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Research Article

ANTIBACTERIAL ACTIVITY OF *CAESALPINIA BONDUC* LEAF, BARK AND SEEDS EXTRACTS AGAINST SELECTED PATHOGENIC BACTERIA.

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Abstract

The present study was aimed to investigate antibacterial potential of dichloromethane, ethyl acetate and methanol and extracts of leaf, bark and seed extracts of *Caesalpinia bonduc* L.(Caesalpinaceae)against the selected human pathogenic bacteria which includes, four gram positive bacteria such as *Streptococcus mutans*, *Bacillus subtilis, Staphylococcus aureus* and *Micrococcus luteus.*The gram negative bacteria includes, *Proteus vulgaris, Klebisiella pneumoniae , Escherichia coli and Schizella Flexneri.* The data pertaining to the experiments, showed that among the plant parts tested, leaf extract exhibited significant activity than bark and seed. Similarly, the methanol extract produced remarkable zone of inhibition than other solvent extracts tested against the selected bacteria. It is inferred that the *C. bonduc* possess high medicinal value, this plant can be utilized as an alternate source to control or to cure the bacterial infection.

Keywords: Antibacterial activity, *Caesalpinia bonduc*, dichloromethane, ethyl acetate, methanol.

Introduction

The knowledge and use of a wide spectrum of medicinal plants have been documented scientifically and thus it has led to the development of drugs to combat various infectious diseases impeding human life and activity. Antimicrobial, especially antibiotic drug resistance is a great challenge to public health despite the existence of a variety of antibiotics in the present scenario (Pfaller and Diekema, 2012). The unregulated use of antimicrobial agents and poor hygienic conditions amongst others, severely affects humans in every corner of life (Eber *et al*., 2010).

Plants as potential antibacterial agents, healing potential of plants have been known for thousands of years. Plants and their medicinal uses were passed down from generation to generation in various parts of the world and has significantly contributed to the development of different traditional systems of medicine. Even today, the World Health Organization (WHO) has estimated that approximately 80-85% of the global population relies on traditional herbal medicines as part of standard health care (Foster *et al*., 2005). Many drugs presently prescribed by physicians are either directly obtained from plants or are synthesized versions of plant products. All these organic compounds are defined as biologically active principles, and generally represent secondary metabolites, or as an intermediate or end product of secondary plant metabolism. These secondary metabolites, apart from determining specific plant traits, showing both biological and pharmacological activity of a plant. Therefore, medicinal properties of plants can be associated with secondary metabolite compounds (Hartmann, 2008).

In vitro experiments clearly proved that plants produce a vast number of secondary metabolites that have antibacterial activity (VanEtten *et al*., 1994; Iwu *et al.,* 1999; Cowan, 1999; Rios & Recio, 2005; Cos *et al.,* 2006). Usually, three molecule families append to have remarkable antimicrobial activity and they are alkaloids, phenolics and terpenes. The polyphenols and phenolics are one of the biggest group of active principles that

have exhibited antimicrobial activity. Important subclasses in this group of compounds include phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (Geissman, 1963; Stern *et al*., 1996; Cowan, 1999).

In the recent past, the search for plant extracts has gained a real concern among scientists for the invention of new compounds which are effective in the ailment of bacterial infection. A great number of reports pertaining to the antibacterial screening of plant extracts have appeared in the literature. Examples of such articles include studies of medicinal plants from different geographical regions: Brazil (Alves *et al*., 2000), Argentina (Salvat *et al*., 2004), India (Perumal *et al.,* 1998; Ahmad and Beg, 2001), China (Zuo *et al*., 2008), Turkey (Sokman *et al*., 1999; Uzun *et al.,* 2004); Greece (Skaltsa *et al.,* 2003), Spain (Rios *et al.,* 1987; Recio *et al.,* 1989), Serbia (Stefanovic *et al.,* 2009a; Stefanovic *et al*., 2009b, Stanojevic *et al*., 2010a; Stanojevic *et al.,* 2010b; Stojanovic-Radic *et al*., 2009; Stefanovic *et al.,* 2011; Stefanovic *et al.,* 2012); Africa (Atindehou *et al*., 2002; Konning *et al*., 2004; Chah *et al*., 2006); Australia (Palombo and Semple, 2001).

