

## Bioactivity of Active Extracts of Leaf of *Bauhinia Racemosa*

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**Abstract:** The leaves of *B. Racemosa* were collected from its natural habitat in Wukari local government area of Taraba state, Nigeria. The method of cold maceration was used in the extraction by serial exhaustive extraction method. The result obtained from antimicrobial activity obtained from the extract of the leaf of *B. racemosa* revealed that not all the extract of leaf of inhibited or exhibited antimicrobial activity against *E. coli*, *Staphylococcus aureus*, *Salmonella spp*, *Candida albicans* and *Aspergillus niger*. The result revealed that almost all of the extract exhibited or inhibited antibacterial against *E. coli*, *Staphylococcus aureus*, *Salmonella spp*, *Candida albicans* and *Aspergillus niger*, except extract of chloroform and acetone which did not show any inhibition on *Salmonella spp* (00.00 mm), extract of chloroform donnot act against *Candida albicans* and *Aspergillus niger*, extract of ethanol and ethyl acetate show zero inhibition on *Aspergillus niger*. Extracts that do not inhibit or exhibit antifungal activity on certain bacterial, have zero zone of inhibitory (00.00 mm).

### 1. INTRODUCTION

Infection diseases are one of the main reasons which cause the death every day. Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries, the use of medicinal plants is very wide spread in many parts of the world because it is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without or minimum side effects. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Subhashini *et al.*, 2010). WHO (2001) defines medicinal plant as herbal preparations produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological processes which may be produced for immediate consumption or as a basis for herbal products. Ojelere *et al.*, (2014) point out that medicinal plants are plants containing inherent active ingredients used to cure disease or relieve pain.

One of the criticisms of herbal medicine is lack of standardization and quality control profiles. In addition, there is evidence of herbs been used in the treatment of diseases and for revitalizing the body system in almost all ancient civilization (Manjula *et al.*, 2013). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. In developing countries majority of the people living in rural areas almost exclusively use traditional medicine in treating all sorts of diseases.

The medicinal properties of plants could be based on the antioxidant, antimicrobial antipyretic effects of the phytochemicals in them (Cowman, 1999). Equally, because information on the use of plant species for therapeutic purpose has been passed from one generation to the next through oral tradition, this knowledge of therapeutic plants has started to decline and become obsolete through the lack of recognition by younger generations as a result of a shift in attitude and ongoing social view the valuable.

The plant *Bauhinia racemosa* is used in traditional medicine for the treatment of various ailments. The stem bark of the plant is an astringent and is used in the treatment of headache, fever, skin diseases and in tumors (Gupta *et al.*, 2004; Kirtikar and Basu, 1975). In Ayurveda the bark is useful for the treatment of malaria, dysentery and diarrhea. The bark and leaves are sweetish and acid,

refrigerant, antipyretic, astringent, vermifugal, cure biliousness, urinary discharges, thirst headache, quartan fever, vatta, anal fistula, tuberculous glands, skin diseases, throat troubles, tumors, diseases of the blood, good in chronic dysentery and diarrhea (Anonymous, 1985; Nadkarni and Nadkarni, 2000). The fruit is acrid and sweet; refrigerant and astringent have been isolated from the roots (Jain *et al.*, 2002; Prabhaker *et al.*, 1994). The polar constituents in the ethanol extract of leaves of *B. racemosa* may be responsible for the antihistaminic activity and *B. racemosa* may therefore have a role in the treatment of asthma (Nirmal *et al.*, 2011).

For proper understanding of these plants, the medicinal value of the plant must be known, the rightful role at which the plants can play and these plants must be subject to scientific proof base on a proven and phytochemical screening and antimicrobial activities. The traditional medicines have some challenges such as lack scientific proof and lack of prescription. This research gives more insight on the scientific data on the antimicrobial activities of *B.Racemosa*. To the best of our knowledge little study little or no work have been done on *B. Racemosa* in this part of the world. This research is useful as it validate that the leaves of *B. Racemosa* are used by traditional practitioner is justifiable are also to be use for medication. However as these research is carried out to justify the traditional uses of plants by carrying out the antimicrobial tests. The aim of this research is to study the determine the bioactive components of the leave of *B.Racemosa*. as a medicinal plant. The major objectives are; to carry out the antimicrobial test on microorganisms such as *Eschrichia coli*, *Stapylococcus aureus*, *Salmonella spp*, *Candida albicans* and *Aspergillus niger*

## 2. MATERIALS AND METHODS

### 2.1. Collection Sample and Preparation

The leaves of *B Racemosa* were collected from its natural habitat in Wukari local government area of Taraba state, Nigeria. The sample were collected freshly and taken for identification by Biological sciences Department of Federal University Wukari. The leaves were then washed thoroughly in running tape water and then with distilled water. The leaves were cut into small bits and dried for two weeks under shade. After drying, the plant materials were ground well using mechanical blender into fine powder. Then the powder was stored in airtight containers with proper labeling and kept in the laboratory for use.

