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Assessment of Antibacterial Activity of some plants of the family Malvaceae

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ABSTRACT:
The present work deals with the assessment of phytochemicals, antioxidant activity and antibacterial activity of methanolic (90%) leaf extracts from a number of plants of the family Malvaceae(**Abutilon indicum (Link) Sweet.,KydiacalycinaRoxb.,Sida acuta Burm.f.,Sida cordifolia L., Thespesia populnia(L.) Sol. Ex Correa.and Urena lobata L.)** against six pathogenic bacterial strains (Gram-positive: Bacillus subtilis MTCC 121, Staphyllococcus aureus ATCC 25923, Staphyllococcus epidermidis MCC3086; Gram-negative: Escherichia coli MTCC 433, Salmonella typhi MTCC 734, Vibrio cholerae NI6961).The extracts were subjected to antibacterial assays like disc diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination.Among the samples Abutilonshowed highest antibacterial activity
against almost all tested bacteria showing highest inhibition zones and lowest MIC and MBC values, which point towards search for novel antimicrobials. The other plant extracts representeddifferential antimicrobial activity against the bacterial strains.

Keywords: Malvaceae, Antibacterial, Abutilon, Kydia, Leaf extract.

I. INTRODUCTION:

Medicinal plants have been used in traditional health care systems since prehistoric times and are still the most important health care source for the vast majority of the population around the world. Nature itself has a source of medicinal agents for thousands of years and an outstanding number of modern drugs have been isolated from natural sources, many based on their use in traditional system of medicines[1,2]. Medicinal plants play a vital role for the development of new drugs. These plants have a wide variety of chemical constituents and some of them have the ability to inhibit the growth of microorganisms[3]. About thirty thousand plant species are used for medicinal purposes[4]. A vast wealth of medicinal plant sources is still underutilization for curing a number of diseases. Moreover, the use of synthetic drugs has many bad side effects on human body as well as the indiscriminate use of synthetic drugs has led to the development of multi-drug resistant microorganisms[5]. This situation has raised our interest in the use of medicinal plants against microorganisms. Some species of Malvaceae family also show antimicrobial activity[6,7].Malvaceae, the mallow family is a major group of angiosperm that is flowering plants. It contains about 244 genera and more than 4225 species[6,7]. It is mostly distributed in tropical region and a few occur in temperate region and includes members of economic values like okra, cotton, durian, cacao and many medicinal as well as ornamental plants.[5]Malvaceae family plants exhibited antimalarial, antimicrobial, antihypertension, antidiabetic, anti-inflammatory and antioxidant activities. Bioactive compounds including indole alkaloids, terpenoids and anthraquinones have been isolated from these plants[6,7].

The aim of the study is to access the antioxidant properties and antibacterial activities of some plants of the family Malvaceae against some Gram-positive and Gram negative bacteria.

II. MATERIALS AND METHODS:

Collection of plant materials:

Fresh plant materials were collected from different places in Kalyani University Campus (Nadia), West Bengal. All the plant samples were identified using previously authenticated samples kept in the herbarium of the Department of Botany, Kalyani University. Details of the plants (used in the experiment) are as follows:

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Serial No.	Scientific Name	Parts used	Sample No.	Common Name
1.	Abutilon indicum	Leaf	S1	Indian mallow, Atibaala
2.	Kydiacalycina	Leaf	S2	Bharanga, Pola
3.	Sida acuta	Leaf	S3	Flannel weed, bala
4.	Sida cordifolia	Leaf	S4	Heart-leaf sida, Bala
5.	Thespesia populnea	Leaf	S5	Portia tree, Indian tulip tree
6.	Urena lobata	Leaf	S6	Caesarweed, Congo jute

Preparation of Methanol Extract:

The plant samples were washed to remove the adherent dust particles & then shade dried for 15 days followed by mechanical grinding to obtain the samples in powdered form. All the powdered samples were extracted with 90% methanol for three days (72 hrs.) in dark condition with occasional shaking. Sample to solvent ratio was kept as 1:20. The filtrates were obtained by filtration using Whatman No. 1 filter papers and concentrated under reduced pressure using rotary vacuum evaporator (Buchi). Thus,extracts were obtained and yield was calculated. These crude extracts were kept at 4^0 C until used for further use.

Determination of antibacterial activity:

In this method the bacteria were used in the assay of antibacterial activity of plant extract. Among the bacterial cultures three were Gram-negative bacteria (Escherichia coli- MTCC 433, Salmonella typhi- MTCC 734, Vibrio cholera NI6961) and another three were Gram-positive bacteria (Bacillus subtilis- MTCC 121, Staphyllococcus aureus- ATCC 25923, Staphyllococcus epidermidis). Mueller-Hinton agar media (pH 7.2 to 7.4) was used as growth medium for the determination of antibacterial activity of sample extracts.

Each time, the required volume of media was prepared and sterilized using autoclave. Spread-plate technique was applied using sterilized cotton swabs to make the bacterial lawn (100 µl inoculum were taken) over the surface of media-containing plates. The inoculums of bacteria were prepared 24 hours before the experiment.

