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Effect of *Croton bonplandianum* & *Acalypha indica* Plant Extracts on Septic Arthritis Causing Microbes

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Abstract: ***Septic arthritis is an acute inflammatory disorder caused by microbial infection, affecting the joint fluid and joint tissues. Today, treatment for infections caused by microorganisms is a major problem with growing antibiotic resistance and slow pace in the discovery of new antibiotics. Perhaps, plant extracts with antimicrobial components are the promising drugs for the future which help in overcoming the crisis aroused due to antibiotic resistance. In the current study, ethyl acetate, chloroform and methanol extracts of two weeds namely Croton bonplandianum (aerial parts and roots) and Acalypha indica (aerial parts) were studied for their in-vitro anti-arthritic activity against septic arthritis causing microorganisms. The ethyl acetate, chloroform and methanol extracts of Croton bonplandianum was found to be effective against all the test organisms including multidrug resistant Pseudomonas aerogenosa. Therefore, it is been identified as the promising source of potent bioactive compounds with anti-arthritic activity.***

Keywords: *Croton bonplandianum,* aerial parts, roots, *Acalypha indica, in-vitro* anti-arthritic activity, septic arthritis, infectious arthritis

1. Introduction

Septic arthritis, also called as infectious arthritis, is caused by a bacterial infection or more rarely by a fungal or viral infection. The condition is typically acute, causing severe joint pain, inflammation, redness, and in some cases fever and chills but may also become chronic[1]. Septic arthritis may affect any joint but most frequently found in the knee, hip, shoulder, wrist, elbow, and finger joints. Usually only one joint will be affected but, in some cases, there may be more than one. This condition needs to be diagnosed and treated quickly because it can destroy joints in a short period [2].

Septic arthritis is of two types based on the type of microorganisms involved in causing the infection [3]. They are: non-gonococcal septic arthritis (caused by other than *Niesseria gonorrhoeae*) and gonococcal septic arthritis (caused by only *Niesseria gonorrhoeae*).

Septic arthritis occurs most often in people who had a recent traumatic joint injury and joint surgery or joint replacement, and/or in people who currently have an infection in their blood (bacteremia or septicemia). Microorganisms can spread from an original site of infection into the blood and then carried into the joint space. Additional risk factors for septic arthritis include age (older than 60 years), having diabetes, a weakened immune system, and/or another condition that affects the joints, such as gout or rheumatoid arthritis. According to Nicolson [4], 50% rheumatoid arthritis patients have *Mycoplasmal* bacterial infections. Infections trigger arthritis however, tooth abscesses may also lead to shoulder bursitis.

Croton bonplandianum Baill (CB) and *Acalypha indica* Linn (AI) weeds belongs to the family *Euphorbiaceae.* It is a large family of flowering plants with 240 genera and around 6000 species. About 115 species are found in India. *Croton bonplandianum* is a shrub with glabresent stem found abundantly in the waste lands of India [5]. In rural areas of Andhra Pradesh people use the latex for arthritic pain and in West Bengal use it for cuts and wounds [6] .

The genus *Acalypha* consists of 23 species. *Acalypha indica* is a weed with medicinal properties used in Ayurveda, Unani and Sidda medicines [7].

The Acalypha leaves are laxative, anthelmintic, emetic, expectorant, and are also useful in treating chronic bronchitis, asthma and consumption. Traditionally, leaves were known to treat scabies and rheumatic arthritis. The leaves decoction is employed in ear-ache as instillation and also as fomentation round the aching ear; and a cataplasm of the bruised leaves is applied to syphilitic ulcers, to maggot-eaten sores and also to relieve the pain of snake- bites. Powder of dry leaves is used in bedsores. In congestive head-ache a piece of cotton saturated with the expressed juice of the plant or leaves inserted into each nostril is said to relieve it by causing hemorrhage from the nose. In cases of obstinate constipation of children, the leaves ground into a paste, made into a ball, and introduced into the rectum to produce free motion. An infusion of the root or the root bruised in water, acts as a cathartic [8].

2. Materials and Methods

All the chemicals used for this experiment are of analytical grade. The chemicals and the media were purchased from Desai chemicals, T.S.N. Colony, Visakhapatnam, Andhra Pradesh, India. The media used for the study are Mueller-Hinton broth, Mueller-Hinton agar, Sabouraud's dextrose broth and Sabouraud's dextrose agar.

