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STUDIES ON ANTIMICROBIAL ACTIVITY OF HIBISCUS SPECIES OF KARNATAKA AGAINST CLINICAL ISOLATES

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ABSTRACT: Antimicrobial activity of the various species of *Hibiscus* like *Hibiscus sabdariff, Hibiscus rosa-sinensis, Hibiscus trionum, Hibiscus cannabinus, Hibiscus mutabilis* against various pathogens like *Staphylococcus aureus, E. coli, Bacillus subtilis, Streptococcus species, Salmonella typhimurium, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcescens*, were studied with the help of agar well diffusion method. The solvent extracts like Methanol, Ethanol, Chloroform, Acetone, Diethyl ether of all the samples were used to measure the antimicrobial activity by using Muller-Hinton agar plates which showed the highest zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa.* In *Hibiscus sabdariff,* Methanol, Chloroform, and Acetone extract showed the highest zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. In *Hibiscus rosa-sinensis*, Methanol and Acetone showed the highest zone of inhibition against *Streptococcus species, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. Chloroform showed the highest zone of inhibition against *Streptococcus species*. In *Hibiscus surattensis*, Chloroform showed the highest zone of inhibition against *E. coli*. Methanol, Chloroform, and Acetone showed the highest zone of inhibition against *Serratia marcescens*. All the pathogens were resistant to ethanol. *E. coli* and *Bacillus subtilis* showed the least zone of inhibition against all the extracts. In all the plant samples, all the pathogens were resistant to Diethyl ether extract except for *Streptococcus species* and *E. coli*, which showed least susceptible to it.

INTRODUCTION: The genus Hibiscus (Malvaceae) consists of more than 300 species of annual or perennial herbs, shrubs or, trees [1]. It is natively distributed from India or Saudi Arabia [2], while evidence [3] was showed by Murdock that *Hibiscus* sometime before 4000 BC was cultivated in western Sudan (Africa) by the black populations.

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To avoid the increasingly growing antibiotic resistance, many natural products such as native or modified proteins have been investigated for their antibacterial actions as possible substitutes for antibiotics [4, 5, 6]. It is an annual, erect, bushy, herbaceous subshrub growing up to 8 ft (2.4 m) tall, having smooth, cylindrical, red stems.

The leaves are long 3 to 5 in (7.5–12.5 cm), having reddish veins in green color, and alternate, long or short petioles. The older plants have, the simpler upper, and the leaves of young seedlings are simpler. The leaves are 3 to 5 or even 7; toothed margins. When they are mature and dry, it split

open, and the capsule turns brown. In the present study anti-microbial activity of various species of *Hibiscus* has been tested against various pathogens like *E. coli, Staphylococcus aureus, Pseudomonas aerugi-nosa, Klebsiella pneumonia, Bacillus subtilis, Salmonella typhimurium, Serratia marcescens* by using various extracts of Methanol, Ethanol, Chloroform, and Acetone.

Klebsiella pneumonia is gram-negative, non-motile bacilli belonging to the family Enterobacteriaceae. The large polysaccharides capsule forms the outermost layer of Klebsiella. They are present in faeces. It causes destructive pneumonia in HIV patients and in the patients undergoing immuno-suppressive therapy. *Bacillus subtilis* are large, Gram-positive, aerobic or facultatively anaerobic. They have the characteristic feature of producing spores resistant to unfavorable conditions. They are often detected in drinking water supplies. Ingestion of organisms through the consumption of foods like rice, vegetables, raw milk, meat products due to the toxins produced by the organisms.

E. coli is present in humans and animals, which is not harmful, whereas it can be pathogenic, causing some serious diseases such as urinary tract infections, bacteraemia, and meningitis. It can cause acute diarrhoea. *Pseudomonas aeruginosa* is a Gram-negative rod, aerobic, flagellated found in faeces, soil, water, and sewage. It causes serious pulmonary infections in patients suffering from cystic fibrosis and immunocompromised patients. *Staphylococcus aureus* is aerobic and anaerobic, non-motile, non-spore-forming. It is present on the skin and mucous membranes of animals. It is present in the Gastrointestinal tract and in sewage. The toxins and extracellular enzymes produced by organisms cause infections. The antimicrobial activity of *Hibiscus* species against pathogens has been studied by the agar well diffusion method by measuring the zone of inhibition against the various extracts treated with pathogens. Agar well diffusion method was used to screen the antibacterial and antifungal activities of different solvent extracts as displayed by Daoud *et al.,* (2015) [7]. In our study, it has found that Gram-positive bacteria like *Streptococcus, Staphylococcus* showed the highest zone of inhibition to the Hibiscus extracts due to the presence of thick-walled peptidoglycan, which undergoes degradation when treated with methanolic extract of *Hibiscus* whereas Gram-negative bacteria like *Bacillus subtilis, Salmonella typhimurium, Serratia marcescens* were resistant to diethyl extract of *Hibiscus* due to the presence of 3 layers around their plasma membrane made up of lipopolysaccharides (LPS).

MATERIALS AND METHODS: The plant samples of different species maintained in the greenhouse of Visveshwarapura College of Science, Bangalore, India, were obtained from the University of Agriculture Science, Bangalore, India. The obtained leaf samples were cleaned with distilled water, dried under shade, powdered, and stored in airtight bottles.