### 2.2. Preparation of Plant Extract

The method of cold maceration was used in the extraction by serial exhaustive extraction method which involves the successive extraction with solvents in order of their increasing polarity from hexane to ethanol to ensure efficient extraction of wide range of compounds present in the leaves. The leaf extracts were prepared by soaking 200g of 400ml hexane for four days with frequent agitation until soluble matter are dissolved. The resulting mixture was filtered using Whatman No.1 filter paper, evaporated and concentrated into solid extracts using rotatory evaporator, kept under room temperature overnight to remove all solvent. The process was repeated for chloroform, ethyl acetate, acetone and ethanol sequentially in order of increasing polarity. The extracts were kept in a desiccator until required for test.

### 2.3. Bioassay

This is the study of antimicrobial activity of the crude or purified extracts against micro-organism. It was used as a guide to determine the active components of the leaves of *B. Racemosa*. The crude extracts were tested for antibacterial and antifungal activities. The test organisms were collected from Bauchi Specialist Hospital, Bauchi State, Nigeria. The antimicrobial assay was carried out using methods described by Ochi *et al.*, (2015) with modifications.

### 2.4. Preparation of varying concentration of extracts

Various concentration of extracts were prepared ranging from 12.5 mg/mL; this was obtained by measuring 1mg of extract and dissolved in 10mL dimethyl sulphur oxide(DMSO),a solvent that dissolved the extract (100mg/mL). A serial dilution of the dissolved extract ((100mg/mL) was carried out into three different bottles containing DMSO to obtain concentrations of 50,25 and 12.5 mg/mL respectively.

### 2.5. Sensitivity test of crude extract of Agar Well Diffusion Method

The organisms used were standardized using Mc-Farland turbidity standard scale 1, to obtain a bacterial cell density of 10<sup>6</sup> colony forming unit milliliter(cfu/mL).The standardized inoculate were uniformly streaked (swabbed) into freshly prepared Mueller Hinton agar and potato dextro agar

plates respectively for the bacterial and fungal growth. For wells were punched on the inoculated plate with a cork borer (8mm in diameter). The wells were properly labeled according to different number of concentration prepared. The well were then fill up with the extracts about 0.2Ml per well. The plates were allowed to stay on the bench for 1hr for extract to diffused on the agar. The Mueller Hinton agar plates for bacterial were incubated at 37°C for three days were the potato dextro agar plates for fungi were incubated at room temperature (drawer)for three days. At the end of incubation period, all the plates were observed for any evidence of inhibition, which will appear as a clear zones that were completely devoid of growth around the wells (zone of inhibition). The diameters of zones were measured with transparent ruler calibrated in millimeter (mm).

**2.6. Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration (MIC) of the extract was determined using tube dilution method. Serial dilution of the extract was carried out in test tubes using Mueller Hinton Broth (MHB)and potato Dextro Broth (PDB) as diluents. The lowest concentration showing inhibition (clear zone) for each organism when the extract was tested during sensitivity test was serially diluted in test tubes containing Mueller Hinton Broth (MHB) and potato Dextro Broth (PDB). Each tube containing the broth and the extract was incubated with the standardized organisms. A tube containing sterile broth (MHB and PDB) without any organism been used as a control. All tubes were then incubated 37°C for 24 hrs. After incubation period, the tube was examined for the presence or absence of growth using turbidity as a criterion. The lowest concentration (dilution) in the series without any visible signs of growth was considered to be the minimum inhibitory concentration (MIC).

**3. RESULTS**

**3.1 Antimicrobial Activities of *Bauhinia Racemosa***

**3.1.1. Mean zone of inhibition of crude extract of *Bauhinia racemosa***

The activity of hexane, chloroform, ethyl acetate, acetone and methanol crude extracts of *Bauhinia racemosa* was tested on five clinical isolates and the result of the mean zone of inhibition is presented in Table 1 below.