Arora *et al*. (2004) evaluated the *in vitro* antibacterial/synergistic activities of *Withania somnifera* extracts. The results show that the methanol extract of leaves shows high activity against *S. typhimurium* than *E. coli*. While in roots E. coli more activity than *S. typhimurium* while hexane extract of both leaves and roots low activity against *S. typhimurium* than *E. coli*. Hussain and Gorsi, (2004) evaluated the antimicrobial activity of *Nerium oleander*. The result in this study shows that the ethanolic extract of leaves of *N.oleander* high antimicrobial activity against all the tested microorganism except *Aspergillus niger*. The results obtained show that the ethanolic extract of the root of *N. oleander* exhibited moderate activity against *Bacillus pumillus* and *S. aureus* while with *E. coli* it was high, whereas against *B. subtilis* low activity was observed. While methanolic extract of *N. oleander* roots revealed marked activity against all the bacteria used. None of the crude extracts showed activity against *A. niger* and chloroformic extracts of leaves and roots of *N.oleander* did not show any appreciable activity against any of the microbes used.

Cursino *et al*. (2005) evaluated the synergistic interaction between ascorbic acid and antibiotics against *Pseudomonas aeruginosa*. Ampicillin and tobramycin with ascorbic acid did not show synergy against any of the 12 isolates of *P. aeruginosa*. Betoni *et al*. (2006) evaluated the synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus*.

Lucija Majhenic *et al.* (2007) reported that antioxidant and antibacterial activities of *Paullinia cupana* seed extracts and found that they possed strong antimicrobial and antioxidant properties, and they can therefore be used as a food additive, cosmetic and pharmaceutical industries. Sandra *et al.* (2007) reported the gram positive bacteria were significantly controlled by essential oil isolated from Daccus carota show to have important phytochemical constituents such as pinnes which are responsible for breaking of cell wall in bacteria.

Abubakar, (2010) evaluated the antibacterial potential of crude leaf extracts of *E. camaldulensis* against some pathogenic bacteria.Anja Klancnik *et al.* (2010) evaluated the diffusion and dilution methods for determining the antibacterial activity of plant extracts and their mixtures. Khoobchandani *et al.* (2010) investigated that the antimicrobial activity of various solvent extracts of *Eruca sativa* and seed oil against-antibiotic resistant Gram-negative (*E. coli*, *P. aeruginosa* and *S. flexneri*) and Gram-positive (*S. aureus* and *B. subtilis*) bacteria.

Derwich *et al*. (2010) evaluated the antibacterial activity and chemical composition of the essential oil from the flowers of Nerium oleander. The data indicated that *Escherichia coli* were the most sensitive strain tested to the oil of *N. oleander* with the strongest inhibition zone (28.89mm).The *P. aeruginosa* was, in general, found to be more sensitive among bacteria with inhibition zone of 18.22mm. Modest activities were observed against *S. aureus*, with inhibition zones of 6.32mm.The component of this oil, 1.8- cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*E. coli*, *P. aeruginosa, S. typhi, S. aureus, S. intermedius* and *B. subtilis*).

Mohamed *et al*. (2010) evaluated the chemical constituents and biological activities of *Artemisia herba-alba*.Mohamed Hajji *et al*. (2010) reported that, *Periploca laevigata* is widely distributed in Tunisia and used in traditional medicine. The chemical composition, and antimicrobial, antioxidant and angiotensin activities of essential oil obtained from the plant exhibited remarkable activity against several fungal strains. Their findings provoked that the essential oil from *P. laevigata* might be a good candidate for further investigations of new bioactive substances.

Olusesan *et al.* (2010) evaluated the preliminary in-vitro antibacterial activities of ethanolic extracts of *Ficus sycomorus* and *Ficus platyphylla* using the same concentration of the two test plants extracts, the zones of inhibition showed by *F. sycomorus* ranged between 11.5 - 21.5 mm while that of *F. platyphylla* was from 17.0 - 22.0 mm against the test organisms.