Solvent Extract Preparation: 5 g of each powdered sample was extracted with 50 mL of methanol for 48 h at room temperature with continuous stirring. The filtration method was employed after 48 h to collect the supernatant. The crude extract of the solvent was obtained by the process of evaporation.

The Antimicrobial analysis method was performed to reveal the antimicrobial activity of the plant samples with the help of the agar well diffusion method. The Solvent extraction was done by the Soxhlet apparatus. Muller-Hinton agar plates were prepared to evaluate the antimicrobial activity of the samples with the following solvent extracts Methanol, Ethanol, Acetone, Chloroform and Diethyl ether against selected human pathogens *viz., Streptococcus sp., Salmonella sp., E. coli, Bacillus sp., Pseudomonas sp., Serratia marcescens, Staphylococcus aureus,* and *Klebsiella pneumonia*. The Muller-Hinton agar plates were spread with 100µl inoculum of each selected pathogen uniformly with the help of a swab. A sterile cork borer is used to punch a well of 6mm in diameter after 5 minutes of incubation. The well was loaded with 80 µl of the concentrated sample. The plates were incubated at 37ºC overnight, and after incubation, plates were observed for the zone of inhibition [8, 9].

Minimum Inhibitory Concentration: The Minimum inhibitory concentration was carried out for the samples showing higher antimicrobial activity against the pathogens. Different concen-trations of the samples were checked for the anti-

microbial activity to determine the concentration at which the samples showed the least activity. Five different concentrations of the samples were used for the determination of the MIC. The chloroform extract of *Hibiscus mutabilis, Piper chaba, Ocimum gratissimum, Ocimum sanctum purple,* and *Ocimum basillicum* were used to determine the MIC as they showed a higher zone of inhibition against the pathogens [9].

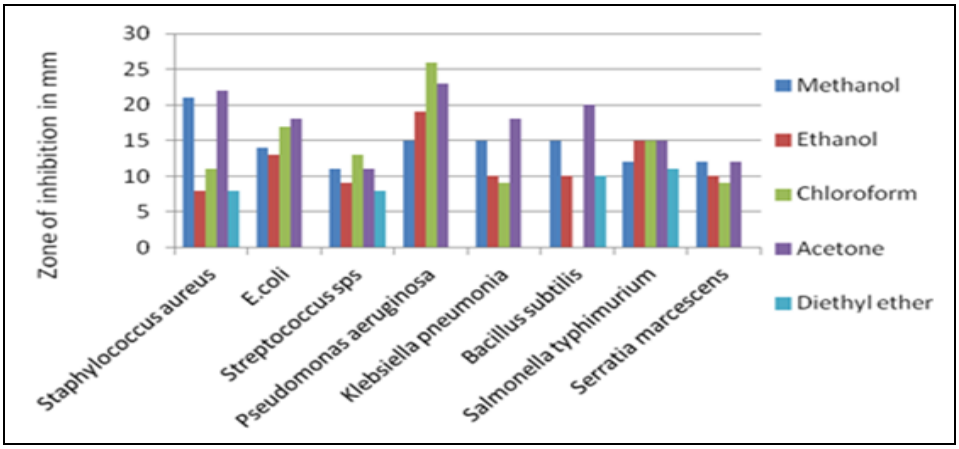
RESULTS AND DISCUSSION: Medicinal plants play an important role in acting against pathogens by treating diseases. Antioxidant assays in foods and biological systems can be classified into 2 groups, those based on the evaluation of lipid peroxidation and those based on the measurement of free radical scavenging power [10]. The agar well diffusion method is used to study the antimicrobial properties of *Hibiscus* plants. The new drug compounds evolved from plants have been contributed to human well-being. The plant extracts have been used for therapeutic purposes due to their antimicrobial properties.

Hibiscus species has been tested its antimicrobial activity by using various solvents of methanol, ethanol, chloroform, acetone, diethyl ether against various pathogens like *Staphylococcus species, E. coli, Streptococcus sp., Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis, Salmonella typhimurium, Serratiamarcescens.*

Hibiscus sabdariff is effective against *Staphylococcus aureus* and *Pseudomonas aeruginosa* but not effective in inhibiting *E. coli, Bacillus subtilis, Serratia marcescens* (**Table 1**, and **Graph 1**).

TABLE 1: EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HIBISCUS SABDARIFF* AGAINST SELECTED HUMAN PATHOGENS USING VARIOUS SOLVENTS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN mm)

Solvents	*S. aureus*	*E. coli*	*Streptococcus sps*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. typhimurium*	*S. marcescens*
Methanol	21	14	11	15	15	15	12	12
Ethanol	8	13	9	19	10	10	15	10
Chloroform	11	17	13	26	9	0	15	9
Acetone	22	18	11	23	18	20	15	12
Diethyl ether	8	0	8	0	0	10	11	0