Plant material

Croton bonplandianum (aerial parts and roots) and *Acalypha indica* (aerial parts) were collected from Hanumanthawaka, Visakhapatnam district of Andhra Pradesh. The plants were identified and authenticated by Prof. M. Venkaiah, Department of Botany, Andhra University, Visakhapatnam. A voucher specimen of each of the plant (BGR/RG/CB-2, BGR/RG/AI-3) was deposited in the herbarium of College of Pharmaceutical Sciences, Andhra University for further reference.

Extraction
Freshly collected plant materials were shade dried (1kg) and coarsely powdered. It was successively extracted with methanol (95%) in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure until a soft reddish brown mass was obtained. The dried methanol extract was suspended in water and fractionated with hexane, ethyl acetate and chloroform.

Phytochemical screening
Qualitative determination of alkaloids[9], flavonoids[10], saponins[11], glycosides[12], tannins, steroids and triterpinoids[13] for aerial parts and roots of *C.bonplandianum* and aerial parts of *Acalypha indica* was performed using standard phytochemical screening procedure.

Preparation of Samples
All the dried extracts and the standard antibiotics chloramphenicol (anti-bacterial) and grisofulvin (anti-fungal) were suspended in dimethylsulfoxide to yield the concentrations 50, 100, 200 and 400µg/ml.

Preparation of media
Mueller-Hinton agar (34g) was weighed and dissolved in 100ml of double distilled water. The final volume was made up to 1000ml. The pH was adjusted to 7.4 using a pH meter. Similarly, the Mueller-Hinton broth was prepared by dissolving 23g of Mueller-Hinton broth media (without agar) in 1000ml distilled water. Similarly, Sabouraud's dextrose agar (47g/l) and Sabouraud's dextrose broth (35g/l) were prepared by dissolving the ingredients in distilled water and adjusting the pH to 5.6.

All the media were autoclaved for 20min at 15 lbs/inch^2 pressure. Immediately after autoclaving, they were allowed to cool in a water bath until a temperature of 45 to 50°C is reached.

Test Organisms:
The arthritis-causing microorganisms used for the experimental were procured from MTCC, IMTECH, *Chandighar*.

Gram Positive Bacteria	Gram Negative Bacteria	Fungal strains
Staphylococcus aureus	*Pseudomonas aerogenosa*	*Candida albicans*
Streptococcus pyogenes	*Pseudomonas aerogenosa* (multi-drug resistant)	
Streptococcus pneumoniae	*Echerichia coli*	
	Klebsiella pneumoniae	
	Salmonella typhimurum	

The test organisms *Pseudomonas aerogenosa* (multi-drug resistant) is resistant to twelve antibiotics namely [ceftriaxone, ciprofloxacin, netilmicin, ceftazidime, amikacin, cefoperazone, gentamicin, sparfloxacin, cefadroxil, lomefloxacin, cefotaxime, chloramphinicol] and was isolated from patient's sample at KG Hospital, Visakhapatnam

Inoculum Preparation
Each of the test microorganisms were inoculated into liquid media (Mueller-Hinton broth) in separate sterile tubes and incubated at 37^{0}C for 18hr. the suspensions were checked to provide approximately 10^{-5} – 10^{-7} CFU/ml.

In-vitro Anti-Arthritic Activity
In-vitro anti-arthritic activity is a modified method of well-plate method[14]. For the study, the author has precisely chosen microorganisms causing septic arthritis. In-vitro anti-arthritic study has been performed for the first time. This method involves two steps. First, all the test extracts were subjected to determine minimum inhibitory concentration (MIC) against all the test organisms individually. Second, they were investigated for their *in-vitro* anti- arthritic activity on agar plates along with standard antibiotics.