➢ **Preparation of disc containing plant extract:**

Plant extracts (200mg/ml) were prepared as stock solution for each sample by dissolving them in suitable volume of methanol. Discs were prepared from filter paper. About 15µl , 30µl and 60µl extract solution was poured into each disc respectively for making the 3mg , 6mg, 12mg concentration. The discs were dried under laminar.

➢ **Disc Diffusion Assay:**

In this method[8] agar plate is spread with inoculum (100 µl), dried and then paper discs impregnated with plant extracts were placed. A paper disc containing the highest amount of solvent used for dissolving the extracts served as a negative control. Discs containing Ampicillin and Streptomycin (5 µg/ disc) were used as a positive control. The bacteria are allowed to grow on the agar media in the incubator(temperature:37ºc) for 24 hours. After 24 hours of incubation, the zone of inhibition was measured by millimeter(mm) scale. The highly effective concentration of plant extract will produce a wide ring of no bacterial growth, while no change in surrounding bacterial concentration indicates less effective sample extracts. The effectiveness of the sample extract can be measured using their zone of inhibitions.

➢ **Determination of Minimum Inhibitory Concentration(MIC):**

The MIC value represents the lowest concentration of plant extract which prevents growth of bacteria after 24 hours incubation by micro-well dilution method[9]. For making these dilutions plant extracts were dissolved in methanol to obtain plant extracts of different concentration (ranging from 1mg/ml-13mg/ml)were prepared. Each plant extracts of different concentration were poured in the respective wells in a 96 well plate with Müeller-Hinton broth (MHB) to obtain the final concentrations.10 µl of inoculum was added in each well obtaining a final volume of 200µl. Media containing extract of different concentrations without inoculum were served as extract control for each concentration. Blank media

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with inoculum was used as inoculum control. The plates were then incubated for 24 hr. at 37ºC with shaking. After 24 hour the absorbance was taken 610 nm using an ELISA plate reader (BioTek). The MIC value was detected as the lowest concentration at which the absorbances remain same as the extract control indicating no bacterial growth.

➢ **Determination of Minimum Bacterial concentration(MBC):**

The MBC represents the lowest concentration of plant extract which killed the bacteria[9]. MBC was performed by taking out a loop full of suspension from each micro-well previously used for the MIC assay and streaked on petri dish contain MHA. MBC is the concentration at which no growth of bacteria was seen after incubating the plates for 24hr. at 37ºC.

III. RESULT AND DISCUSSION

Antibacterial activity:

Table 1: Growth inhibition zones (mm) showing antibacterial activity of the extracts against different bacterial strains.

Plant Samples	Concentration of extract (mg/disc)	Bacteria					
		Gram Positive			Gram Negative		
		B. subtilis	Staphylococcus aureus	Staphylococcus epidermidis	E. coli	V. Cholerae	Salmonella typhi
Abutilon	3	11.15± 1.77	ND	ND	11.65± 1.63	ND	ND
	6	13.35± 2.05	5.95± 0.07	ND	15.45± 1.63	6.80± 0.42	5.89± 1.12
	12	15.10 ± 2.40	8.25± 1.06	6.00± 0.28	15.75± 0.21	7.45± 0.35	6.67± 0.67
Kydia	3	5.75± 0.07	ND	5.60± 0.42	16.35± 0.07	7.45± 0.21	ND
	6	7.40± 0.71	5.90± 0.42	6.55± 0.78	16.30± 0.57	8.50± 0.00	ND
	12	ND	8.45± 1.91	7.85± 1.34	18.75± 2.33	9.00± 2.40	6.45± 0.09
S. acuta	3	7.42± 0.45	ND	ND	7.49± 0.33	ND	ND
	6	8.61± 0.23	6.91± 1.03	5.59± 1.31	8.78±1.23	6.90± 0.49	ND
	12	9.13± 0.71	8.43± 0.04	6.45± 0.73	10.12± 0.79	7.48± 0.65	6.09± 0.19
S. cordifolia	3	7.34± 0.79	ND	ND	7.03± 0.75	ND	ND
	6	8.04± 0.64	5.59±0.67	5.69±0.65	8.12± 1.03	6.90± 0.33	ND
	12	9.03± 0.79	7.31± 0.29	6.33± 0.06	9.45± 0.69	7.04± 0.78	5.78± 0.52
Thespesia	3	ND	ND	ND	ND	ND	ND
	6	ND	ND	ND	ND	ND	ND
	12	5.75± 3.13	ND	ND	7.03± 0.78	ND	ND
Urena	3	5.55± 1.28	ND	ND	8.70± 0.28	ND	ND
	6	6.00± 0.08	ND	ND	10.7± 1.84	ND	ND
	12	6.80± 2.65	6.55± 1.06	5.60± 0.14	13.5± 0.71	6.30± 0.28	ND

All the sample extracts were loaded on the discs at different concentrations (3mg/ disc, 6mg/ disc and 12mg/ disc and vehicle control discs were loaded with the exact solvent volume (60µl MeOH) used to prepare the disc of the highest concentration. Among the samples,Abutilon and Kydia showed the highest antibacterial activity against almost all tested bacteria except Salmonella typhi against which no sample extract showed satisfactory inhibition zones.Thespesia extract

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showed the least inhibition zones among the extracts.