**Table1.** Mean zone of inhibition of crude extract of *Bauhinia racemosa*

Test Organism	Conc. mg/ml	HEBR	CEBR	EAEBR	AEBR	EEBR	Positive (+) control streptomycin	Negative Control DMSO
<i>E-Coli</i>	200	18.00	18.00	20.00	20.00	18.00	32.00	00.00
	100	16.00	17.00	19.00	19.00	17.00	28.00	00.00
	50	14.00	18.00	17.00	19.00	15.00	28.00	00.00
	25	12.00	15.00	15.00	17.00	13.00	27.00	00.00
<i>St. aureus</i>	200	18.00	18.00	17.00	17.00	17.00	17.00	00.00
	100	16.00	15.00	15.00	15.00	15.00	15.00	00.00
	50	14.00	14.00	13.00	13.00	13.00	13.00	00.00
	25	12.00	13.00	11.00	13.00	10.00	10.00	00.00
<i>Salmonella Spp</i>	200	19.00	00.00	17.00	00.00	20.00	30.00	00.00
	100	16.00	00.00	16.00	00.00	18.00	28.00	00.00
	50	14.00	00.00	14.00	00.00	17.00	28.00	00.00
	25	12.00	00.00	13.00	00.00	15.00	26.00	00.00
<i>Candida albican</i>	200	23.00	00.00	22.00	22.00	23.00	<b>Fulcin (+)ve</b> 28.00	00.00
	100	21.00	00.00	20.00	20.00	21.00	26.00	00.00
	50	19.00	00.00	18.00	18.00	18.00	24.00	00.00
	25	17.00	00.00	17.00	17.00	17.00	23.0	00.00

		17.00						
<i>Aspergillus niger</i>	200	21.00	00.00	00.00	22.00	00.00	30.00	00.00
	100	19.00	00.00	00.00	20.00	00.00	28.00	00.00
	50	17.00	00.00	00.00	18.00	00.00	26.00	00.00
	25	16.00	00.00	00.00	16.00	00.00	24.00	00.00

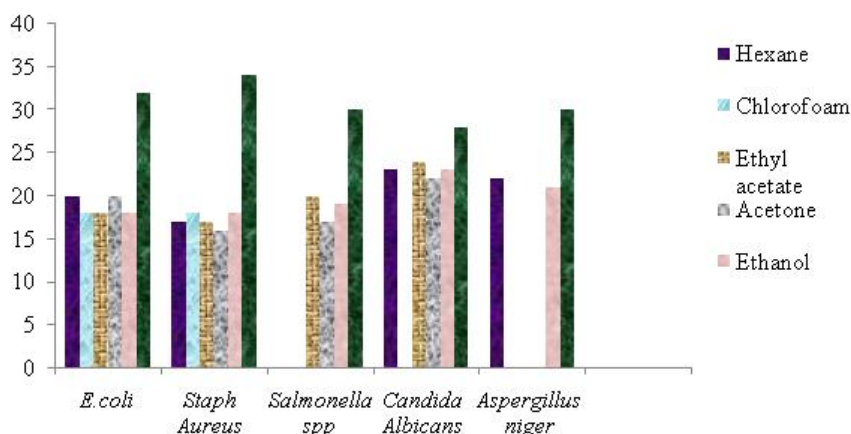


Figure1. Histogram of antimicrobial activities of *B. racemosa*

Table3. Minimum Inhibitory Concentration (MIC) of crude extract of *Bauhinia racemosa*

S/N	Organism	MIC Hexane (mg/ml)	MIC Chlorofoam (mg/ml)	MIC Ethyl acetate (mg/ml)	MIC Acetone (mg/ml)	MIC Ethanol (mg/ml)
1	25	25	25	25	25	25
2	25	25	25	25	25	25
3	25	25	25	25	25	25
4	25	25	25	25	25	25