Saravanan *et al*. (2010) evaluated the antibacterial activity of all sati on pathogenic bacterial. The results indicated the aqueous extract of garlic inhibited the growth of both Gram positive and gram negative tests bacterial cultures. Karthick Raja Namasivayam *et al.* (2014) reported that biofilm represents the most prevalent type of virulent factor of most of the pathogenic

microorganism and involved in crucial development of clinical infection and exhibit resistance to antimicrobial agents.

Amel Ali Sulieman *et al.* (2015) reported that traditional medicines became a main source of primary health in different part of Sudan as result of cost effectiveness and viability of antibiotic in addition of antibiotic resistance and their side effect. Razieh Ahmadi *et al.* (2015) evaluated the antimicrobial properties of essential oil from Iranian plant, *Thymus kotschyanus* on yeast, Gram-positive and Gram-negative pathogenic bacteria and essential oil also showed high antimicrobial activity against two medically important pathogens *C. albicans* and *B. cereus*.

Schum*et al.* (2015) studied the *Aframomum angustifolium* from Uganda. The ether and methanol extracts of the ripe pod of *A. angustifolium* were screened for antibacterial activity against *S. aureus* and *E. coli.* Presence of various phytochemicals especially flavonoids and terpenoids in the test plant shown to have potential antibacterial activity. Similarly, alkaloid and saponin present in *Cestrum aurentiacum* was analyzed for its antimicrobial properties by Sivaraj *et al.* (2015).

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potentiality of leaf, bark and seed extracts of *Caesalpinia bonduc* towards the control of selected bacteria.

Materials and Methods

Collection of plant materials

In the present study, leaf, bark and seed of *Caesalpinia bonduc* was collected from Melvenniyour village, Kalvarayan hills 11º 45’ northern latitude and 78º 58 Villupuram district, Tamil Nadu, India. The collected samples were air dried at room temperature (28±2°C; RH 75±5%) and powdered using electrical blender. The extracts were prepared by soaking 100g of dried powder in 500ml ofdichloromethane, ethyl acetate and methanol successively for 24h by cold extraction methods. The extracts were filtered using Whatman filter paper No.1, then the crude extracts were individually concentrated in vacuum at 40°C in a rotary evaporator.

Extraction methods

The dried leaves, bark and seed (100g) were powdered mechanically using commercial electrical stainless steel blender and extracted sequentially with dichloromethane, ethyl acetate and methanol by soaking method separately until exhaustion. The extract was concentrated under reduced pressure of 22-26 mmHg at 45°C by rotary vacuum evaporator and the residue obtained was allowed to complete drying of the solvent. Then they were transferred to an amber vial, labeled, covered with silver foil and kept in refrigerator at 4°C until use. The method prescribed by Vogel, (1978).

General laboratory techniques for the preparation of media, inoculation and maintenance of culture were as follows:

Antimicrobial assay

In vitro antimicrobial evaluation of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts were carried out against 8 bacterial strains, which includes 4 Gram-positive bacteria (*Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus and Streptococcus mutans*) and 4 Gram-negative bacteria (*Escherichiacoli*, *Klebsiella pneumoniae, Proteus vulgaris* and *Schigella flexneri*). The bacterial strains were obtained from the Institute of Basic Medical Sciences (IBMS), University of Madras, Taramani Campus, Chennai, India. An inoculums of each bacterial strain was suspended in 5 ml of nutrient broth and incubated for 24 h at 37°C. A lapful bacteria were taken from the stock cultures and dissolved in 0.1 ml of saline.

Plant extracts were screened for antimicrobial activity using the Disc Diffusion Assay (Ericsson and Sherris, 1971). The Petri plates (9 cm dia.) were pre-seeded with 10 ml of Muller Hinton Agar and stock culture was streaked thoroughly to ensure uniform distribution of the micro-organisms. Sterile paper discs (5 mm diameter) containing 100µg/ml of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts were screened for antibacterial activity.

Simultaneously, paper discs dipped with pure respective organic solvents were used as positive controls. The Petri plates were then pre-incubated for 3 h at 5°C to permit maximum diffusion of the extracts into the media. Cefalexin and Gentamycine (10µg/ml) was used as negative control against gram positive and gram negative bacteria respectively (Hailu Tadeg et*. al.,* 2005; Karman *et al*., 2004; Nancy *et al*., 2000; Zaidan *et al*., 2005) were used as reference standards. After the incubated period the zone of inhibition (diameter) was measured with a scale and the data were tabulated.