Table 2: Minimum Inhibitory Concentration (MIC; mg/ml) and Minimum Bactericidal Concentration (MBC; mg/ml)

Plant Samples	Bacteria											
	Gram Positive						Gram Negative					
	B. subtilis		Staphylococcus aureus		Staphylococcus epidermidis		E. coli		V. Cholerae		Salmonella typhi	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Abutilon	3	4	5	6	12	14	2.5	3.5	6	7	ND	ND
Kydia	4	4.5	5.5	6	5	6	2	3	3.5	4.5	ND	ND
S. acuta	7	8	11	12	11	12	7.5	8	13	14	ND	ND
S. cordifolia	7	8.5	12	12.5	12	12.5	8	8.5	13	14	ND	ND
Thespesia	13	14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Urena	7	9	10	11	12	13	5	7	10	11	ND	ND

ND= Not detected upto 15 mg/ ml

Abutilon and Kydiaalso showed lower MIC and MBC than other tested samples, indicating their higher antibacterial potentiality.

High antibacterial activity of different parts of these two extracts was also reported previously[10,11]. But we have evaluated a comparison among them. Different Sida sp. were also reported to be antibacterial in nature[12,13]. Our study also depicted substantial antibacterial properties of Sida acuta and Sidacordifolia.

IV. CONCLUSION:

Plant-obtained antioxidants and antimicrobial representatives are inhabiting the limelight day by day as they are harmless and consistent. The study offered a relativevaluation of the antibacterial efficacies of six plants belonging to the family Malvaceae using their leaf methanolic extracts. Findings from the study revealed significant antibacterial activities in all the plants at varying degrees Among these plants, Abutilonindicum and Kydiacalycinaleaf methanolic extract showed the highest antibacterial. It is intended from the study that the potent antioxidant and antibacterial properties of these leaves can be exploited for the seclusion of phytochemicals to be used in therapeutics as antimicrobial(s) of plant origin.

Conflict of Interest: There are no conflicts of interest among the authors.

REFERENCES:

[1]. Tiwari, A.K. and Madhusudanarao, J., 2002. Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects.Current Science, 83(1), pp.30-8.

[2]. Tariq, A., Mussarat, S. and Adnan, M., 2015. Review on ethnomedicinal, phytochemical and pharmacological evidence of Himalayan anticancer plants. Journal of ethnopharmacology, 164, pp.96-119.

[3]. Iwu, M.W., Duncan, A.R. and Okunji, C.O., 1999. New antimicrobials of plant origin. Perspectives on new crops and new uses. ASHS Press, Alexandria, VA, pp.457-62.

[4]. Pushpangadan, P., Dan, V.M., Ijinu, T.P. and George, V., 2013. Phytochemical characterization of medicinal plant in herbal drugs. International E-Publication.Chemistry, Biochemistry and Ayurveda of Indian Medicinal Plants, pp. 1-12.

[5]. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clinical microbiology reviews, 12(4), pp.564-82.

[6]. Christenhusz, M.J. and Byng, J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa, 261(3), pp.201-17.

[7]. Das, U. and Islam, M.S., 2019. A review study on different plants in Malvaceae family and their medicinal uses. American Journal of Biomedical Science and Research, 3, pp.94-7.
[8]. Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M., 1966. AntibioticSusceptibility Testing by a Standardized Single Disk Method, Am. J. Clin. Pathol., 36, 493-6.
[9]. Klančnik A., Piskernik S., Jeršek B. and Možina S.S., 2010. Evaluation of Diffusion and Dilution Methods to Determine the Antibacterial Activity of Plant Extracts, J. Microbiol. Methods, 81(2), 121-6
[10]. Kaushik, D., Khokra, S.L., Kaushik, P., Sharma, C. and Aneja, K.R., 2010. Evaluation of antioxidant and antimicrobial activity of Abutilon indicum. Pharmacologyonline, 1, pp.102-8
[11]. Pavithra, G.M., Naik, A.S., Siddiqua, S., Vinayaka, K.S., Kekuda, P.T.R. and Mukunda, S., 2013. Antioxidant and antibacterial activity of flowers of Calycopteris floribunda (Roxb.) Poiret, Humboldtiabrunonis Wall and KydiacalycinaRoxb. International Journal of Drug Development and Research, 5(2), pp.301-10.
[12]. Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simpore, J., Colizzi, V. and Traore, A.S., 2005. Antibacterial activity of alkaloids from Sida acuta. African journal of biotechnology, 4(12), pp. 122-8.
[13]. Venkatachalam, D., Thavamani, S., Sebastian, A.C., Anju, V.B., Mathew, C., Leon, D., Thomas, J. and Muhammed, M.M., 2019. Evaluation of antimicrobial activity of Sida cordifolia leaf extract. South Asian Journal of Research in Microbiology, 4(1), pp.1-7.