

Antibacterial And Antifungal Properties Of Bougainvillea Spectabilis Andtecoma Stans Plant Extracts Against Human Pathogens

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Abstract:

The use of various herbal remedies and preparations are described throughout human history representing the origin of modern medicine. Herbal medicine is also called botanical medicine or phyto-medicine, and is defined as the use of plant to prevent or treat illness. Medicinal plants constitute an important component of flora and are widely distributed all over India. The pharmacological evaluation of substances from plants is an established method for the identification of lead compounds towards the development of novel and safe medicinal agents. The importance of medicinal plants and traditional health systems in solving the health care problems of the world is gaining increasing attention. As this renewed interest in medicinal plants continues to surge, research on these botanical treasures is experiencing an explosive growth on the global stage. However, this zealous pursuit of knowledge sometimes exacts a toll on the very ecosystems and indigenous populations in the countries of origin. This study was conducted to determine the antimicrobial and antifungal activity of Bougainvillea spectabilis and Tecoma stans grown wildely in Jamnagar geographical location. This study concluded that both plants show positive outcome towards antibacterial activity while negative outcome at antifungal activity. Both of these plants are having antibacterial compounds which can be extracted by methanol. The study on antimicrobial values ofBougainvillea spectabilisandTecoma stans, will help us, more effectively and efficiently manner for pharmacological purposes.

Keywords: Bougainvillea spectabilis, Tecoma stans, antibacterial and antifungal activity

Introduction

Medicinal plantshave been defined by World Health Organisation (WHO) as plants that contain properties or compounds those can be used for therapeutic purposes or synthesize metabolites to produce useful drugs. Medicinal plants are widely distributed in India and constitute an important component of flora. The pharmacological evaluation of metabolites from plants is an established method for the identification of lead compounds which can be utilized for the development of novel and safe medicinal agents. The importance of medicinal plants and traditional health systems are gaining cumulative attention in solving the health care glitches of the world. Since, this resurgence of interest, the research towards medicinal plants is growing phenomenally at the international level.Likewise, traditional medical practice has become an integral part of the culture in most of the developing countries (Chopra et al., 1992; Ghani 2003). In the annals of history, the roots of medicinal concoctions traced back to the embrace of nature's bounty, predominantly stemming from florabe it the unadorned essence of raw botanical specimens or the distilled essence found within primitive extracts and intricate blends. In our contemporary understanding, it is surmised that myriad plant species, reaching into the thousands, have been celebrated across diverse cultures for their curative properties.

Species under study Bougainvillea spectabilis commonly known as bougainvillea is linked with the Nyctaginaceae family (Hannah Joyce R. Caliling, 2020). The plant is sometimes referred to as "Paper Flower", the reason being its bracts are thin and papery (Jiraungkoorskul, 2017). The floral arrangement in this plant is at leaf axile and in clusters of three. The propagation of the plant Bougainvillea is done by cutting, layering, and budding. (Singh, 2015)The leaf of this thorny woody perennial vine, contains various active components such as furanoids, saponins, flavonoids, quinones, phenols, sterols, triterpenoids, glycosides, tannins, and small quantities of sugars(Pratibha Chauhan, 2016). The chemical constituent which largely contributes to its medicinal significance is 3-o-methylchironinositol (D-pinitol) isolated from the leaves of Bougainvillea spectabilis(Sikandar Khan Sherwani, 2013) The vital constituents which the therapeutic properties are bougainvinones, pinitol, quercetagetin, contribute to quercetin, and terpinolene(Jiraungkoorskul, 2017). Apart from its medicinal properties the plant species also has been recently studied as an alternative to Wright stain in blood smear preparation. Extract of the bracts of the plant was macerated with 100% methanol for about 72 hours before carrying out the staining procedure to achieve the crude extract (Hannah Joyce R. Caliling, 2020). The plant additionally serves as a phytomonitor and helps in quantifying foliar dust fall. The active monitoring of dust fall at critical locations helps keep an account of the air pollution(Nitesh C.J., 2017)

