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THE EFFECT ON ANTIMICROBIAL ACTIVITY OF *THESPESIA POPULNEA* AND *ABUTILON INDICUM* AGAINST CLINICAL MICROBES

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ABSTRACT: Traditional medicine is an important source of potentially useful compounds for the development of phytotherapetic agent. Antimicrobials of plant origin have enormous therapeutic potential in the treatment of infectious diseases while simultaneously migitigiting many of the side effects that are often associated with synthetic antimicrobials. In the present investigation suggested that the effect of antimicrobial activity of *Thespesia populnea and Abutilon indicum* against clinical microbes were performed. In the experiments of the test plant *T. populnea and Abutilon indicum* leaf extract with different solvent of aqueous, methanol, and diethyl etherwere treated against the bacteria like *Bacillus cereus, E. coli, K.pnemoniae, Pseudomonas aeuroginosa* and *Staph. aureus,* and fungi such as *Aspergillus flavus, A.niger. A.terreus, Penicillium* sp. and *Fsarium solani* were performed respectively. However the antimicrobial properties of *Thespesia populnea* and *A.indicum* leaf with methanolic and diethyl ether extract of maximum zone inhibition and excellent performance when compared to other solvent of aqueous extract. It can be concluded that the plant used to discover natural products that may serve as lead for the development of new biomedical applications.

KEYWORDS: *T. populnea and A.indicum*bacteria, fungi, antimicrobial activity.

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1.INTRODUCTION

As we move into the 21st century, we observe a big change in the attitude of physicians, researchers and the general public towards prophylaxis and therapeutics originating from plant drugs. Nearly all-major pharmaceutical houses are back into research on plant products [18]. Medicinal and aromatic plants constitute a major source of natural compounds which are widely used in medicinal products, cosmetics and paints that are of paramount importance in everyday life. Hence the ethnobotanical approach is currently being applied to the search for new drugs using plants which are being used by traditional societies. Plants are a potential source of antimicrobial compounds and several researchers throughout the world are investigating the antimicrobial activity of medicinal plants which are utilized in the traditional or alternative healthcare systems. [26,7]. Screening of medicinal plants for therapeutically active bio-molecules including those with antimicrobial properties has gained an unprecedented importance in the recent years. World Health Organization (WHO) has recently shown genuine interest in promoting the development and utilization of indigenous medicinal plant resources in the developing countries so as to extend safe and effective healthcare to maximum number of population on those countries [5]. Emerging antibiotic resistant infections are one of the most serious problems the medical professionals face today. Due to the immense cost of discovery and regulatory uncertainties, large pharmaceutical companies are hesitant to commit to antibiotic discovery programs. The tens of millions of unwarranted deaths per year. Recently, considerable attention has been paid to utilize eco-friendly plant based products for prevention and cure of different human diseases since they are safe and effective. Studies are conducted to shed light on the antibacterial activity of some indigenous medicinal plants. Nonetheless, the investigations have primarily been restricted to screening only. In order to promote herbal drugs there has to be an evaluation of therapeutic potentials of drugs [4]. The screen the antibacterial and antifungal activities of *Thespesiapopulnea*, by using modern scientific approaches and innovative scientific tools. *Abutilon indicum* (Family: Malvaceae) is extensively grown in Bangladesh., India, Pakistan, Srilanka. The plant is considered as antibacterial, astringent, anthelmintic, carminative and diuretic. It is used locally for high fever, colds, tuberculosis, bronchitis, mumps, diabetes, hernia, hemorrhoids, diarrhea and various types of worm infections. Many chemical constituents have been isolated from *Cuscutare flexa* such as Cuscutin, quercitin, coumarin, amarbelin, myricetin and oleanolic acid. *A. indicum* leaves are used in the treatment of toothache, lumbago, antifertility and liver disorders. Bark and root are used as antidiabetic, aphrodisiac [10] nervine tonic and diuretic. The plant extracts and their products for antimicrobial activity have shown that a potential source of novel antibiotic prototypes of higher plants [1]. Antimicrobial activity in seeds has been reported*;* the methanolic extract of *A. indicum* exhibited some estrogenic potential of antifertility substances. Gossypetin – 8 and 7- glycosides and cynidin 3- rutinoside are also isolated by