Bacterial and fungal cultures each of 20µl was added to the petri plates containing 25ml of Mueller-Hinton agar and spread using L-shaped glass rod. Four mm diameter wells were made with metal borer on the agar plate into which appropriate concentrations of the chemotherapeutic agents (25µg/ml) and the plant extracts (*C. bonplandianum* with 50, 100µg/ml and *A.indica* with 200, 400, 800µg/ml) were added. Each extract was tested in triplicate. The agar plates inoculated with bacteria were incubated at 37^{0}C for 24hr and the agar plates inoculated with fungi were incubated at 28^{0}C for 72hr. The bioactive or anti-arthritic agents diffuse from the well through the agar medium to an extent so that the growth of the added microorganism was inhibited entirely around the well producing a clear zone. The anti-arthritic activity is expressed as the diameter of zone of inhibition in millimetre, measured with a zone reader. The diameter of zone of inhibition is calculated by the formula:

Zone of inhibition (mm) = D – d
Where,
D= diameter of zone of inhibition
d = diameter of the well (4mm).

3. Results

The phytochemical screening of the hexane, ethylacetate, chloroform and methanol extracts of *C.bonplandianum* aerial parts and roots are as shown in the table (Table 1). Both hexane extracts are positive for steroids only. Aerial parts chloroform and methanol extracts are rich in steroids, terpenes, alkaloids, flavonoids and glycosides while ethyl acetate extract was positive for terpenes, flavonoids and tannins only. All the root extracts are positive for steroids. Ethyl acetate is positive for glycosides, chloroform for alkaloids and methanol for alkaloids, flavonoids, tannins and glycosides.

The phytochemical screening of the hexane, ethyl acetate, chloroform and methanol extracts of *A.indica* is shown in the table (Table 2). Flavonoids and tannins are present in the chloroform and methanol extracts. In addition to these the methanol extracts were positive for steroids, saponins and glycosides.

Table 1: Preliminary Phytochemical screening of aerial parts and root extracts of *C. bonplandianum*

Croton bonplandianum		Aerial parts extracts				Root extracts			
S. No.	TESTS	H	E	C	M	H	E	C	M
1	Steroids	+	-	+	+	+	+	+	+
2	Terpenes	-	+	+	+	-	-	-	-
3	Saponin	-	-	-	-	-	-	-	-
4	Steroidal Saponins	-	-	-	-	-	-	-	-
5	Terpenoidal Saponins	-	-	-	-	-	-	-	-
6	Alkaloids	-	-	+	+	-	-	+	+
7	Flavonoids	-	+	+	+	-	-	-	+
8	Tanins	-	+	-	-	-	-	-	+
9	Glycosides	-	-	+	+	-	+	-	+
10	Carbohydrates	-	-	-	-	-	-	-	-

Table 2: Preliminary Phytochemical screening of aerial parts extracts of *A. indica.*

Acalipha indica aerial parts					
S. No.	TESTS	H	E	C	M
1	Steroids	-	-	-	+
2	Terpenes	-	-	-	-
3	Saponin	-	-	-	+
4	Steroidal Saponins	-	-	-	-
5	Terpenoidal saponins	-	-	-	-
6	Alkaloids	-	-	-	-
7	Flavonoids	-	-	+	+
8	Tanins	-	-	+	+
9	Glycosides	-	-	-	+
10	Carbohydrates	-	-	-	-

Note: H- hexane, E- ethyl acetate, C- chloroform, M- methanol

The minimum inhibitory concentration of the chloroform and methanol extracts of the aerial parts and root of *C. bonplandianum* was 50µg/ml against Gram's positive, Gram's negative bacteria and fungi. The methanolic extracts had exhibited significant zone of inhibition against all the test organisms. The MIC for *S. pyogenes* and *S. pneumoniae* against chloroformic aerial parts extract was 400µg/ml. The rest of the organisms were sensitive at 50µg/ml. The ethyl acetate, extracts of both aerial parts and root had shown MIC at 100µg/ml against *S. aureus, S. pyogenes, S. pneumoniae, P. aerogenosa* and *K. pneumoniae* and 50µg/ml against *Salmonella typhimurum*, *Escherichia coli* and *Candida albicans*. The ethyl acetate extracts were effective against multidrug-resistant *P. aerogenosa* at 400µg/ml. Whereas, the methanol and chloroform extracts were effective against the multidrug-resistant *P. aerogenosa* at 50µg/ml. A significant zone of inhibition was observed against both bacteria and fungi. The MIC of the hexane extracts was greater than 800µg/ml. The hexane extracts were ineffective against all the test organisms at a concentration of 800µg/ml.

