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**ORIGINAL ARTICLE**

# The Screening of Phytoconstituents, Antibacterial and Antifungal Activities of *Brysocarpus Coccineus* Schum and Thonn. Stem (Connaraceae).

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Abdulmumeen A. HAMID and Olapeju O. AIYELAAGBE: The Screening of Phytoconstituents, Antibacterial and Antifungal Activities of *Brysocarpus Coccineus* Schum and Thonn. Stem (Connaraceae).

## ABSTRACT

The uses of *Brysocarpus coccineus* stem in traditional African medicine have led to the antibacterial and antifungal activities of the hexane, ethylacetate and methanol stem extracts of the plant evaluation using six pathogenic bacteria and six fungi. The extracts inhibited the 12 test organisms to different degrees. Hexane, ethylacetate and methanol successive extracts of *Brysocarpus coccineus* stem effectively inhibited the growth of *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* at concentrations between 12.5 and 200mg/ml, while the extracts showed lower inhibition on *Staphylococcus aureus, Salmonellae typhii and Klebsiellae pneumonae*. The sensitivity of test bacteria was concentration dependent, activity being higher at higher concentrations of the three extracts. Ethylacetate and methanol exhibited higher antifungal properties on *Rhizopus stolon* and *Epidermophyton floccosum*, while hexane also inhibited the growth of *Rhizopus stolon, Epidermophyton floccosum, Tricophyton rubrum* and *Aspergillus niger* with activity comparable to that of the reference drug tioconazole. Preliminary phytochemical screening of the three extracts revealed the presence of saponins, reducing sugar, steroids, glycosides, flavonoids and anthraquinones.

***Key words:***

## Introduction

Medicinal plants have played essential roles since the early stage of life in the treatment of all kinds of diseases. African flora is very rich in these medicinal plants. The bacterial and fungal infections are also prevalent in our society and this leads to various diseases like gonorrhea, syphilis, pneumonia and different skin disorders. The search for new, safer and cheaper drugs especially from plants, to treat and cure these diseases is on the increase. *Brysocarpus coccineus* Schum and Thonn. (Connaraceae) is a climbing shrub found in Africa [1]. The plant especially its leaf is used in traditional medicine for the treatment of veneral diseases, impotency, diarrhoea, jaundice, piles, dysentery, earache, sore of mouth and skin, tumour, wounds, stomatitis, rheumatism, swellings and urinary disorders [2,3,4,8]. The pharmacological properties of *Brysocarpus coccineus* as antioxidant, anti-inflammatory, analgesic, antidiarrhoeal and antipyretic have been established [5,6,7,8,9].

Further, the uterotonic, molluscidal, hepatoprotective, anxiolytic and sedative activities of various extracts of the plant have also been reported [10,11,12,13]. A coumaroyl derivative, 4-hydroxycoumarin, flavonoids and flavones have been isolated from the leaves of *Brysocarpus coccineus* by Vickery and Vickery [14]. The presence of quercetin, quercetin 3-O-α-arabinose and quercetin 3-O-β-D-glucose from the bioactive ethylacetate and n-butanol soluble parts of ethanol extracts of *Brysocarpus coccineus* was also

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established by Ahmadu *et al*.,[15].

From the previous work, no scientific investigations have been reported on biological activities of the stem of *Brysocarpus coccineus*. Hence we now study the preliminary phytoconstituents, antibacterial and antifungal activities of *Brysocarpus coccineus* stem.

## Materials and methods

*Collection and Authentication of the Plant Material:*

The plant material of *Brysocarpus coccineus* was collected from Ibadan area of Oyo State, Nigeria, in the month of November 2009. Botanical identification and authentication was done by Mr. A.W. Ekundayo of the Forestry Research Institute of Nigeria (FRIN), Ibadan where a voucher specimen was deposited with the herbarium file number FHI108801.