Results and Discussion

Antibacterial activity of different solvents extracted from *C. bonduc* leaf tested against selected human pathogenic bacteria are shown in table 1. On perusal, of he dataclearly indicates that dichloromethane produced the remarkable zone of inhibition against gram negative bacteria, *K. pneumoniae* (20mm followed by a gram positive *S. aureus* (18mm). Finally, *B.subtilis*, *E.coli* and *M. luteus*showed similar response (15mm) to the dichloromethane extract of *C. bonduc* leaf.

Besides, the ethyl acetate extract produced 22mm zone of inhibition among the gram positive bacteria *B. subtilis* followed by *M. luteus* (20mm) and *S. aureus* (19mm). Whereas, among gram negative bacteria 21mm zone of inhibition was noted against *S.flexneri* followed by *K.pneumoniae, E. coli and P. vulgaris* with 18mm, 17mm and 13mm zone of inhibition respectively. Interestingly, methanol extract showed remarkable antibacterial activity against all the bacteria except *S. mutans*. The bacterial growth inhibitory trend was observed as follows: *B.subtilis* and *S. flexneri* (31mm)>*M. luteus* (30mm)>*K. pneumoniae* (29mm)>*Proteus vulgaris* (28mm) >*S. aureus* (27) >*E. coli* (26mm).

Table:1.Antibacterial activity of different solvent extracts of *Caesalpinia bonduc* leaf tested against selected human pathogenic bacteria.

Microorganism tested		Zone of inhibition(mm) diameter			
		Organic solvents tested			
		PC*	DCM	EA	ME
Gram positive	*Bacillus subtilis*	10	15	22	31
	Micrococcus luteus	12	15	20	30
	Staphylococcus aureus	11	18	19	27
	Streptococcus mutans	-	-	-	-
Gram negative	*Escherichia coli*	8	15	17	26
	Klebsiella pneumonia	9	20	18	29
	Proteus vulgaris	13	12	13	28
	Schigellaflexnari	11	17	21	31

PC*= Positive Control *i.e.*,Cefalexin 10µg and Gentamycin 10µg for gram positive and gram negative bacteria respectively,**DCM** = Dichloromethane extract; **ME** = Methanol extract.

Antibacterial activity of different solvents extracted from *C. bonduc* bark tested against selected human pathogenic bacteria is shown in table 2. It was observed that, dichloromethane produced the remarkable zone of inhibition against gram negative bacteria, *S. flexnari* (20 mm) followed by *E.coli* (18mm), *S.aureus* (15 mm), *B. subtilis* (12 mm) and *M. luteus* (11 mm) to the dichloromethane extract of *C. bonduc* bark.

Table:2. Antibacterial activity of different solvent extracts of *Caesalpinia bonduc* bark tested against selected human pathogenic bacteria.

Microorganism tested		Zone of inhibition(mm) diameter			
		Organic solvents tested			
		PC*	DCM	EA	ME
Gram positive	*Bacillus subtilis*	11	12	14	26
	Micrococcus luteus	-	11	10	30
	Staphylococcus aureus	13	15	11	24
	Streptococcus mutans	-	-	-	-
Gram negative	*Escherichia coli*	6	18	14	28
	Klebsiella pneumonia	11	-	12	27
	Proteus vulgaris	12	-	14	26
	Schigella flexnari	11	20	14	30

PC*= Positive Control *i.e.*,Cefalexin 10µg and Gentamycin 10µg for gram positive and gram negative bacteria respectively,**DCM** = Dichloromethane extract; **ME** = Methanol extract;

Besides, the ethyl acetate extract produced 14mm zone of inhibition *B. subtilis* followed by *S.aureus* (11 mm) and *M. luteus* (11mm) among the gram positive bacteria. Whereas, among gram negative bacteria like *E.coli, P. vulgaris and S. flexneri* showed similar response (14 mm) zone of inhibition was noted respectively. Interestingly, methanol extract showed remarkable antibacterial activity against all the bacteria except *S. mutans*. The bacterial growth inhibitory trend was observed as follows: *M. luteus* and *S. flexneri* (30mm)> *E. coli* (28 mm)>*K.pneumoniae* (27mm)>*B. subtilis* and *P. vulgaris* (26mm) >*S. aureus* (24 mm).