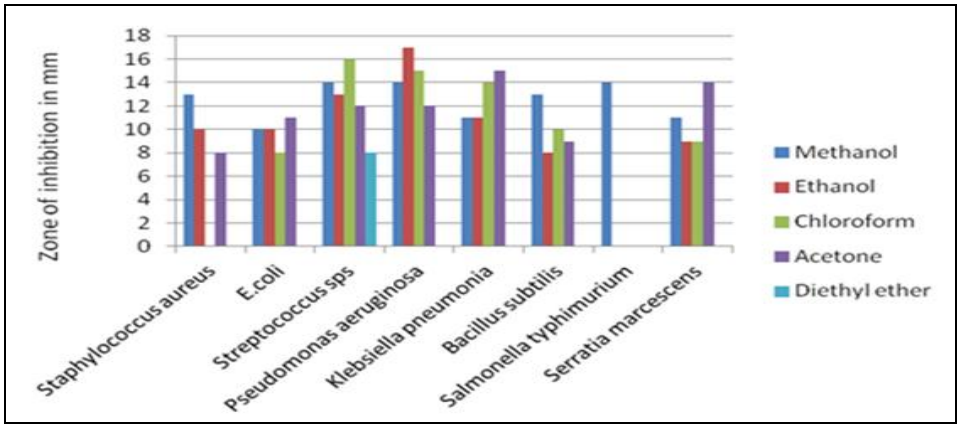
GRAPH 1: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS SABDARIFF*

Eunkyung Jung *et al*., (2013) [11] has studied the antimicrobial activity by using the ethanol extract of *Hibiscus sabdariffa* against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli.* Ethanolic extract of *Hibiscus sabdariffa* inhibited effectively *Bacillus subtilis,* and *Staphylococcus aureus* at a higher concentration than the water extract of *Hibiscus sabdariffa,* whereas paper disc method was used to determine the inhibition of *E. coli* by the water extract of *Hibiscus sabdariffa* at the concentration of 25 and 50 mg mL^{-1}.

The *Hibiscus rosa-sinensis* showed an effective zone of inhibition against *Streptococcus* species and *Pseudomonas aeruginosa*, whereas *Salmonella typhimurium* showed resistance in all the extracts except for methanol which showed an effective zone of inhibition (**Table 2**, **Graph 2** and **Fig. 1**).

TABLE 2: EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HIBISCUS ROSA-SINENSIS* AGAINST SELECTED HUMAN PATHOGENS USING VARIOUS SOLVENTS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	*S. aureus*	*E. coli*	*Streptococcus sps*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. typhimurium*	*S. marcescens*
Methanol	13	10	14	14	11	13	14	11
Ethanol	10	10	13	17	11	8	0	9
Chloroform	0	8	16	15	14	10	0	9
Acetone	8	11	12	12	15	9	0	14
Diethyl ether	0	0	8	0	0	0	0	0