A perennial shrub and a member of Bignoniaceae family, Tecoma stans is an evergreen shrub that grows rapidly and is commonly known as Yellow-Elder due to its bell-shaped yellow flowers. The leaf showcases opposite or subopposite phyllotaxy and the appearance of the leaf is odd-pinnately compound (Watson, 1993) The plant species act as invaders in a natural grassland ecosystem and they modify the biodiversity of the region by abolishing natural resources. Its drought resistance nature and easy maintenance makes it a preferred plant for ornamental gardens(Bhat, 2019). Yellow-Elder is native to Northern America and extends up to Mexico and Central America. The plant species was later introduced to several other parts of the world like southern Africa, India, and Hawaii.Tecoma stans has monoterpene alkaloids as constituents specifically Tecomine 1 and Tecostanine 2 which largely contribute to the hypoglycaemic behavior of the plant extract(Luca Costantino, 2003) Other than this phytosterols, triterpenes, flavonoids, phenols, saponins and iridoid glycosides were also identified from the leaves and roots of this plant(Sunitha Katta, 2016).

The present study examined the antibacterial and antifungal activities of plant extracts (Bougainvillea spectabilis and Tecoma stans) against selected clinical pathogenic strains of bacteria and fungi. The extraction procedure for both plant's extracts are same where methanol, water, hexane, and ethyl acetate solvents were used and evaluated on the basis of antibacterial and antifungal activity.

Materials and Methods

Collection and Identification of Plant Materials: Bougainvillea spectabilisand Tecoma stansplantwere used during this studywere collected during 2019 to 2021 fromJamnagar district Geographical region of Gujarat.Identification of these plants were done visually by comparing plant parts with available pictures on public database.

Preparation of Crude Extracts: All plant materials were collected and then sun dried for 3 days. After completion of sun drying, material was grinded by mixer grinder (Make: Phillips) and fine power was made. These powder of both plants were analysed for water content, ash content, carbon, hydrogen, nitrogen and sulphur content. This fine powder was used for extract preparation using multiple solvents. 20 gm fresh plant materialwas used for extraction with 60 ml solvents. Extraction was carried out in Soxhlet Extractor with 4 different solvents which are water, methanol, hexane and ethyl acetate. The filtrate was evaporated to dryness under reduced pressure using a rotary vacuum evaporator (BUCHI). The concentrated crude extracts obtainedfrom evaporator was dried inside oven (Thermo) at 60°C for 6 hrs and dried extract powder was stored at 4°C (Dieudonné Lemuh et: al; 2015).

Determination of moisture content

This method relies on measuring the mass of water in a known mass of sample. The moisture content is determined by measuring the mass of sample before and after the water is removed by evaporation. **% moisture** = {(W1-W2)/W1} X 100

Here, W1 isinitial weight and W2 isdried weight of the sample before and after drying, respectively. The basic principle of this technique is that water has a lower boiling points than the other major component within samples e.g. lipids, proteins, carbohydrate and minerals.

In brief, first weigh the petridish and taken 3 g of sample,put this sample in petridish. Then the petridish with sample was dried in the hot air oven at 105°C for 6 hrs. After completion of 6 hrs drying the petridish is taken out from the hot air oven, cooled down to room temperature and weighed. This process is continued every after 1 hr until the weight became constant. After achieving constant weight moisture content was measured in duplicate as per above mentioned formula (Anju Paul et: al; 2018).

Determination of ash content

Ash is a residue which remained after heating of organic waste/organic matter in the presence of oxidising agents, which provides a measure of the total amount of ash within a sample. Analytical techniques for providing information about the total ash are based on the fact that the ash can be distinguish from all the other components within a sample in measureable way.

The ash content of the different plant biomass was determined by a muffle furnace according to ISO 1171-1981.Weigh the crucible at first step. Then take around 3 g of sample and put it in crucible and then it is heat for 2 hours at 550°Cin muffle furnace. Then it is taken out cooled and weighed. After getting final weight ash content was calculated in duplicate as per below mentioned formula (Anju Paul et: al; 2018).

% Ash= (W4/W3) X 100

Here, W3is initial weight and W4is weight after ashing of sample.