*Preparation of Plant Extracts:*

The whole plant of *Brysocarpus coccineus* was separated into leaves and stem, air-dried and weighed (stem, 1100g and leaves, 534g). The dried stem was successively extracted in hexane, ethylacetate and methanol for 10 days respectively using cold extraction method. The resultant hexane (6g), ethylacetate (9g) and methanol (8g) extracts were obtained by evaporation and stored in the refrigerator for further use.

*Phytochemical Studies:*

The Preliminary phytochemical screening of the hexane, ethylacetate and methanol extracts of *B. coccineus* stem was done using standard procedures [16,17,18,19,20].

*Antimicrobial Assay*:

*Microorganisms:*

Cultures of six human pathogenic bacteria made up of four gram negative and two gram positive were used for the antibacterial assay. Namely; *Salmonella typhii* (UCH 4801), *Escherichia coli* (UCH 00260), *Pseudomonas aeruginosa* (UCH 1102) and *Klebsiellae pneumonae*(UCH 2894) (gram negative), *Bacillus subtilis* (UCH 74230) and *Staphylococcus aureus* (UCH 2473) (gram positive). For the Antifungal assay, six fungi were also utilized.These were; *Candida albicans, Aspergillus niger, Rhizopus stolon, Penicillum notatum, Tricophyton rubrum and Epidermophyton floccosum*. All the microorganisms used were clinical strains from the Department of Medical Microbiology (University College Hospital, Ibadan) and screened in the Laboratory of Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria.

*Media:*

Nutrient agar, Sabouraud dextrose agar, nutrient broth and tryptone soya agar were used in this study. Hexane, ethylacetate and methanol were also used in solubilizing the extracts and as negative controls in the assays.

*Antimicrobial Agents:*

Gentamycin (10µg/ml) and Tioconazole (0.7mg/ml) were included as standard reference drugs in the study.

*Antimicrobial Activity Determination:*

*Agar Diffusion-pour Plate Method (Bacteria):*

An overnight culture of each organism was prepared appropriately from its stock and inoculated each into the sterile nutrient broth of 5ml, each incubated for 18-24hrs at 37$^{o}$C. From overnight culture, 0.1ml of each organism was taken and put into the 9.9mls of sterile distilled water to get (1:100) $10^{-2}$ of the dilution of the organism.

From the diluted organism ($10^{-2}$), 0.2ml was taken into the prepared sterile nutrient agar cooled to about 40-45$^{o}$C, then poured into sterile Petri dishes and allowed to solidify for about 45-60mins. Using a sterile cork-borer of 8mm diameter, the wells were made according to the number of the test tubes for the experiment. For this work 8 wells were made. The graded concentrations of the extracts were put into the wells accordingly including the controls. The studies were done in duplicates to ascertain the results obtained. The plates were left on the bench for about 2hrs to allow the extract diffuse properly into the nutrient agar i.e. pre-diffusion. The plates were incubated for 18-24hrs at 37$^{o}$C.

*Agar Diffusion-surface Plate Method (Fungi):*

A sterile Sabouraud dextrose agar was prepared accordingly and aseptically poured into the sterile plates in duplicates and solidified properly. 0.2ml of the (1:100) $10^{-2}$ of the organism was spread on the surface of the agar using a sterile Petri-dish cover to cover all the surface of the agar. Eight wells were bored using a sterile cork-borer of 8mm diameter. The graded

concentrations of the extracts were put into the well accordingly including the controls.. All the plates were left on the bench for 2hrs to allow the extract diffuse properly into the agar i.e. pre-diffusion. The plates were incubated at 25ºC for 72hrs [21,22,23].

## Results and discussions

The results of the phytochemical screening of the hexane, ethylacetate and methanol extracts of *Brysocarpus coccineus* stem are presented in Table1. Preliminary phytochemical screening revealed the presence of reducing sugar, steroids, glycosides, flavonoids and anthraquinones in all the extracts. There was presence of saponins in ethylacetate and methanol extracts of *Brysocarpus coccineus* stem but absent in hexane extract of the plant. However, tannins and alkaloids were not found in all the three extracts of *Brysocarpus coccineus* stem.