GRAPH 2: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS ROSA-SINENSIS*

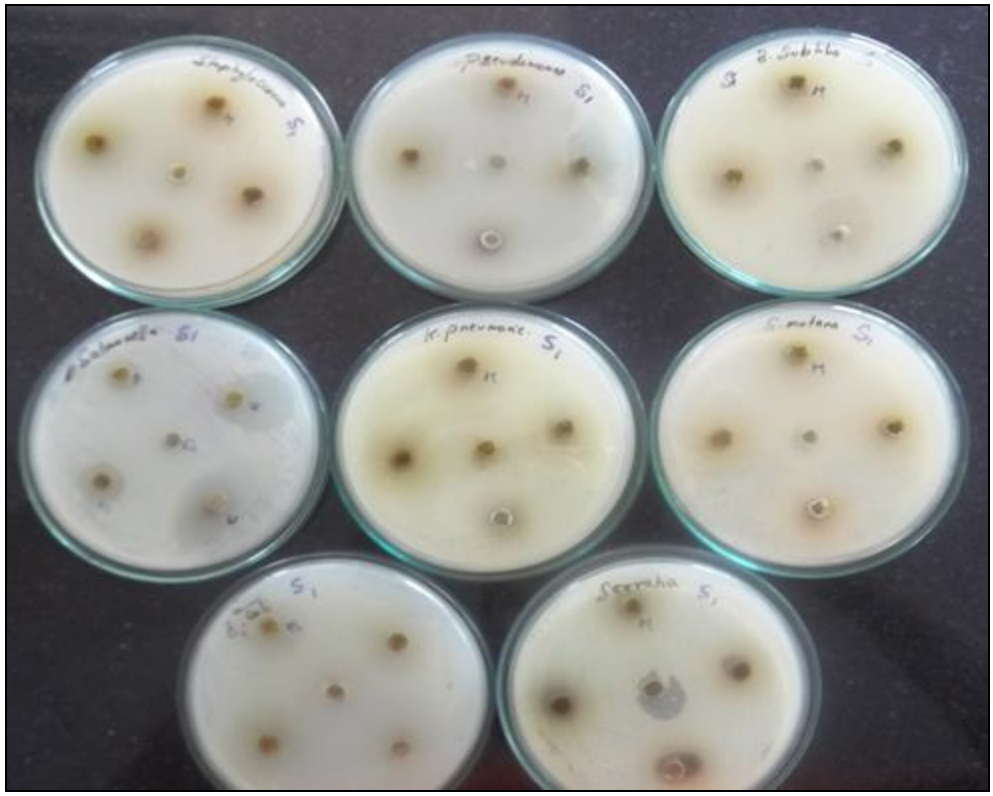


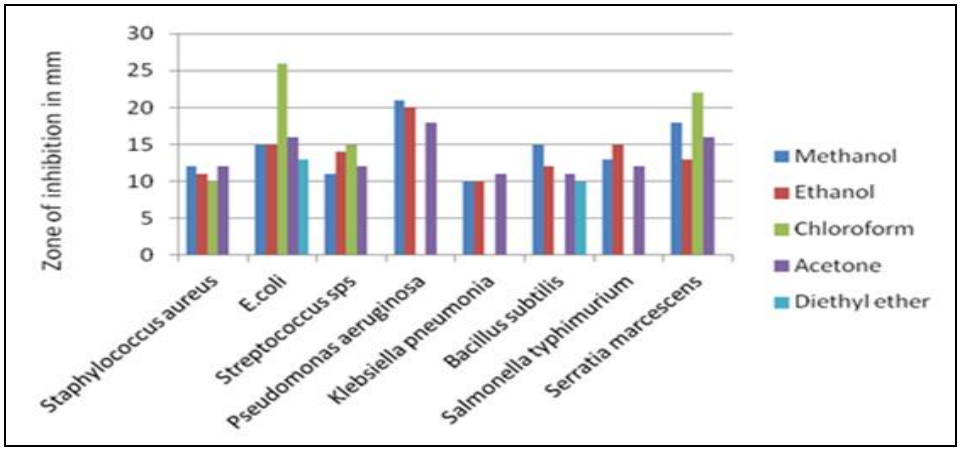
FIG. 1: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS ROSA-SINENSIS* AGAINST VARIOUS PATHOGENS

P. Ruban *et al*., (2012) [12] showed *Bacillus subtilis* and *Escherichia coli* got inhibited at the maximum concentration of (17+-2.91), (14.90+-1.71) mm in the cold extract of *Hibiscus rosasinensis*. The highest zone of inhibition illustrated against *Bacillus subtilis, Escherichia coli* as (18.86+-0.18), (18.00+-1.63) mm in the methanol extract whereas the highest zone of inhibition illustrated against *Salmonella* species at (20.40+-1.54) mm in the ethanol extract.

The *Hibiscus surattensis* showed the highest zone of inhibition against *pseudomonas aeruginosa* and *Escherichia coli*, whereas the chloroform and diethyl extract of *Hibiscus surattensis* was not effective in inhibiting *Klebsiella pneumonia, Bacillus subtilis, Salmonella typhimurium*. The diethyl extract of *Hibiscus surattensis* was not effective against all the pathogens except for the *E. coli*, which showed the least zone of inhibition of 13 mm. The maximum zone of inhibition of 26 mm was illustrated in the chloroform extract against *E. coli* and the maximum zone of inhibition of 22mm was illustrated against *Serratia marcescens* (**Table 3**, **Graph 3** and **Fig. 2**).

TABLE 3: EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HIBISCUS SURATTENSIS* AGAINST SELECTED HUMAN PATHOGENS USING VARIOUS SOLVENTS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	*S. aureus*	*E. coli*	*Streptococcus sps*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. typhimurium*	*S. marcescens*
Methanol	12	15	11	21	10	15	13	18
Ethanol	11	15	14	20	10	12	15	13
Chloroform	10	26	15	0	0	0	0	22
Acetone	12	16	12	18	11	11	12	16
Diethyl ether	0	13	0	0	0	10	0	0



GRAPH 3: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS SURATTENSIS*

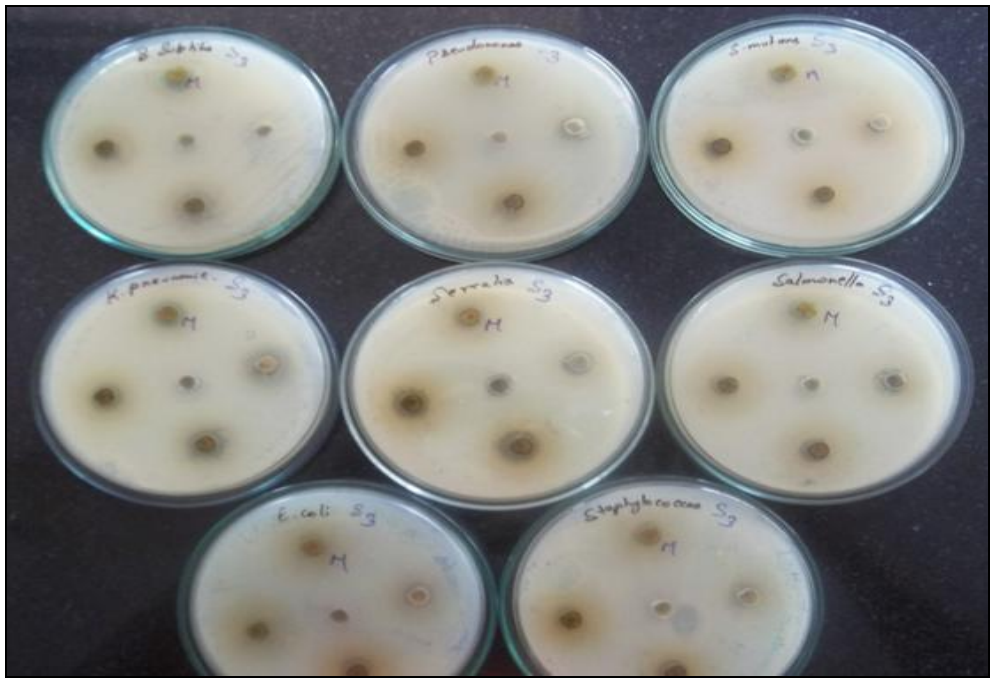
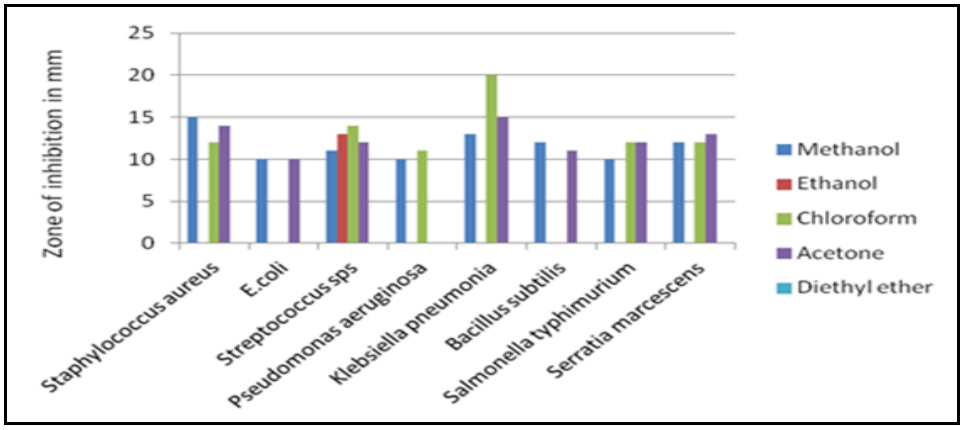