Elemental Analysis

The elemental analyser uses a combustion method to convert carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O) elements into simple gases. These gases were measured using thermal conductivity detector (TCD). 10 mg of plant biomass samples were weighed onaluminium boat, placed into elemental furnace, and burnt in a pure oxygen

environment at 1150°C. The elemental composition (CHNS) of dried microalgal biomass was determined using vario MACRO cube CHNS/O analyser (Elementar) while O was calculated on deduction basis. Weight per cent of each element was analyzed and calculated in duplicate (Nishant Saxena et: al; 2020).

Determination of antimicrobialactivity using disc diffusion method

The extracts from the plant biomass of Bougainvillea spectabilisand Tecoma stans plants were evaluated for their antimicrobialactivity using disc diffusion method. The extracts in the 5% concentration were tested against pathogenic organisms such asStaphylococcusaureus, Bacillus subtilis, Aspergillus niger and Candida albicans. All these strains were procured from National Collection of Industrial Microorganism (NCIM) Pune and their specific strain codes are 2079, 2045, 1004 and 3102respectively (Fig.1). All these strains were inoculated, preserved and revived as per the shared procedure by NCIM using respective media/broth for the antimicrobial study(Anju Paul et: al; 2018).

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Fig.- 1: Strain Identification and Procurement Details

Media Preparations

Nutrient Agar (NA): Weighed 28 g of Nutrient agar and mixed with 1000 ml of distilled water. Put the solution in water bath till agar gets dissolved completely.

Potato Dextrose Agar (PDA): Weighed 39 g of potato dextrose agar and mixed with 1000 ml of distilled water. Put the solution in water bath till agar gets dissolved completely.

Malt Glucose Yeast Peptone Agar (MGYP): Weighed 41.4 g of malt glucose yeast peptone agar and mixed with 1000 ml of distilled water. Put the solution in water bath till agar gets dissolved completely.

All required goods used in antimicrobial activity (i.e. Petri dish, test tubes, filter paper dishes, distilled water and pipette) and media (i.e.NA, PDA and MGYP) were autoclaved at 121°C (15 psi) for 15 minutes.

Preparation of 5% Extract Solution:Weighed accurately 5.0 gm of each extract and diluted with 100 ml of distilled water. 4 extracts of Bougainvillea spectabilis and Tecoma stans were prepared using water, methanol, hexane and ethyl acetate which were nominated as BW, BM, BH, BE, TW, TM, TH & TE respectively.

All materials used in this disc diffusion assay and all types of culture media were sterilized by standard autoclave cycle at 121°C & 15 lbs for 30 minutes.

Serial Dilution:All the strains were grown in respective media and fresh dilutions were prepared for this study. 2 scoop full of each strain was mixed properly in sterilised distilled water, from that 1ml is pipetted out and was poured into another test tube containing 9 ml sterilised distilled water. This method is continued till 10⁵ dilutions and final diluted solution was used for evaluating antimicrobial activity. Extract specific solvent was used as a negative control (-ve) and cefpodoxime proxetil (200 mg) antibiotic was used as positive control (+ve) during antibacterial study. In case of antifungal activity negative controls were same while Fluconazole (400 mg) was used as a positive control.

All positive controls tablets were dissolved in 10 mL& 20 mL respectively to make final solution concertation of 2% with respective diluent and then 0.1 mL volume of both positive controls were used. 0.1ml of 5% herbal extract, 0.1ml of dilution liquid containing microorganism and 15 ml nutrient agar. One glass petridish was marked in 4 different areas

for each extract where herbal extract was in duplicate, while positive and negative control was in singlet form. This type of 4 plates were prepared for antibacterial activity of one plant and 4 separate plates were prepared for antifungal activity of the same plant. Similarly, separate set of plates was prepared for each activity of each plant using 0.1 ml of 5% herbal extract, 0.1ml of dilution liquid containing microorganism and 15 ml nutrient agar (Table -1). After the agar gets solidified, the plates were placed in incubator (at 38°C) for 48 hours. After 48 hours, plate count was performed to determine the antimicrobial activities of Herbal extract.

Bacterial strains were incubated at 37.0 ± 2.5 °C for 48 hrs and fungal strains were incubated at 25.0 ± 2.5 °C for 5 days. After completion of incubation days zone of inhibition was measured. All estimations were done in duplicate and mean values were reported with STDEV.