Antibacterial activity of different solvents extracted from *C. bonduc* seed tested against selected human pathogenic bacteria is shown in table 3. The dichloromethane produced the remarkable zone of inhibition against gram negative bacteria, *S. flexneri* (16 mm) followed by *B. subtilis* and *P. vulgaris* (11mm), *S. aureus* and *E. coli* (9mm) and *K.*

pneumonia (6mm).Besides, ethyl acetate extract by *S. aureus* (21mm), *B. subtilis* (17mm) and *M. luteus* (13mm) among the gram positive bacteria. Whereas, among gram negative bacteria 22mm and 20mm zone of inhibition was noted against *E. coli* followed by *S. flexneri*. Interestingly, methanol extract showed remarkable antibacterial activity against all the bacteria.The bacterial growth inhibitory trend was observed as follows: *E. coli* (30mm)> *P. vulgaris* (29mm)> *S. flexneri* (28mm)>*S. mutans* (27mm) >*S. aureus* (26mm) >*B. subtilis* (25mm)>*K. pneumonia* produced 24mm zone of inhibition *S. aureus* followed (21mm) >*M. luteus* (18mm). In the last decades, there has been particular interest in the use of naturally abundant antimicrobial agents from plants. Antimicrobial agents are chemical compounds derived from herbs, shrubs and or whole plants (Burt, 2004). Basically, there are two ways to control or inhibit the growth of microorganisms, *i.e.* through physical or chemical agents, where choice is made on the basis of the situation.

Table:3. Antibacterial activity of different solvent extracts of *Caesalpinia bonduc* seed tested against selected human pathogenic bacteria.

Microorganism tested		Zone of inhibition(mm) diameter			
		Organic solvents tested			
		PC*	DCM	EA	ME
Gram positive	*Bacillus subtilis*	9	11	17	25
	Micrococcus luteus	-	-	13	18
	Staphylococcus aureus	11	9	24	26
	Streptococcus mutans	5	-	21	27
Gram negative	*Escherichia coli*	5	9	22	30
	Klebsiella pneumonia	6	6	-	21
	Proteus vulgaris	12	11	-	29
	Schigella flexnari	15	16	20	28

PC*= Positive Control *i.e.,*Cefalexin 10µg and Gentamycin 10µg for gram positive and gram negative bacteria respectively,**DCM** = Dichloromethane extract; **ME** = Methanol extract; **WA** = Water extract.

In more specific, antimicrobial agents are categorized based on the spectrum of action, namely narrow and broad spectrum. Narrow spectrum antimicrobial agents can only inhibit the growth of either Gram positive or Gram negative bacteria, whereas a broad spectrum antimicrobial agent can inhibit both Gram positive and negative bacteria. Nevertheless, most of the antibiotics have no longer effective to control bacterial diseases due to the occurrence of antibiotic resistance. Therefore, scientists around the world were struggling to find for alternative, preferably from the natural resources. There have been many studies reported in plants (Abdolhamid Bamoniri *et al*., 2010; Khusro *et al*., 2013 ; Kyu Hwan Park *et al*., 2013 ; Lillian Barros *et al*., 2013), especially medicinal plants as potential antimicrobial reservoir. As the results from different studies need to be comparable, we examined the antimicrobial activity of plant extracts of *Caesalpinia bonduc* by disc diffusion method against the selected gram-positive and gram-negative bacteria the results obtained from the present investigations are leading into deep exploration of type of bioactive principles present in the plant extracts. Further studies on fraction and identification of compounds from C. bonduc are under process.

Conclusion

In the present investigation, among the three solvent extracts, the methanol extract of *C. bonduc* leaf showed strong bioefficacy against the medically important pathogenic bacteria. Further, the ability of the *C. bonduc* can be possibly utilized in pharmacology in the near future.

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