FIG. 2: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS SURATTENSIS* AGAINST VARIOUS PATHOGENS

In *Hibiscus trionum*, the chloroform extract showed the highest zone of inhibition of 20mm against *Klebsiella pneumonia*, and *E. coli* showed resistance against all the extracts. All the pathogens showed resistance to the ethanol and diethyl extract. The Acetone and Methanol extract showed a maximum zone of inhibition of 14 and 15mm, respectively, against *Staphylococcus aureus* (**Table 4**, **Graph 4** and **Fig. 3**).

TABLE 4: EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HIBISCUS TRIONUM* AGAINST SELECTED HUMAN PATHOGENS USING VARIOUS SOLVENTS (THE VALUES REPRESENTS THE ZONE OF INHIBITION IN MM)

Solvents	*S. aureus*	*E. coli*	*Streptococcus sps*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. typhimurium*	*S. marcescens*
Methanol	15	10	11	10	13	12	10	12
Ethanol	0	0	13	0	0	0	0	0
Chloroform	12	0	14	11	20	0	12	12
Acetone	14	10	12	0	15	11	12	13
Diethyl Ether	0	0	0	0	0	0	0	0



GRAPH 4: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS TRIONUM*

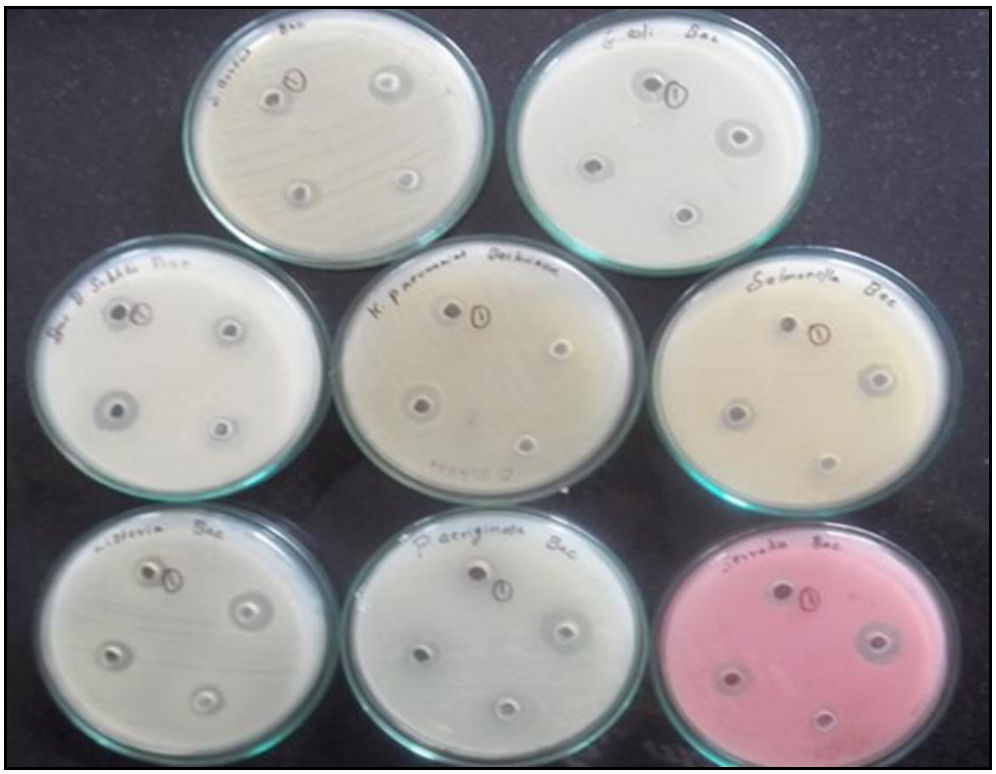
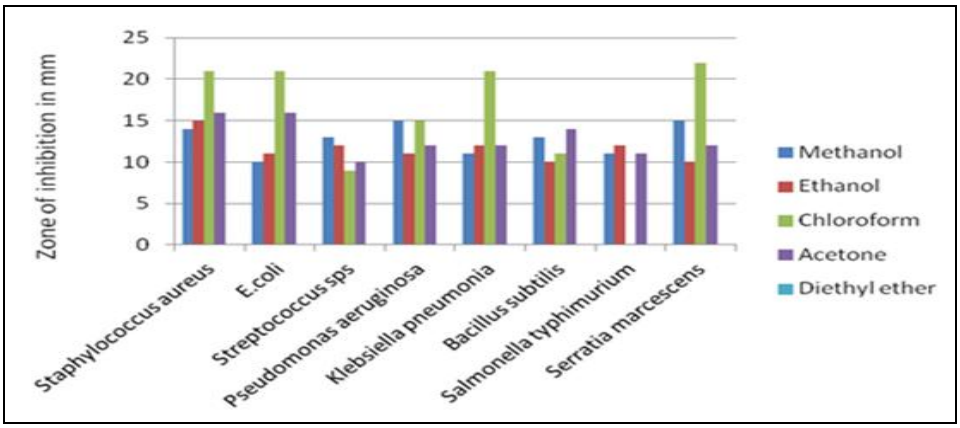