Plant Name	Activity Name	Extract Name	
	Ť	Bougainvillea water extract (BW)	
		Bougainvillea methanol extract (BM)	
	Antibacterial activity against	Bougainvillea hexane extract (BH)	
Bougainvillea spectabilis	Bacillus substilis (2045)	Bougainvillea ethyl acetate extract (BE)	
		Tecoma water extract (TW)	
		Tecoma methanol extract (TM)	
	Antibacterial activity against	Tecoma hexane extract (TH)	
Tecoma stans	Staphylococcus aureus (2079)	Tecoma ethyl acetate extract (TE)	
		Bougainvillea water extract (BW)	
		Bougainvillea methanol extract (BM)	
	Antifungal activity against	Bougainvillea hexane extract (BH)	
Bougainvillea spectabilis	Aspergillus niger (1004)	Bougainvillea ethyl acetate extract (BE)	
		Tecoma water extract (TW)	
		Tecoma methanol extract (TM)	
	Antifungal activity against	Tecoma hexane extract (TH)	
Tecoma stans	Candida albicans (3102)	Tecoma ethyl acetate extract (TE)	

 Table-1 Details of Antibacterial & Antifungal Activity of Plant's Extracts

Results & Discussion:

Fine powder of both plants (Bougainvillea and Tecoma) were made as per the process mentioned in material method section. This was the raw material for this study hence its quality evaluation was done by analysing key parameters such as moisture, ash, carbon, hydrogen, nitrogen and sulphur content.

Analytical Parameters	Bougainvilleaspectabilis	Tecoma stans
N %	1.48	0.08
STDEV	0.01	0
С %	43.85	41.34
STDEV	0.03	0.2
Н %	6.11	6.61
STDEV	0.05	0.05
S %	0.05	0
STDEV	0	0
0 %	41.3	48.45
STDEV	0.07	0.13
Moisture %	6.71	7.73
STDEV	0.30	0.16
Ash %	7.21	3.52
STDEV	0.21	0.06

Table-2 Quality Evaluation of Raw Material (Plant's Powder)

Moisture content was observed 6.71 % inBougainvilleaplant and 7.73% in Tecomawhile ash content was witnessed 7.21% and 3.52% (on dried basis) respectively. More than double ash content was found in Bougainvilleaplant as compared to Tecoma. Similarly, sulphur content also found more in Bougainvillea as compared to Tecoma which is 0.05%. Sulphur and ash contents are directly proportional to each other since sulphur contributes to ash content.

CHNOcontent of Bougainvilleawas observed 43.9%. 6.1%, 1.38% and 41.3% respectively while in case of Tecoma41.3%, 6.61%, 0.08 and 48.5% respectively. All contents were observed more in Bougainvilleaas compared to Tecomaexcept moisture, hydrogen and oxygen contents. Hydrogen and oxygen are directly proportional to moisture content which has been proved with this data (Table-2)..

Measurement of antibacterial activity:

The all four extracts of Bougainvillea (BW, BM, BH & BE) and Tecoma (TW, TM, TH & TE) were tested against twopathogenic strains of bacteria. As shown in figure 3 and 5, the clear zone of inhibition of BW, BM, BH & BE were visible against clinical pathogenic bacterial strains and similar observations were reported in case of TW, TM, TH & TE as well which is clearly visible in figure 7 and 9. Out of these BM and TM were the most effective against the following Gram-positive bacteria: S. aureus, B. substiliswith a range of inhibition zones (20.5 –25 mm and 20-21.5 mm respectively).

AllBougainvillea plant extracts BW, BM, BH and BE showed zone of inhibition against Bacillus substilis which were 2.5, 25, 3.5 and 1.5 mm respectively (Fig.2). Maximum zone of inhibition was observed in methanol extract of Bougainvillea which were around 78% of positive control. No zone was visible in any negative control which concludes these solvents don't have any antibacterial activity.

Similarly, against Staphylococcus aureus, zone of inhibition were observed 2.0, 20.5, 2.5 and 2.0 mmcorrespondingly (Fig.4). Here also methanol extract showed maximum zone of inhibition which were around 75% of positive control. Full bacterial growth was visible in all negative controls. This data clearly concluded that Bougainvilleaplant grown in Jamnagar district region has antibacterial compounds which can be extracted by methanol.