Sebastian.The seeds are reported as laxative the ethno-medicobotanical investigations in Kerala for use of leaves of *A.indicum* in malarial fever, cold, cough, chest pain. Gallic acid shows analgesic activity in animal models. The public health is of antimicrobial and antifungal resistance due to severe exploitation of synthetic antibiotics. The synergism assay conducted on bacteria using well-known antibiotics such as ampicillin, oxytetracycline, chloramphenicol and fungal using well known antibiotics such as penicillin, ketazole majority of the bacteria showed resistance to the employed antibiotics. Large pharmaceutical companies and industries are hesitant to develop novel antibiotic drugs due to the emerging of antibiotic resistant microbes [17]. The carried out by antibacterial activity of Leaf, Bark, Flower and Seedextracts of *Thespesia populina* against the tested organisms using agar disc diffusion method while Anti-fungal activity of Leaf, Bark, Flower and Seed extracts of *Thespesia populnia* L. against the tested organisms using agar disc diffusion method. Bacterial strains used were GramNegative bacteria were *E. coli, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia*, while Gram positive *Streptococci pyogenes, Staphylococci* and *Bacillus cereus* for studies. Fungal cultures were (*Aspergillus flavus, Aspergillus niger, Fusarium oxyforum, Colletotrichum falcetum, Rizopus stolonifer* and *Mucarpiritorus*) using different organic solvents like Methanol (Polar Solvent), Chloroform (Non-Polar) and Aqueous solvent [12].

2. MATERIALS AND METHODS

Collection of plant materials

Healthy plants of *Thespesia populnea* L. and *Abutilon indicu*m L. were collected from Gopal nagar, Thanjavur, Tamilnadu, India. The leaf materials were cleaned and free from dirt particles and shade dried.

Preparation of plant extracts

Soxhlet method used for extraction of crude materials One gram of *Thespesia populnea* L. and *Abutilon indicu*m L. powder leaves blended with 50 ml of different solvents separately (aqueous, methanol and diethyl ether) for different periods with agitation at room temperature. After the extracts were allowed to filtration by using a 0.45 Millipore filter paper. The plant extracts concentrated using a rotary evaporator at 40°C under reduced pressure. Finally the extracts were allowed to weigh and store at -20°C till their usage in the different tests.

Agar well – diffusion method

Agar well – diffusion method was followed for determination of antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria (*Bacillus cereus, E.coli, K.pneumoniae, P. aeuroginosa* and *S. aureus*) and fungi (*Aspergillus flavus, A. niger, A. terreus, Penicillium* sp. and *Fusarium* sp.) were determined agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About different solvent leaves extracts of *T. populnea and*

Abutilon indicum added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions of the plates were incubated in an upright position at 37± 2°C for 24 hrs for bacterial and 28± 2°C for fungi. The organic solvents (aqueous and methanol) were acted as a negative control results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity.

3. RESULTS AND DISCUSSION

The ethanolic crude extract of *T. populnea* flowers exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in the sample possesses antibacterial activity. The disc diffusion method showed the zone of inhibition for 10 mg/ml against *S. typhi, E. coli, E. faecalis* and *B. cereus* respectively when compared with standard drug Chloramphenicol showing 22 mm, 19 mm, 23 mm and 25 mm zone of inhibition respectively. Then it is evident from the data presented in the sample possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 10 mg/ml as for 20 mg/ml as 9 mm and 0 mm, for 30 mg/ml as 19 mm and 18 mm and for 40 mg/ml as 23 mm and 26 mm against *C.lunata,* and *C. albicans* respectively when compared with standard drug Fluconazole showing 25 mm and 20 mm of inhibition respectively. The above result shows that the activity of ethanolic crude extracts of *T. populnea* flowers shows significant antibacterial and antifungal activities and also the possession of antimicrobial activities against a number of microorganisms [25]. The antimicrobial investigation by agar well diffusion method showed that the plant *T.populne* and *A.indicum* with aqueous solvent extract has an effective activity comparable with the other plants. The effect of different concentration of 25, 50, 75, and 100µl *T. populne* leaf extract was treated against *Bacillus cereus, E.coli, K. pneumonia, P. aeroginosa* and *S. aureus* controlled by this method. The maximum antibacterial activity was 20.9±6.89, 07.3±2.44, 18.0±6.00, 17.6±5.87 and 06.3±2.11mm and *A. indicum* 13.5±1.03, 15.2±4.06, 17.5±4.30, 21.1±5.23, and 12.2±4.21zone of inhibition recorded against *Bacillus cereus, E.coli, K. pneumonia, P. aeroginosa* and *S. aureus* treated respectively (Table-1). Shahla *et al*. [19] studied that thephytochemical screening and antibacterial activity of *Citrullus colocynthis* against *Staphylococcus aureus* were analysed. The antimicrobial properties of medicinal plants against microbes, the significant antimicrobial activity of active extracts was compared with the other extracts against bacteria and fungi [6,11, 16, 17, 24]. The effect of antibacterial activity of *T. populnea* with methanolic extract was excellent properties observed. The maximum zone inhibition was 12.1±4.02, 14.0±4.67, 13.3±4.44, 12.0±4.00 and 10.00±3.33mm and extraordinary properties zone of inhibition *A. indicum* methanolic extract 21.6±7.03, 17.1±5.66,