FIG. 3: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS TRIONUM* AGAINST VARIOUS PATHOGENS

In *Hibiscus cannabinus*, the highest zone of inhibition illustrated against *Serratia marcescens, Klebsiella pneumonia, E. coli,* and *staphylococcus aureus* in the chloroform extract of 22 mm, 21mm respectively. All the pathogens showed resistance to the Diethyl ether extract, whereas the least zone of inhibition of 10mm and 11mm illustrated in the methanol extract against *E. coli* and *Salmonella typhimurium,* respectively (**Table 5**, **Graph 5** and **Fig. 4**).

TABLE 5: EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HIBISCUS CANNABINUS* AGAINST SELECTED HUMAN PATHOGENS USING VARIOUS SOLVENTS (THE VALUES REPRESENT THE ZONE OF INHIBITION IN MM)

Solvents	*S. aureus*	*E. coli*	*Streptococcus sps*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. typhimurium*	*S. marcescens*
Methanol	14	10	13	15	11	13	11	15
Ethanol	15	11	12	11	12	10	12	10
Chloroform	21	21	9	15	21	11	0	22
Acetone	16	16	10	12	12	14	11	12
Diethyl ether	0	0	0	0	0	0	0	0



GRAPH 5: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS CANNABINUS*

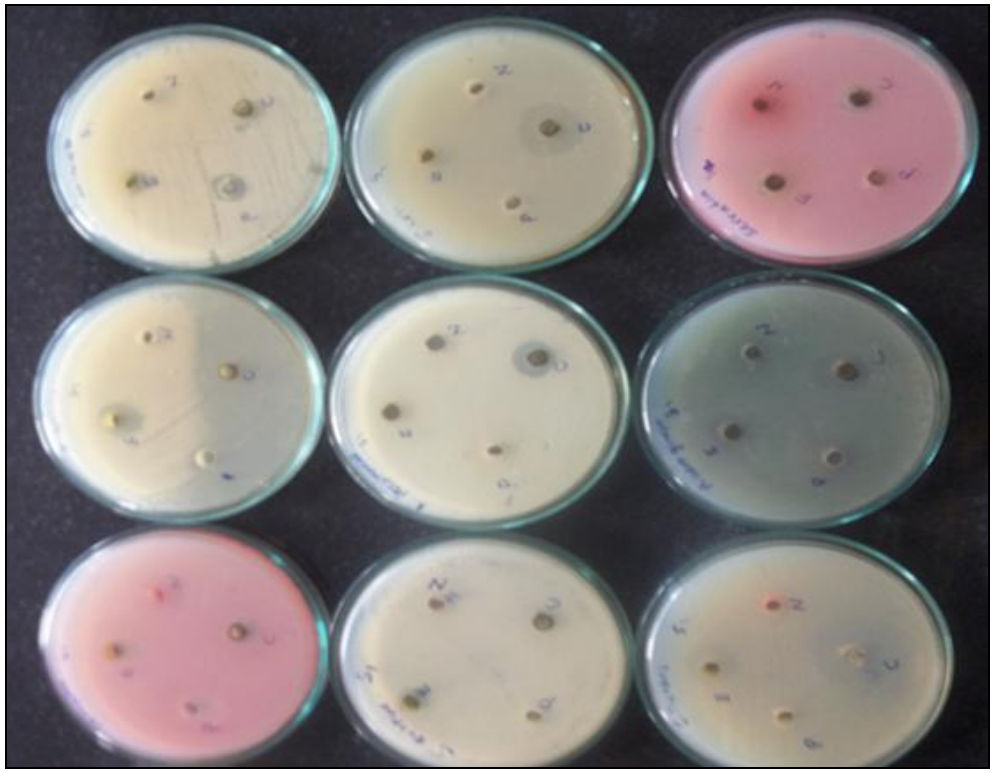
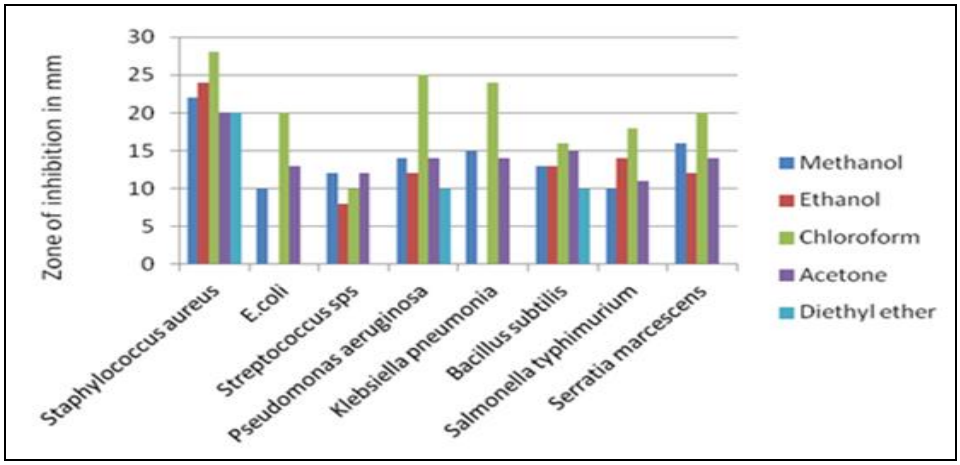