The chloroform and methanolic extracts of *A. indica* aerial parts had shown no significant effect on the test organisms except *S. typhimurum* and *C. albicans*. The MIC for the above organisms was 200 µg/ml. However, the MIC of hexane and ethyl acetate extracts was greater than 800µg/ml. However, the hexane and ethyl acetate extracts were ineffective against all the test organisms even at a higher concentration of 800µg/ml.

In *in-vitro* anti-arthritic activity, on comparison with the standard antibiotics chloramphenicol and grisofulvin a significant concentration dependent zone of inhibition against most of the test organisms was exhibited by the chloroform and methanol extracts of *C. bonplandianum* aerial parts and roots. Whereas *A.indica* extracts were effective only against *Salmonella typhimurum* and *Candida albicans* only.

Table 3: Minimun inhibitory concentration of *C. bonplandianum* against arthritis-causing organisms.

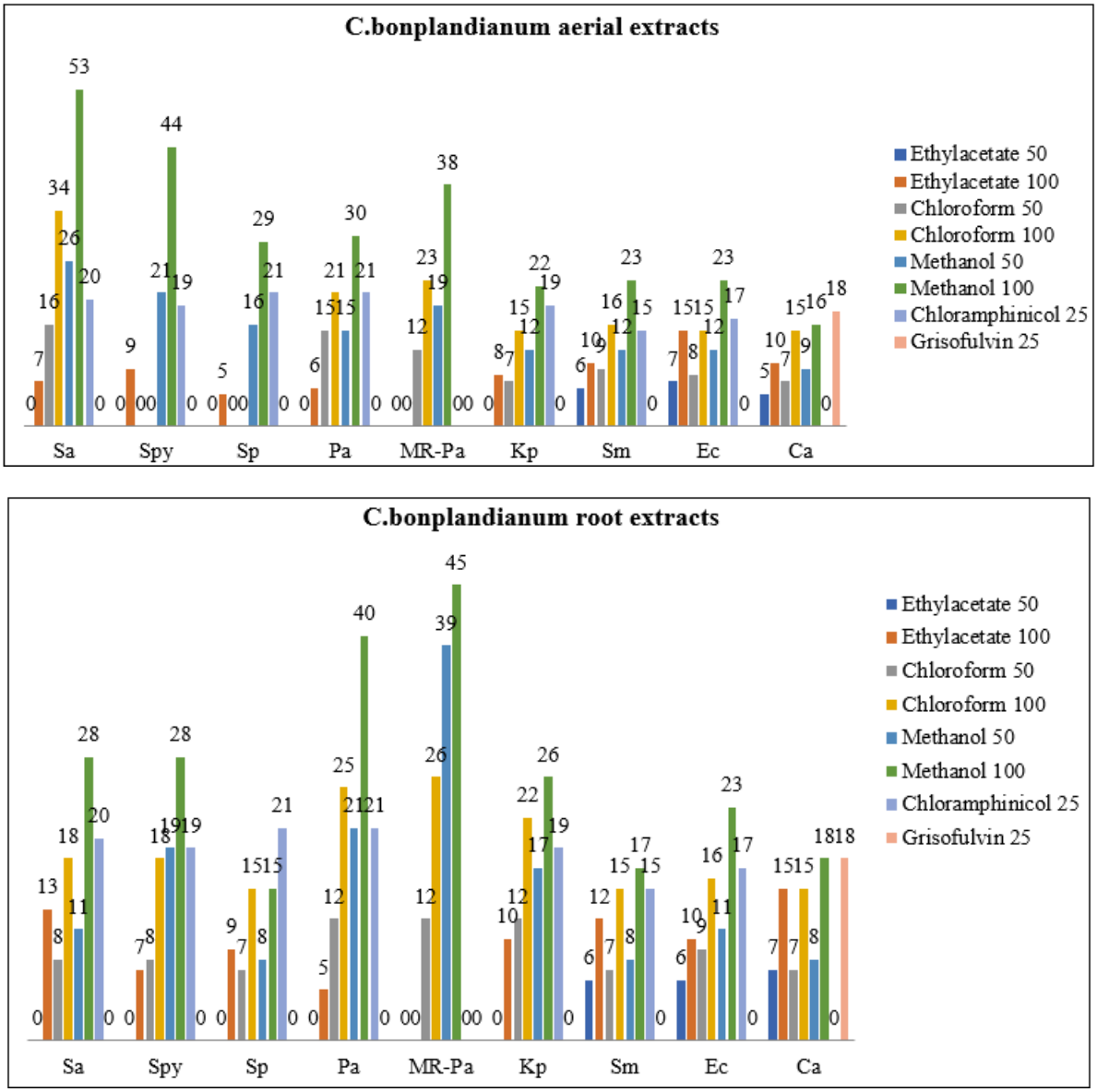
Extract	Minimum inhibitory concentration (MIC) µg/ml								
	Sa	Spy	Sp	Pa	MR-Pa	Kp	Sm	Ec	Ca
CB Aerial parts									
Ethyl acetate	100	100	100	100	400	100	50	50	50
Chloroform	50	400	400	50	50	50	50	50	50
Methanol	50	50	50	50	50	50	50	50	50
CB Root									
Ethyl acetate	100	100	100	100	400	100	50	50	50
Chloroform	50	50	50	50	50	50	50	50	50
Methanol	50	50	50	25	25	50	25	50	50
Grisofulvin	-	-	-	-	-	-	-	-	25
Chloramphinicol	25	25	25	25	-	25	25	25	-
DMSO (control)	-	-	-	-	-	-	-	-	-