18.3±6.04, 18.3±6.04 and 17.3±5.66mm with respective bacterial samples treated and minimum zone of inhibition was 08.3±2.78mm and recorded against *E.coli* at 25µl concentration of plant extract leaf respectively (Table – 2). The effect of antibacterial activity of *T. populnea* leaf extract of diethyl ether extract has excellent zone of inhibition was *T. populnea* plant extract against bacteria *Bacillus cereus, E.coli, K.pneumoniae, P. aeuroginosa* and *S. aureus* (19.0±6.33, 06.6±2.22, 16.3±5.44, 18.6±6.20, and 14.3±4.78mm more significant results observed and *A. indicum* leaf extract also 24.3±8.04, 14.3±4.66, 18.6±6.24, 14.3±4.66 and 13.6±4.33mm that higher concentration of 75 and 100µl excellent performance when compared with lower concentration of 25µl 13.1±4.33, 05.2±1.66, 13.2±4.33, 11.3±3.66 and 12.3±3.02mm zone of inhibition recorded with respective bacterial samples was respectively (Table-3). According to the antifungal properties of the *T.populnea* leaf with aqueous solvent were observed in 100µl concentration of leaf extract against *A.niger* (11.6±3.87) and *A. indicum* leaf extract was observed 100µl concentration of leaf extract against *A. niger* (21.6±7.00) followed by other fungi and minimum zone f inhibition at *T. populnea* plant extract 25µl concentration against *A.flavus* (4.33±1.44mm) and *A. indicum* plant extract 25µl concentration *Fusarium* sp. (07.1±2.33mm) zone of inhibition observed respectively (Table – 4). Efficacy of antifungal enhancement of *T.populnea* with methanolic extract against fungi like *Aspergillus flavus, A.niger, A.terreus, Penicillium* sp. and *Fusarium* sp. was treated. It was 16.7±5.56mm (*Aspergillus flavus*) zone of inhibition recorded at 100µl concentration of leaf extract respectively. *A. indicum* 27.6±9.00mm *A. terreus* whereas other concentration and suppression of antifungal activity was depending upon the active doses of the crude leaf extract with subjective. The minimum zone of inhibition at 25µl concentration *T. populnea* extract was 05.0±1.67mm (*A.flavus*) and *A. indicum* minimum zone of inhibition 50µl 08.0±2.66mm (*A.flavus*) recorded respectively (Table-5). Udayakumar and Hazeena Begum [29] reported that *T. populnea* leaf extracts were found to be effective against *E. coli* with an inhibition zone of 10 mm and *S. typhi* with 11 mm zone. The effect of antifungal activity of *T.populnea* leaf with diethyl ether extract was performance against fungi like *Aspergillus flavus, A. niger, A. terreus, Penicillium* sp. and *Fusarium* sp. with 7.67±2.53, 9.00±3.00, 3.05±1.42, 2.36±1.14, and 9.00±3.04mm and *A. indicum* leaf extract 100µl (16.4±5.33, 11.2±3.06, 17.6±5.64, and 16.3±5.33mm zone of inhibition measured respectively. It may be due to the plant extract with extraction solvent was main active principles of plant with recording antimicrobial properties when compared to other solvents (Table – 6). According to the previous study, *P. amarus* a plant related with *Phyllanthus urinaria* when extract with ethanol may inhibit the activity of *Salmonella typhi* [24, 9, 20, 2]. The antimicrobial activity of all the Leaf extracts was examined against Gram positive and Gram-negative bacteria and fungal strains by measuring zone of inhibition. The antimicrobial activity was performed by Agar disc diffusion method at concentration level of 2.5, 5.0, 7.0, 10µg/ml respectively. The *Abutilon indicum* leaf extract showed high activity against *Staphylococcus aureus* at very low