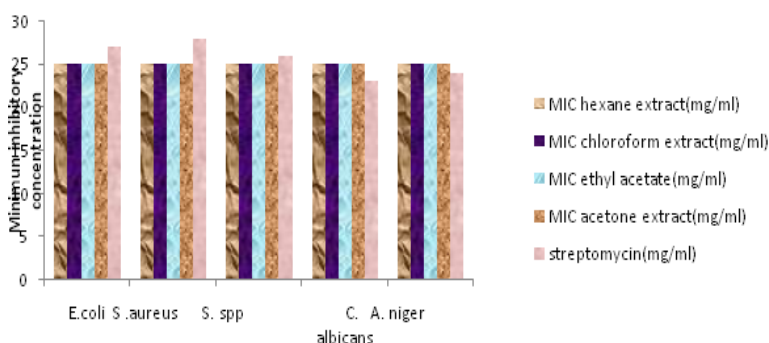


Figure2. Histogram of Minimum Inhibitory Concentration of *B. racemosa*

#### 4. DISCUSSION

The result of antimicrobial activity as shown in Table 1 show the activities of the crude hexane, chloroform, ethyl acetate, acetone and ethanol obtained from extract of leave of *B racemosa* were tested on five clinical isolates; *E. coli*, *Stapylococcus aureus*, *Salmonella spp*, *Candida albicans*, *Aspergillus niger*, while streptomycin and fucin were used as control drugs. The minimum inhibitory activity (MIC) of the extracts of *B. racemasa* against tested microbes ranges from 25 to 200 mg/ml in all the extracts against the tested bacteria. The result revealed that almost all of the extract exhibited or inhibited antibacterial against *E. coli*, *Stapylococcus aureus*, *Salmonella spp*, *Candida albicans* and *Aspergillus niger*, except extract of chloroform and acetone which did not show any inhibition on *Salmonella spp* (00.00mm), extract of chloroform donnot act against *Candida albicans*

and *Aspergillus niger*, extract of ethanol and ethyl acetate show zero inhibition on *Aspergillus niger*. Extract that do not inhibit or exhibit antifungal activity on certain bacterial, have zero zone of inhibitory (00.00 mm) as represented in Table 1 above.. Ethanol extract exhibited the highest activity for *Candida albicans* test (24 mm zone diameter of inhibition), followed by Hexane and acetone on the same organism (*Candida albicans*), (with both having 23mm zone diameter of inhibition). Acetone and chloroform extract did not show any activity against *Salmonella spp*, chloroform extract did not demonstrate any activity against *Candida albicans* and *Aspergillus niger* and also both ethanol and ethyl acetate extracts did not act against *Aspergillus niger*. This result is presented in figure 1 below.

The crude extracts of acetone extract inhibited or exhibited antifungal activity against *E. coli*, *Stapyllococcus aureus*, *Candida albicans* and *Aspergillus niger* with a diameter that ranged between 13 – 23 mm with the highest zone of inhibition of 23 followed by 22 mm at minimum inhibitory concentration (MIC) at 200mg/ml, but did not show inhibition on *Salmonella spp*. Chloroform extract inhibited or exhibited antifungal activity against only two organism (*E. coli*, and *Stapyllococcus aureus*), with a minimum zone of inhibition ranges from 13-18mm but did not show inhibition on *Salmonella spp*, *Candida albicans* and *Aspergillus niger*. Ethanol extract inhibited or exhibited antifungal activity against almost all the test organism except on *Aspergillus niger*, with the minimum zone of inhibition ranging from 10-24mm with the highest inhibition on *Candida albicans* (24mm), ethyl acetate just like the ethanol extract inhibited or exhibited antifungal activity against *E. coli*, *Stapyllococcus aureus*, *Salmonella spp*, *Candida albicans* with the minimum zone of inhibition ranging from 11-22mm with the highest inhibition on *Candida albicans* (22mm). However, hexane extract show inhibited on all the test organisms *E. coli*, *Stapyllococcus aureus*, *Salmonella spp*, *Candida albicans* and *Aspergillus niger* ranging from 12-21mm with the highest effect on *Aspergillus niger* (21mm). The least activity is observed in ethanol extract with 10mm zone of inhibition against *Stapyllococcus aureus*, followed by 11mm observed in ethyl acetate extract on *Stapyllococcus aureus* which occur at 25mg/ml minimum inhibitory concentration (MIC).

### 5. CONCLUSION

The result obtained from antimicrobial activity obtained from the extract of the leave of *B. racemosa* revealed that not all the extract of leaf of inhibited or exhibited antimicrobial activity against *E. coli*, *Stapyllococcus aureus*, *Salmonella spp*, *Candida albicans* and *Aspergillus niger*. Precisely *Salmonella spp* which acetone and chloroform extract donnot show any antimicrobial activity on as well as chloroform extract not acting againats both *Candida albicans* and *Aspergillus niger*. Ethanol and ethyl acetate extract did not act against *Aspergillus niger*. Conclusively, the higher the zone of inhibitory the stronger the antimicrobial activity of such extract on that particular organism. Zero zone of inhibitory implies that the extract has no any antimicrobial activity on that organism, from the result obtained in table 4.2 it can be concluded that ethanol extract has the highest antimicrobial activity on *Candida albicans* (24mm minimum zone of inhibition).

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