International Research Journal of Engineering and Technology (IRJET) e-ISSN: 2395-0056
Volume: 06 Issue: 05 | May 2019 www.irjet.net p-ISSN: 2395-0072

Phytochemical Screening and Effect of pH in the Antimicrobial Activity of Different Solvent of Hibiscus Mutabilis

Nahid Usmani Mirza[1], R. S. Nigam[2], O.P. Rai[3]

[1]Astt. Prof , Department of Chemistry, AKS University,Satna (M.P.)
[2] Dean, Department of Chemistry, AKS University, satna(M.P.)
[3]Prof. Department of Chemistry, Govt. P.G. College, Satna (M.P.)

ABSTRACT :- Hibiscus Mutabilis is reported for bioactive constituent. A quantitative assessment of antimicrobial activities was carried out by determining the minimum inhibitory and microbicidal concentrations (MICs and MMCs) of the various solvent extracts like 70% ethanol, acetone and ethyl acetate against some selected bacteria such as two Gram positive bacteria Bacillus subtilis, Staphylococcus aureus and two Gram negative bacteria Escherichia coli, Pseudomonas. On treating the extract of Hibiscus Mutabilis flower leave with different pH it was found that antimicrobial activity was increasing on increasing pH.From our study, we can conclude that ethanol extract has more prevailing and sustainable antibiotic properties than other solvents extrac.. Preliminary phytochemical screening of Hibiscus Mutabilis showed the presence of alkaloids, glycosides, steroids, phenols, tannins, flavonoids and saponins in the crude drug.

Keyword- Antibacterial, Alkaloids, Hibiscus Mutabilis; Phytochemical; MICs and MMCs,

INTRODUCTION-

The genus Hibiscus (Malvaceae) comprises about 275 species in the tropics and sub-tropics (Dasuki, 2001), Within the Malesian region, 43 species is found. Hibiscus Mutabilis Linn. Is a large bushy shrub belonging to family Malvaceae and commonly known as changeable rose or cotton rosemallow. Plants of Hibiscus are widely planted as ornamentals and are used in traditional medicine. the species studied, leaves and flowers of H. mutabilis, believed to have emollient and cooling effect, are used to relieve swellings and skin infections (Dasuki, 2001).They are used as an antiseptic for boils and ulcers. The sap from flowers is used as coloring agent.

Plants contain secondary metabolites; include alkaloids, glycosides, terpenoids, phenols, tannins, flavonoids and saponins (Edema and Alaga, 2012). Furthermore, there is growing interest in the chemical composition of plants towards discovery of more effective bio-therapeutic agents (Roja and Rao, 2002).

The aim of this work to find out the optimum condition of $\mathbf{P_H}$ at which Hibiscus Mutabilis Linn extracts is more effective against Bacillus subtilis, Staphylococcus aureus Escherichia coli, and Pseudomonas species of bacteria.

EXPERIMENTAL SECTION

Material and methods-The Hibiscus Mutabilis flower leaves are collected from the herbal garden of AKS university satna [M.P.] and authenticated in department of biotechnology of AKS university satna.

Selected bacterial species –
Gram-positive bacteria - Bacillus subtilis, Staphylococcus aureus .
Gram-negative bacteria - Escherichia coli, Pseudomonas.

Preparation of extract- The fresh flower leaves were washed with distilled water and air dried to constant weight. Extract was prepared by soxhlet extraction method. About 100 gm of powdered material was uniformly packed into a thimble and run in soxhlet extractor. It was exhaustible extracted with 70% ethanol; acetone and ethyl acetate for the period till the solvent in the siphon tube of extractor become colorless. After that extracts were filtered and filtrate were concentrated by evaporation to make the final volume one – fourth of the original volume and stored at air tight bottles.

Test for phytochemical constituents – Freshly prepared extracts were subjected to standard phytochemical analysis for different constituents.

Table.1 Qualitative screening of phytochemicals in ethanol, acetone and ethyl acetate flower extracts of Hibiscus mutibulis.