concentration (2.5µg/ml) compared to *E.coli,* leaf extract showed high activity against *Candida parapsilosis* at a very low concentration (2.5µg/ml) compared to *Aspergillus niger*. The zone of inhibition had calculated in cm *Ampicillin* (antibacterial), *Itraconazole* (antifungal) as standard drug at a concentration of 200µg/ml.LB Agar was used as culture media for antibacterial and potassium dextrose agar was used as culture media for antifungal activity. The plant extract shows the growth inhibition produced by the leaf extracts of *A.indicum* on 6 species of bacteria. The activities can be referred as either less, moderate or highly active based on the zone of inhibition that ranges from 9- 12mm, 12 – 16mm or >16mm respectively [8]. The leaf extract of *Abutilon indicum* was found to be highly active against *S. aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi* (25, 25, 17,18mm). The chloroform extract was moderately active against all organisms tested except for *Klebsiella pneumoniae* (8mm). Aqueous extract was found topossess no activity against any of the bacteria tested. The of the plant extract tested against various bacteria were in concordant with the positive control (Chloramphenicol).The comparative study of the antibacterial activity of the different extracts of *Abutilon indicum* along with negative and positive control revealed that it possesses good antibacterial potential.Kumar et al. [9] also reported antifungal activity against *Candida albicans* (MTCC 10231) by the leaf extract of *T.populnea*. Shekshavali and Hugar [21] also reported the presence of antifungal property of *T.populnea* bark extracts against *Candida albicans* and *A. flavus*. The previous study reveals that different extracts from the *Abutilon indicum* leaves were not shown statistically significant to inhibit microorganisms [21, 29]

Table 1: Effect of antibacterial activity of *Thespesia populnea* and *Abutilon indicum* leaves with aqueous extract against bacteria

Name of bacteria	Zone of inhibition (mm)							
	Thespesia populnea				*Abutilon indicum*			
	25 µl	50 µl	75 µl	100 µl	25µl	50 µl	75 µl	100 µl
Bacillus cereus	12.3±4.11	16.6±5.56	14.7±4.89	20.9±6.89	12.6±2.33	12.4±1.04	13.5±1.03	12.1±3.12
E. coli	11.4±3.77	12.3±4.10	09.0±3.00	07.3±2.44	11.2±3.33	10.2±3.05	15.2±4.06	14.4±3.03
Klebsiellapneumoniae	11.7±3.37	16.7±5.53	12.2±4.11	18.0±6.00	20.4±7.33	14.3±8.19	17.5±4.30	11.5±7.05
Pseudomonas auroginosa	10.3±3.43	11.3±3.77	14.0±4.67	17.6±5.87	14.1±6.21	11.4±5.04	21.1±5.23	15.2±9.03
Staph. aureus	05.0±1.67	08.0±2.67	10.0±3.33	06.3±2.11	16.5±2.33	16.2±1.03	12.2±4.21	18.3±1.16

Standard deviation ±error

Table 2: Effect of antibacterial activity of *Thespesia populnea* and *Abutilon indicum* leaves with methanolic extract against bacteria

Name of bacteria	Zone of inhibition (mm)							
	Thespesia populnea				*Abutilon indicum*			
	25 µl	50 µl	75 µl	100 µl	25µl	50 µl	75 µl	100 µl
Bacillus cereus	12.5±4.20	14.4±4.87	14.5±4.80	13.7±4.53	12.1±4.02	16.6±5.33	15.3±5.12	21.6±7.03
E. coli	08.3±2.78	10.3±3.44	12.2±4.11	14.0±4.67	12.3±2.02	14.2±4.66	18.3±6.04	17.1±5.66
K.pneumoniae	14.3±4.78	13.0±4.33	09.0±3.00	13.3±4.44	10.6±3.33	14.3±4.66	14.2±4.66	18.3±6.04
P.auroginosa	10.0±3.33	12.0±4.00	09.6±3.22	12.0±4.00	05.6±1.66	08.2±2.66	12.3±4.00	18.3±602
Staph. aureus	05.3±2.00	07.0±2.00	06.3±2.11	10.0±3.33	06.6±2.00	11.3±3.66	14.3±4.66	17.3±5.66

Standard deviation ±error

Table 3: Effect of antibacterial activity of *Thespesia populnea* and *Abutilon indicum* leaves with diethyl ether extract against bacteria