Table 4: Anti-arthritic activity of the aerial parts and root extracts of *C. bonplandianum*

CB aerial conc. (µg/ml)	Sa	Spy	Sp	Pa	MR-Pa	Kp	Sm	Ec	Ca
Ethylacetate 50	0	0	0	0	0	0	6	7	5
Ethylacetate 100	7	9	5	6	0	8	10	15	10
Chloroform 50	16	0	0	15	12	7	9	8	7
Chloroform 100	34	0	0	21	23	15	16	15	15
Methanol 50	26	21	16	15	19	12	12	12	9
Methanol 100	53	44	29	30	38	22	23	23	16
Chloramphinicol 25	20	19	21	21	0	19	15	17	0
Grisofulvin 25	0	0	0	0	0	0	0	0	18

CB root conc. (µg/ml)	Sa	Spy	Sp	Pa	MR-Pa	Kp	Sm	Ec	Ca
Ethylacetate 50	0	0	0	0	0	0	6	6	7
Ethylacetate 100	13	7	9	5	0	10	12	10	15
Chloroform 50	8	8	7	12	12	12	7	9	7
Chloroform 100	18	18	15	25	26	22	15	16	15
Methanol 50	11	19	8	21	39	17	8	11	8
Methanol 100	28	28	15	40	45	26	17	23	18
Chloramphinicol 25	20	19	21	21	0	19	15	17	0
Grisofulvin 25	0	0	0	0	0	0	0	0	18

Note: Sa-*Staphylococcus aureus*, Spy- *Streptococcus pyogenes*, Sp- *Streptococcus pneumonia*, Pa- *Pseudomonas aerogenosa*, MR-Pa- multi-drug resistant *Pseudomonas aerogenosa*, Kp- *Klebsiella pneumonia*, Sm- *Salmonella typhimurum*, Ec- *Escherichia coli*, Ca- *Candida albicans*



Graph 1: Anti-arthritic activity of the aerial parts and root extracts of *C. bonplandianum*

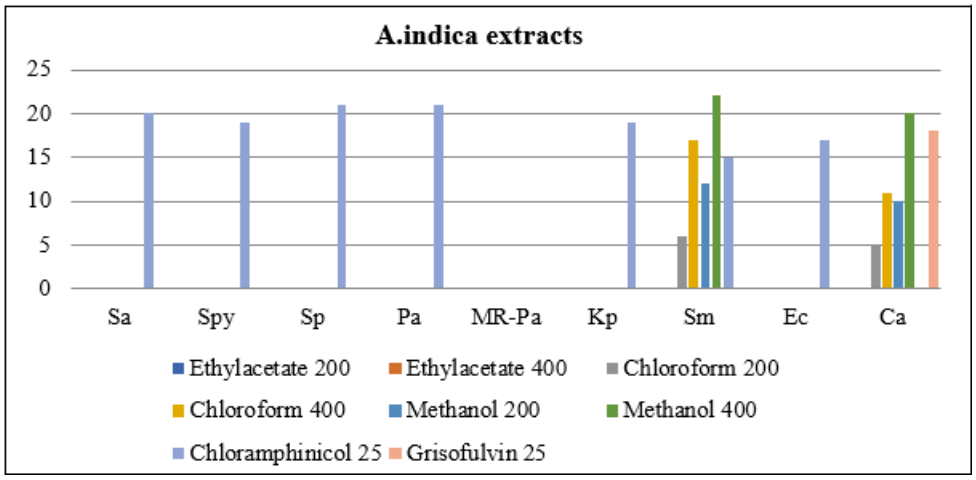
Table 7: Minimun inhibitory concentration of *A. indica* against arthritis-causing organisms