FIG. 4: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS CANNABINUS* AGAINST VARIOUS PATHOGENS

In *Hibiscus mutabilis*, Chloroform extract showed the highest zone of inhibition against *Staphylococcus aureus* (28mm), *Pseudomonas aeruginosa* (25mm) whereas all the pathogens except *Staphylococcus aureus* showed resistance to all the extracts of Diethyl ether (**Table 6**, **Graph 6** and **Fig. 5**).

TABLE 6: EVALUATION OF ANTIMICROBIAL ACTIVITY OF *HIBISCUS MUTABILIS* AGAINST SELECTED HUMAN PATHOGENS USING VARIOUS SOLVENTS (THE VALUES REPRESENT THE ZONE OF INHIBITION IN MM)

Solvents	*S. aureus*	*E. coli*	*Streptococcus sps*	*P. aeruginosa*	*K. pneumonia*	*B. subtilis*	*S. typhimurium*	*S. marcescens*
Methanol	22	10	12	14	15	13	10	16
Ethanol	24	0	8	12	0	13	14	12
Chloroform	28	20	10	25	24	16	18	20
Acetone	20	13	12	14	14	15	11	14
Diethyl ether	20	0	0	10	0	10	0	0



GRAPH 6: THE GRAPHICAL REPRESENTATION OF ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS MUTABILIS*

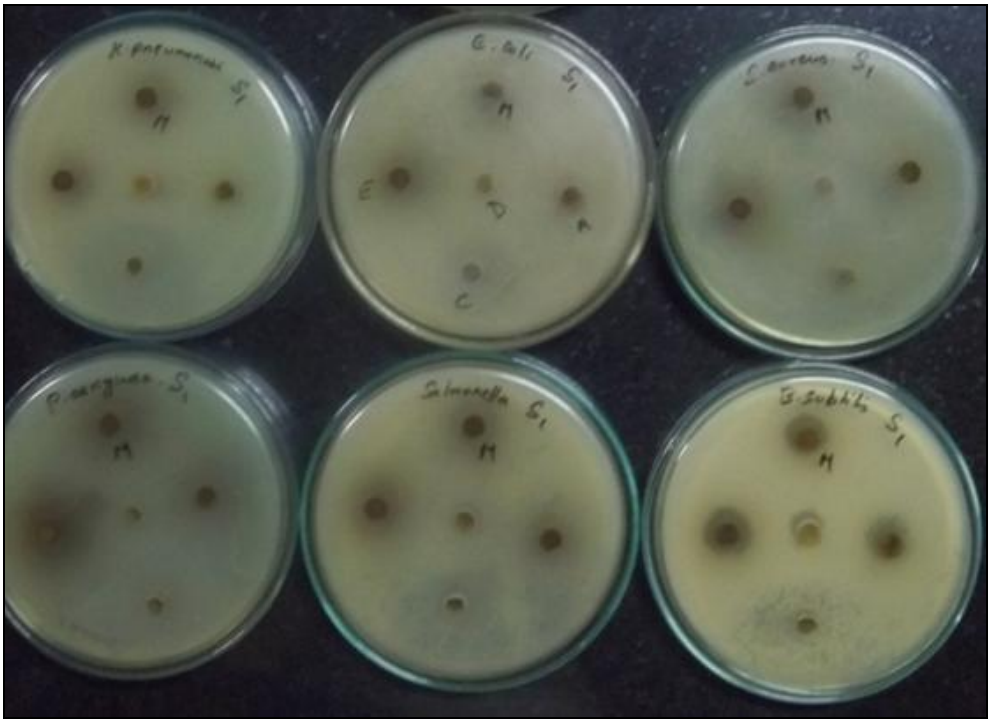


FIG. 5: MULLER-HINTON AGAR PLATE SHOWING ZONE OF INHIBITION BY USING VARIOUS SOLVENT EXTRACTS OF *HIBISCUS MUTABILIS* AGAINST VARIOUS PATHOGENS