The result of antibacterial activities of the hexane, ethylacetate and methanol extracts at concentrations ranging from 6.25 to 200mg/ml was presented in Table 2.

The bacteria used were clinical strains of *Staphylococcus aureus* and *Bacillus subtilis* (gram positive), *Escherichia coli*, *Pseudomonas aeruginosa*, *klebsiellae pneumonae* and *salmonellae typhii* (gram negative).

All the bacteria strains were sensitive to the three extracts at concentrations between 12.5 and 200mg/ml, but hexane extract exhibited higher inhibition on *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* than both ethylacetate and methanol extracts of *Brysocarpus coccineus*. Meanwhile, all extracts also inhibited the growth of *Staphylococcus aureus* and *salmonellae typhii*, while the inhibition of *klebsiellae pneumonae* by the extracts was very low.

Further, the sensitivity of the test bacteria to all the extracts were concentration dependent, activity being higher at higher concentrations of the extracts.

The result of the antifungal activities of the hexane, ethylacetate and methanol extracts at concentrations ranging from 6.25 to 200mg/ml was presented in Table 3. six clinical strains of fungi were used in our study. *candida, albicans, Aspergillus niger, Rhizopus stolon, penicillum notatum, Tricophylonrubrum* and *epidermophyton floccosum.* The six test fungi were sensitive to all extracts.

Hexane, ethylacetate and methanol extracts of the stem of *Brysocarpus coccineus* exhibited higher antifungal properties on *Aspergillus niger, Rhizopus stolon, penicillum notatum, Tricophyton rubrum* and *Epidermophyton floccosum* with activity comparable to that of the reference drug tioconazole against *Rhizopus stolon, Tricophyton rubrum, Epidermophyton floccosum and Aspergilus niger* for hexane extract, and *Rhizopus stolon* and *Epidermophyton floccosum* for both ethylacetate and methanol extracts.

However, all extracts showed lower inhibition on *Candida albicans.*

**Table 1:** Phytochemical constituents of the hexane, ethylacetate and methanol extracts of *Brysocarpus coccineus* stem.

| Secondary metabolites | Extracts (whole plant) | | |
|---|---|---|---|
| | Hexane | Ethylacetate | Methanol |
| Alkaloids | - | - | - |
| Saponins | - | ++ | ++ |
| Tannins | - | - | - |
| Reducing sugars | ++ | ++ | ++ |
| Steroids | ++ | ++ | ++ |
| Glycosides | ++ | ++ | ++ |
| Flavonoids | ++ | ++ | ++ |
| Anthraquinones | ++ | ++ | ++ |

Key
- Absent ++ present

**Table 2:** Antibacterial activities of the hexane, ethylacetate and methanol extracts of *Brysocarpus coccineus* stem.

| Extracts | Extract conc/Ref./ Control (mg/ml) | Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | S.a | E.coli | B.sub | Ps.a | Kleb | Sal. |
| Hexane | 6.25 | - | - | - | - | - | - |
| | 12.5 | - | 10 | - | - | - | - |
| | 25 | - | 12 | 12 | 10 | - | - |