AL Extract	Minimum inhibitory concentration (MIC) µg/ml								
Aerial parts	Sa	Spy	Sp	Pa	MR-Pa	Kp	Sm	Ec	Ca
Ethyl acetate	>800	>800	>800	>800	>800	>800	>800	>800	>800
Chloroform	>800	>800	>800	>800	>800	>800	200	>800	200
Methanol	>800	>800	>800	>800	>800	>800	100	>800	100
Grisofulvin	-	-	-	-	-	-	-	-	25
Chloramphinicol	25	25	25	25	-	25	25	25	-
DMSO (control)	-	-	-	-	-	-	-	-	-

Table 8: Anti-arthritic activity of the aerial parts of *A. indica.*

AL conc. (µg/ml)	Sa	Spy	Sp	Pa	MR-Pa	Kp	Sm	Ec	Ca
Ethylacetate 400	0	0	0	0	0	0	0	0	0
Ethylacetate 800	0	0	0	0	0	0	0	0	0
Chloroform 200	0	0	0	0	0	0	6	-	5
Chloroform 400	0	0	0	0	0	0	17	-	11
Methanol 200	0	0	0	0	0	0	12	-	10
Methanol 400	0	0	0	0	0	0	22	-	20
Chloramphinicol 25	20	19	21	21	0	19	15	17	0
Grisofulvin 25	0	0	0	0	0	0	0	0	18

Note: Sa-*Staphylococcus aureus*, Spy- *Streptococcus pyogenes*, Sp- *Streptococcus pneumonia*, Pa- *Pseudomonas aerogenosa*, MR-Pa- multi-drug resistant *Pseudomonas aerogenosa*, Kp- *Klebsiella pneumonia*, Sm- *Salmonella typhimurum*, Ec- *Escherichia coli*, Ca- *Candida albicans*



Graph 2: Anti-arthritic activity of the aerial parts of *A. indica.*

4. Discussion

The Minimum inhibitory concentration of the test extracts was determined prior to the in-vitro anti-arthritic activity to determine the concentrations of the extracts for the activity. The anti-arthritic activity of plant extracts was observed using cup-plate method by measuring the diameter of zone of growth inhibition against some arthritis-causing microorganisms.

Both the plants *C. bonplandianum* aerial parts and root extracts[15] *A. indica* leaves[16] and aerial parts[17] were reported to have anti-inflammatory activity. Comparatively, *C. bonplandianum* had exhibited significant effect against all the test organisms than *A.indica*. A significant zone of inhibition by *A.indica* against *Candida albicans*, supports the traditional claim of using leaf extracts for skin diseases[8]. Highly significant zone of inhibition was exhibited by the methanol and chloroform extracts of *C. bonplandianum* against most of the tested arthritis-causing organisms (except *Streptococcus pyogenes* and *Streptococcus pneumonia*). They are also effective against *Pseudomonas aerogenosa* (MR-Pa) resistant to twelve antibiotics.

In the study amongst all the extracts *C. bonplandianum* methanol root extracts had exhibited greater effect which might be due to the combination of various bioactive compounds like steroids, alkaloids, flavonoids, tannins and glycosides[18]. The bioactive components like steroids, terpenoids, alkaloids, tannins, flavonoids and glycosides are classified as compounds with antimicrobial properties[19]. Therefore, the bioactive compounds of *C. bonplandianum* can be worth isolated to treat infectious or septic arthritis.

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