Fig.- 2: Antibacterial Activity of Bougainvellea Extracts on Bacillus substilis



Fig.- 3: Picture Showing Antibacterial Activity of BW, BM, BH & BEagainst Bacillus substilis



Fig.- 4: Antibacterial Activity of Bougainvellea Extracts on Staphylococcus aureus



Fig.- 5: Picture Showing Antibacterial Activity of BW, BM, BH & BE against Staphylococcus aureus

All Tecoma plantextracts TW, TM, TH and TE showed zone of inhibition against Bacillus substilis which were 7, 21.5, 1.5 and 1.0 mm respectively (Fig.6). Maximum zone of inhibition was observed in methanol extract of Tecoma which were around 79% of positive control. No zone was visible in any negative control which concludes these solvents don't have any antibacterial activity.

While, against Staphylococcus aureus, TM and TH didn't display any zone of inhibition only TM and TE indicatedsome zone of inhibition which were observed 20 and 1.5 mm respectively (Fig.8). Here also methanol extract showed maximum zone of inhibition which were around 76% of positive control. Full bacterial growth was visible in all negative controls. This data clearly concluded that Tecomaplant grown in Jamnagar district region has antibacterial compounds which can be extracted by methanol.



Fig.- 6: Antibacterial Activity of Tecoma Extracts on Bacillus substilis



Fig.- 7: Picture Showing Antibacterial Activity of TW, TM, TH & TEagainst Bacillus substilis



Fig.- 8: Antibacterial Activity of Tecoma Extracts on Staphylococcus aureus



Fig.- 9: Picture Showing Antibacterial Activity of TW, TM, TH &TE against Staphylococcus aureus

Measurement of antifungal activity:

The all four extracts of Bougainvillea (BW, BM, BH & BE) and Tecoma (TW, TM, TH & TE) were tested against both the organisms (Aspergillus niger&Candida albicans). Very small zone of inhibition was observed in methanol extract of Bougainvillea and Tecomaplant against both organism.

All Bougainvillea plantextracts BW, BM, BH and BE didn't show any zone of inhibition against Aspergillus niger except BM. Maximum zone of inhibition was observed 2.5 mm only in methanol extract of Bougainvillea which were around only 10% of positive control (Fig. 10). No zone was visible in any negative control which concludes these solvents don't have antifungal activity.

Similarly, against Candida albicans, zone of inhibition was observed 1.5 mm (Fig. 11) in TM extract. Here also methanol extract reflected maximum zone of inhibition which were around 7% of positive control. Full growth was visible in all negative controls. This data clearly concluded that antifungal compounds can't be extracted by water, methanol, hexane and ethyl acetate from Bougainvillea plant grown in Jamnagar district region.



Fig.- 10: Antifungal Activity of Bougainvellea Extracts on Aspergillus niger



Fig.- 11: Antifungal Activity of BougainvilleaExtracts on Candida albicans

AllTecoma plantextracts TW, TH and TE didn't show any zone of inhibition against Aspergillus niger except TM. Maximum zone of inhibition was observed 2.0 mm only in methanol extract of Tecoma which was around only 8 % of positive control. While positive control showed significant zone of inhibition of 23 mm against Aspergillus niger(Fig. 12). No zone was visible in any negative control which concludes these solvents don't have antifungal activity.

Similarly, against Candida albicans, zone of inhibition was observed 2.5 mm in TM extract. Here also methanol extract showed maximum zone of inhibition which were around 10 % of positive control. While positive control indicated significant zone of inhibition of 25 mm against Candida albicans(Fig. 13). Full growth was visible in all negative controls. This data clearly concluded that antifungal compounds can't be extracted by water, hexane and ethyl acetate from Tecoma plant grown in Jamnagar district region.