**Table 2:** Continue.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | 50 | 10 | 14 | 14 | 12 | - | 12 |
| | 100 | 12 | 16 | 16 | 14 | - | 14 |
| | 200 | 14 | 24 | 20 | 18 | 10 | 16 |
| | Hexane | - | - | - | - | - | - |
| | Gentamycin | 36 | 34 | 34 | 36 | 34 | 36 |
| Ethylacetate | 6.25 | - | - | - | - | - | - |
| | 12.5 | - | - | - | - | - | - |
| | 25 | - | 10 | 12 | 10 | - | - |
| | 50 | - | 12 | 14 | 12 | - | - |
| | 100 | 10 | 16 | 16 | 14 | - | 12 |
| | 200 | 14 | 18 | 18 | 16 | 10 | 14 |
| | Ethylacetate | - | - | - | - | - | - |
| | Gentamycin | 36 | 34 | 34 | 36 | 34 | 36 |
| Methanol | 6.25 | - | - | - | - | - | - |
| | 12.5 | - | 10 | - | - | - | - |
| | 25 | - | 12 | 12 | 10 | - | - |
| | 50 | 10 | 14 | 14 | 12 | - | 10 |
| | 100 | 12 | 16 | 16 | 14 | - | 12 |
| | 200 | 14 | 20 | 18 | 16 | 10 | 14 |
| | Methanol | - | - | - | - | - | - |
| | Gentamycin | 36 | 34 | 36 | 36 | 34 | 34 |

**Table 3:** Anfungal activities of the hexane, ethylacetate and methanol extracts of *Brysocarpus coccineus* stem.

| Extracts | Extract conc/Ref./ Control (mg/ml) | Diameter of well = 8 mm Diameter of zone of inhibition of bacteria(mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | C.a | A.n | Rhiz | Pen | T.r | E.f |
| Hexane | 6.25 | - | - | - | - | - | - |
| | 12.5 | - | - | 10 | - | - | - |
| | 25 | - | - | 12 | 10 | - | - |
| | 50 | 10 | 10 | 16 | 12 | 10 | 10 |
| | 100 | 12 | 12 | 18 | 14 | 12 | 16 |
| | 200 | 14 | 18 | 20 | 16 | 18 | 20 |
| | Hexane | - | - | - | - | - | - |
| | Tioconazole | 28 | 22 | 26 | 24 | 22 | 22 |
| Ethylacetate | 6.25 | - | - | - | - | - | - |
| | 12.5 | - | - | - | - | - | - |
| | 25 | - | - | 10 | - | - | 10 |
| | 50 | - | 10 | 14 | 10 | 10 | 12 |
| | 100 | 10 | 12 | 18 | 12 | 12 | 16 |
| | 200 | 12 | 15 | 24 | 16 | 14 | 20 |
| | Ethylacetate | - | - | - | - | - | - |
| | Tioconazole | 24 | 24 | 23 | 24 | 23 | 22 |
| Methanol | 6.25 | - | - | - | - | - | - |
| | 12.5 | - | - | 10 | - | - | - |
| | 25 | - | - | 12 | 10 | - | - |
| | 50 | 10 | 10 | 14 | 12 | 10 | 10 |
| | 100 | 12 | 12 | 18 | 14 | 12 | 14 |
| | 200 | 14 | 16 | 26 | 16 | 16 | 18 |
| | Methanol | - | - | - | - | - | - |
| | Tioconazole | 24 | 24 | 24 | 24 | 24 | 22 |
| | **Key** | | | | | | |
| S.a | *Staphylococcus aureus* | | | | | | |
| E.coli | *Escherichia coli* | | | | | | |
| B.sub | *Bacillus subtilis* | | | | | | |
| Ps.a | *Pseudomonas aeruginosa* | | | | | | |
| Kleb | Klebsiellae pneumonae | | | | | | |
| Sal | Salmonellae typhii | | | | | | |
| C.a | *Candidas albicans* | | | | | | |
| A.n | *Aspergillus niger* | | | | | | |
| Rhiz | *Rhizopus stolon* | | | | | | |
| Pen | *Penicillum notatum* | | | | | | |
| T.r | *Tricophyton rubrum* | | | | | | |
| E.f. | *Epidermophyton floccosum* | | | | | | |

## *Conclusions*

The antibacterial and antifungal activities of all extracts (hexane, ethylacetate and methanol) further confirm the ethnomedicinal use of *Brysocarpus coccineus* to treat veneral diseases, diarrhea, rheumatism, impotency, mouth and skin sores, urinary disorder etc. [2,3,4,8].

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