S.No	Phytochemicals/secondary metabolite	70% ethanol extract	Acetone extract	ethyl acetate extract
1	Carbohydrates	+	+	+
2	Proteins and amino acids	+	+	+
3	Alkaloids	-	-	-
4	Glycosides	+	+	+
5	Saponin	-	-	-
6	Anthraquinone	-	-	-
7	Cardiac glycoside	-	-	-
8	Flavonoids	+	+	+
9	Phenolic	+	+	+
10	Tannins	+	+	+
11	Steroids	+	+	+
12	Fats & fixed oils	+	+	+
13	Thiamine	+	+	+

The agar well diffusion assay for antibacterial activity of different spices-

The agar well diffusion method was carried out to study the antibacterial activity of extracts of *Hibiscus Mutabilis*. Nutrient agar medium was prepared to grow the test micro organisms of Bacillus subtilis, Staphylococcus aureus, Escherichia coli and *Pseudomonas*. The test bacterial culture 0.1 ml was inoculated in a Petri plate containing nutrient agar and spread evenly with sterile metal spreader under sterilized conditions. A hole of 7 mm diameter was made in a nutrient agar plate containing test organisms by using sterilized cork borer and 0.1 ml of extracts 70% ethanol, acetone and ethyl acetate of *Hibiscus Mutabilis* were poured in those well using a micropipettes. Then these inoculated nutrient agar plates were incubated at 37°C for 24 hrs in the incubator. After incubation the zone of inhibition were observed from the size of diameter and measured in mm.

Antibiotic Streptomycin-

The streptomycin susceptibility test discs are used as an antibiotic standard in this study.

Effect of pH on antibacterial activity of extracts of *Hibiscus Mutabilis* - To find out the effect of pH each extracts [70% ethanol, acetone and ethyl acetate] having concentration 100µg/ml were taken in three set of test tubes and 1N HCl

added drop wise drop with some time interval, until the pH of extract is 2 - 5 [pH is determined by digital pH meter].Now for making the medium alkaline (8-9 pH) by increment in pH in every extract is done by using 1N NaOH in three separate test tubes and extracts were then allowed to soaks for some time interval after that period of acid base treatment the extracts were again neutralized with using 1N HCl and 1N NaOH and then every extracts were tested for antibacterial activity by using agar disc diffusion method.

Table 2: Zone of inhibition of streptomycin

Bacteria	Zone of inhibition
Bacillus subtilis	11.23mm
Staphylococcus aureus	13.20mm
Escherichia coli	15.46mm
Pseudomonas	13.55mm

Table 3: Antibacterial activity of Hibiscus Mutabilis leaves with several solvent extracts of in form of Zone of inhibition (mm).

		Zone of inhibition in (mm)		
Species of Bacterial strain	Dose of extract	ethyl acetate extract	Acetone extract	70% ethanol extract
Bacillus subtilis	100µl.	2.45mm	3.65mm	7.42mm
Staphylococcus aureus		3.51mm	5.01mm	9.31mm
Escherichia coli		4.24mm	4.98mm	11.48mm
Pseudomonas		2.99mm	3.12mm	8.21mm

Table 4: Effect of pH in antibacterial activity of Hibiscus Mutabilis flower leaves with several solvent extracts

Species of Bacterial strain	Dose of extract	Effect of pH			Zone of inhibition in (mm) after 24hr
		Original pH	Acidic medium	Basic medium	
Bacillus subtilis	100µl.	7.60	5.2	9	5.22mm
Staphylococcus aureus		7.95	5.2	9	. 6.12mm
Escherichia coli	.	7.85	5.2	9	8.42mm
Pseudomonas		7.65	5.2	9	4.25mm

Results and Discussion: - The present study revealed that ethanol was the better extractive solvent for antibacterial activity of flower leave extracts of *Hibiscus Mutabilis* against the selected strain of bacteria and the maximum zone of inhibition 11.48mm was recorded from 100µl of 70% ethanol extract for *Escherichia coli* at the alkaline medium (9pH) after 24hr and minimum zone of inhibition was recorded as 2.45 mm for 100µlof ethyl acetate for *Bacillus* subtilis after

same time period at acidic medium (2.5 pH).As the pH of the medium get vary from acidic(2.5) to alkaline(9.0) the antibacterial activity of solvent extract of *Hibiscus Mutabilis* also increases.

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