Fig.- 12: Antifungal Activity of Tecoma Extracts on Aspergillus niger



Fig.- 13: Antifungal Activity of Tecoma Extracts on Candida albicans

This is the indicator of antibacterial activity of the extract and also the dose dependence of the activity. The results of the antibacterial assay indicate that the crude extracts of Bougainvillea and Tecomaare active against the tested bacterial strains while inactive against fungal strains. Amongst all the extracts tested the methanol extract proved to be the most effective. It was observed by many scientist that plants are an important source of pharmacophore and it can be function as new chemotherapeutic agents. The first step to develop a chemotherapeutic agent from plants would be the assay of in vitro antibacterial and antifungal activity. The active extracts can be used to identify the active compounds which are directly responsible for the antibacterial activities from the plants. Multi drug resistance has seen in pathogenic bacteria in recent years. This issue has developed interest to search new antibacterial agents from any natural sources. Many studies also reported that multi drug resistance has been displayed by gram negative bacteria P. aeruginosa to many antibiotics. But, the extracts especially the polar ones show a good activity against P. aeruginosa. The antibacterial agents from natural sources also eliminate the side effects of synthetic or semi synthetic antibacterial agents. The antibacterial activity of any extract from natural sources varies with various organisms. The zones of inhibitions ranged from 10 mm to 27 mm. The results obtained in the present study showed that methanol has the capability to extract more antibacterial compounds as compared to other solvents. Hence methanol extracts of Bougainvillea and Tecoma possesses maximum antibacterial activity against Staphylococcus aureus and Bacillus substilisas compared to other solvents. Similarly, Dieu-Hien Truong at; al: (2019) concluded that methanol extracted maximum antibacterial compounds from plant material. Among solvents tested, methanol resulted in the highest extraction yield (33.2%), followed by distilled water (27.0%), ethanol (12.2%), acetone (8.6%), chloroform (7.2%), and dichloromethane (4.9%).(Dieu-Hien Truong at; al: 2019).

The antibacterial activity thus varies if solvents of varying polarity are being used because the solubility of these compounds may be different in these solvents. Hence, it is evident from the results, antibacterial properties depends on extracting solvents methanol extract showed significant zone of inhibition while other extracts showed negligible. The efficacy of these extracts is also contingent upon their aptitude to disperse and permeate within the assay's chosen medium. Even though these extracts aren't pristine compounds, the outcomes gleaned undeniably underline their formidable potential. Thus, these extracts can be further purified to generate antibacterial compounds which has the capability tocreate a huge opportunity todevelop phytomedicine against these microbes. In all these extracts are safer and less prone to development of drug resistance in bacteria since it occurs naturally. The above mentioned pathogens cause a number of life threatening diseases which can be managed by the use of synthetic antibiotics which have their own side effects. Due to these increasing problems which are associated with drug resistance in bacteria and the more cost of synthetic antibiacterial agents, other alternatives are required for pharmaceutical companies.

In case antifungal activity no zone of inhibition was visible in aqueous, hexane and ethyl acetate extract of Bougainvillea and Tecoma. Only methanolic extract showed very small zone of inhibition against Aspergillus niger and Candida albicans. Saeed Ahmad and Muhammad Akram also performed similar study. They prepared plants extracts using similar solvents such as methanol, aqueous, hexane and ethyl acetate and also evaluated their antifungal activity against Aspergillus niger&candida albicans (Saeed Ahmad and Muhammad Akram 2019).

CONCLUSION

The inhibition zone assay revealed primarily two types of observations which were [discs without any surrounded clear or inhibition zones which could be attributed to the absence of any inhibitory activity and clear inhibition zone representing the bacteriostatic or bactericidal action of the tested plant extract.

The extracts evaluated in the study were exhibited antibacterial activity against tested organisms. The results obtained indicated that the methanol could be a potential solvent to extract antibacterial compounds from Bougainvillea and Tecoma. While no solvent is found suitable to extract any antifungal compound from the Bougainvillea and Tecoma grown at Jamnagar location.

These findings support the traditional knowledge of local users about their selection of plant samples as antimicrobial agents and the use of these plants for antibacterial activity. The results of the present study also support the medicinal usage of the studied plants and suggest that the plant extracts possess compounds with antibacterial properties that has the capability to use as antimicrobial agents in the development of new drugs for the treatment of respective infectious diseases.

On the basis of the data obtained in the present study, it is concluded that the methanol fractions obtained from Bougainvillea and Tecoma contain certain components which contribute to their antibacterial activities. Generally presence of polyphenolic compounds in the fraction contributes towards antibacterial capacity and therefore the study provides preliminary pharmacological support for utilizing them in therapeutic usage.

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