Name of bacteria	Zone of inhibition (mm)							
	Thespesia populnea				*Abutilon indicum*			
	25 µl	50 µl	75 µl	100 µl	25µl	50 µl	75 µl	100 µl
Bacillus cereus	19.0±6.33	14.6±4.86	18.7±6.20	18.0±6.00	13.1±4.33	15.6±5.00	17.3±5.66	24.3±8.04
E. coli	06.6±2.22	14.3±4.77	10.0±3.33	12.3±4.11	5.02±1.66	7.63±2.33	12.3±4.02	14.3±4.66
K.pneumoniae	16.3±5.44	17.0±5.67	14.3±4.78	13.6±4.56	13.2±4.33	14.3±4.66	15.3±5.04	18.6±6.24
P.auroginosa	18.6±6.20	12.3±4.00	15.6±5.20	13.3±4.44	11.3±3.66	10.3±3.33	12.6±4.00	14.3±4.66
Staph. aureus	14.3±4.78	07.7±2.56	12.3±4.11	06.0±2.00	12.3±3.02	12.3±4.01	12.4±4.03	13.6±4.33

Standard deviation ±error

Table 4: Effect of antifungal activity of *Thespesia populnea* leaves with aqueous extract against fungi

Name of fungi	Zone of inhibition(mm)							
	Thespesia populnea				*Abutilon indicum*			
	25 µl	50 µl	75 µl	100 µl	25µl	50 µl	75 µl	100 µl
Aspergillusflavus	4.33±1.44	5.33±1.78	07.6±2.56	-	07.1±2.33	10.4±3.33	07.3±2.33	11.6±3.66
A.niger	-	5.00±1.67	07.3±2.44	11.6±3.87	10.3±3.33	15.3±5.00	20.3±6.66	21.6±7.00
A. terreus	6.67±2.22	-	10.3±3.44	-	22.3±7.33	18.6±6.00	07.6±2.33	18.6±6.00
*Penicillum*sp	8.00±2.67	7.33±2.44	-	10.0±3.33	18.2±6.21	21.3±7.23	22.3±7.33	19.6±6.33
*Fusarium*sp.	-	9.00±3.00	-	09.3±3.44	06.6±2.33	06.3±2.10	-	08.3±2.66

(-) absent Standard deviation ±error

Table 5: Effect of antifungal activity of *Thespesia populnea* leaves with methanolic extract against fungi

Name of fungi	Zone of inhibition(mm)							
	Thespesia populnea				***Abutilon indicum***			
	25 µl	**50 µl**	**75 µl**	**100 µl**	**25µl**	**50 µl**	**75 µl**	**100 µl**
Aspergillusflavus	05.0±1.67	2.09±6.00	08.0±2.67	16.7±5.56	09.3±3.21	08.0±2.66	11.3±3.66	14.3±4.66
A.niger	06.0±2.00	8.20±2.67	07.3±2.78	13.6±4.55	21.2±7.00	15.3±5.10	12.3±4.00	22.3±7.33
A. terreus	-	6.12±2.00	06.0±2.00	10.0±3.33	16.3±5.33	22.3±11.2	25.4±8.33	27.6±9.00
*Penicillum*sp	-	6.09±2.00	07.3±2.44	06.7±2.22	15.6±5.33	20.6±6.64	25.3±8.33	25.6±8.33
*Fusarium*sp.	10.3±3.43	-	11.3±3.78	14.0±4.67	-	33.3±1.00	07.6±2.33	08.0±2.66

(-) absent Standard deviation ±error

Table 6: Effect of antifungal activity of *Thespesia populnea* leaves with diethyl ether extract against fungi

Name of fungi	Zone of inhibition(mm)							
	Thespesia populnea				***Abutilon indicum***			
	25 µl	**50 µl**	**75 µl**	**100 µl**	**25µl**	**50 µl**	**75 µl**	**100 µl**
Aspergillusflavus	-	06.0±2.00	07.7±2.53	10.3±3.44	19.3±6.33	15.3±5.00	-	16.4±5.33
A.niger	06.7±2.2	10.3±3.02	09.0±3.00	05.8±1.44	-	11.4±3.66	17.6±5.66	11.2±3.06
A. terreus	04.1±1.44	-	03.5±1.42	04.3±1.44	15.1±5.04	-	14.3±4.66	17.6±5.64
*Penicillum*sp	07.3±1.04	-	02.6±1.14	04.5±1.44	12.3±4.03	-	13.4±4.33	16.3±5.33
*Fusarium*sp.	06.7±2.20	-	09.0±3.04	13.6±4.56	-	08.2±2.66	-	-

(-) absent Standard deviation ±error

4. CONCLUSION

In conclusion, the results of this study revealed that methanol extracts of *T. populnea* and *A.indicum* leaves exhibited strong antimicrobial activity against bacterial and fungal strains tested. The inhibitory effect of this plant will be helpful to pharmacological industry for the preparation of herbal products after further scientific validation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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