acuminata* was seen for *bacillus*. where as all the four extract have a significant result i.e., 12.53, 18.08, 14.12, 15.54.

In which *beta vulgaris* have a higher antibacterial activity for *bacillus*

From table no: 6 All these extract donot have any antifungal activity.

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