Vandana H. Barve *et al*.,[13] illustrated antimicrobial activity of *Hibiscus mutabilis* on microbial cultures *viz*., *Staphylococcus aureus* (NCIM 2079 ATCC 6538P), *Salmonella typhi* (NCIM 2501 ATCC

23564), *Proteus vulgaris* (NCIM 2857), *Bacillus subtilis* (NCIM 2063 ATCC 6633), *Klebsiella pneumonia* (NCIM 2957) and *Escherichia coli* (NCIM 2931 ATCC 25922) by using methanolic extract and ethyl acetate. The Agar diffusion method is used to determine antimicrobial activity. The activity is measured by the zone of inhibition of the extracts against the pathogens like *Bacillus subtilis* were higher compared with the standard drug. The methanolic extract (8mg) and ethyl acetate extract (8mg) showed the zone of inhibition of 15mm and 13mm respectively, compared to standard Trimethoprim-sulfamethoxazole combination showed a zone of inhibition of 14mm. The native Cowpea seed proteins 7S and 11S were reported to strongly inhibit the *in-vitro* growth of *Pseudomonas aeruginosa* ATCC 26853 and *Salmonella typhimurium* ATCC 14028 [14].

Additionally, Soybeans glycinin basic subunit was able to inhibit methicillin-vancomycin intermediate Staphylococcus aureus (MRSA-VISA) while soy glycinin was competent to impede Bacillus spore germination [15, 16]. Anthocyanins are the polyphenolics that are responsible for the red to purple color in plants. They are members of the flavonoid group of phytochemicals [17]. Approximately 85% of anthocyanins were delphinidine-3-sambubioside, which is the principal source of the antioxidant capacity of roselle extract [18].

In the current study, *Hibiscus* species have been tested for antimicrobial activity by the agar well diffusion method. All the pathogens showed resistance to the Diethyl ether extract of all the *Hibiscus* species. Acetone extract of *Hibiscus sabdariff* and Chloroform extract of *Hibiscus Cannabinus* is effective in inhibiting the *Staphylococcus aureus* at the zone of inhibition of 22mm and 21mm, respectively (**Table 1** and **Table 5**).

In *Hibiscus rosa-sinensis*, Ethanol extract showed the highest zone of inhibition against *Pseudomonas aeruginosa* (17mm). *Salmonella typhimurium* showed resistance to all the extracts of *Hibiscus rosa-sinensis* except methanol which showed the least zone of inhibition of (14mm) **Table 2**. In *Hibiscus Surattensis*, *E. coli* got inhibited at the highest zone of inhibition of (26mm) to the Ethanol extract. *Pseudomonas aeruginosa, Klebsiella pneumonia, Bacillus subtilis* showed resistance to the chloroform extract of *Hibiscus surattensi* **Table 3.** In *Hibiscus trionum*, *E. coli* showed resistance to all the extracts except methanol which showed the least zone of inhibition (10mm). The chloroform extract of *Hibiscus trionum* showed the highest zone of inhibition (20mm) against *Klebsiella pneumonia* **Table 4**. Therefore, the potential of medicinal plants is determined by the zone of inhibition in the Agar well diffusion method indicating the antimicrobial activity of plants.

From the above study, it is clear that Methanol, Chloroform, and Acetone extracts of *Hibiscus mutabilis* **Table 6** have shown the highest zone of inhibition against *Staphylococcus aureus, Pseudomonas aeruginosa,* by the disc diffusion method, which evaluated the antibacterial properties of *Hibiscus mutabilis.*

Bacillus subtilis, Salmonella typhimurium has shown the least zone of inhibition against the leaf extracts of all the *Hibiscus* species except for the *Hibiscus sabdariff* **Table 1,** indicating these pathogens are resistant to all *Hibiscus* plants. Therefore, organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa* were sensitive thereby got inhibited against the leaf extracts of *Hibiscus mutabilis* **Table 6**.

Agar well diffusion method is used to determine the antimicrobial activity of *Hibiscus* plants by measuring the zone of inhibition around the discs where pathogens are inoculated, which is very useful in finding the applications of these medicinal plants.

Natural colorants may be promising active biological agents. For example, Phycocyanins were found to have many biological activities [19, 20, 21]. The biological importance of red beet is based on its high red pigment content (betalain), which displays excellent values, meeting some applications in food and pharmaceutical products. Among many plants accumulating betalains, only red beet and prickly pear (*Opuntia ficus indica*) are approved for food and pharmaceutical applications [22]. The anthocyanin-rich blueberry extract was capable of inhibiting the growth, adhesion, and biofilm formation of all of the following:- *Pseudomonas aeruginosa, E. coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* [23].

Roselle can be utilized either as a distinct functional food or as an active ingredient in other functional food potentially applicable in the treatment of various degenerative diseases [24]. Therefore, *Hibiscus sabdariffa* is a safe medicinal plant, having medical compounds with nutritional and medicinal properties [25].

CONCLUSION: The leaf extract of *Hibiscus* plants has been obtained by using various solvents to determine the antimicrobial activity against various pathogens.

The plant extract exhibits a strong antimicrobial activity that has thrown the limelight on the pathogens, which can be inhibited effectively to treat various diseases caused by them and also has led the way to fight against pathogens that are resistant to many drugs. Thus, phytomedicine has been successful in fighting against pathogens and has significance in pharmacology.

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