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An Assessment of the Antimicrobial Properties of Extracts of Various Polarities from *Chasmanthera dependens*, *Emilia coccinea* and *Cuscuta australis*, Herbal Medications for Eye Diseases

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Abstract: An assessment of the antimicrobial properties of extracts from the stem of *Chasmanthera dependens*, the leaves of *Emilia coccinea* and the whole plant of *Cuscuta australis* plants which are used in the management of eye diseases in Nigeria is presented. The 50% methanol extracts were extracted sequentially with hexane, ethyl acetate and butanol to give fractions of various polarities which were tested for antimicrobial properties against various microorganisms including fungal yeast, Gram-positive and Gram-negative bacteria, namely *Candida albicans*, *Bacillus subtillis*, *Citrobacter* sp., *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Salmonella typhimurium*, *Serratia marcescens*, *Shigella flexnerii*, *Staphylococcus albus*, *Staphylococcus aureus* and *Staphylococcus aureus* ATCC 2593. The hexane fractions of all the plants were not found to possess antimicrobial activity on any of the microorganisms tested. The ethyl acetate fractions of *C. dependens* and *E. coccinea* were found to possess significant antimicrobial activity against several of the microorganisms while that of *C. australis* was active against only three of the microorganisms. The butanol fraction of *E. coccinea* showed significant antimicrobial activity against several of the microorganisms while those of *C. dependens* and *C. australis* were respectively active against only two and three of the microorganisms. The results support the use of *E. coccinea* as a potent anti-diarrhoeal agent and the uses of the plants as antimicrobials in herbal medicine.

Key words: *Chasmanthera dependens*, *Emilia coccinea*, *Cuscuta australis*, anti-diarrhoeal agent, antimicrobial activity, eye diseases

INTRODUCTION

Before the advent of orthodox medicine, plants were largely the sources of medications for diseases. Several of these plants were efficacious and have been verified in laboratories to possess pharmacologically-active constituents. For examples, extracts from *Annona senegalensis* tree were found to be active against tumor growth (Weniger *et al*., 1986), while cardiac glycosides have been isolated from *Rauvolfia vomitoria* (Iwu and Court, 1982). Several plants are used as herbal medications for the treatment of eye diseases in South-Western Nigeria and a review of some of these plants has been documented (Ogunlesi *et al*., 2008). Extracts from the leaves of *Chasmanthera dependens*, *Emilea coccinea* and the whole plant of *Cuscuta australis* are among the plants used for treating eye infections in Nigeria.

Chasmanthera dependens (Hoschst), commonly called Chasmanthera, belongs to the family Menispermaceae. It has been found useful in the treatment of several diseases and medical conditions. In addition to the use of the sap in treating eye infections (Ogunlesi *et al*., 2008), the roots are used in herbal preparations as diuretics, antigonococcal and for the management of fractures (Odugbemi, 2008). The methanolic extract of the dried leaves has been reported to exhibit anti-inflammatory and analgesic effects on laboratory animals (Morebise *et al*., 2001). Phytochemical investigation of the stem led to the isolation of quaternary alkaloids, non-phenolic alkaloids (Ohiri *et al*., 1982) as well as furanoid diterpene and 8-hydroxylcolombin from the bark (Iwu *et al*., 1999).

Emilea coccinea, commonly known as scarlet tassel flower, belongs to the family of Compositae. The leaves are eaten raw or stewed. In herbal medicine, the leaves and roots are used in the management of craw-craw, abscesses of the breast, yaws, lice, fever, ringworm, syphilis, hernia, gonorrhea, measles, cough, jaundice and snakebite (Odugbemi, 2006). Some of these bioactivities have been confirmed in the laboratory. These include anti-diarrhoeal, antimicrobial and fungicidal activity (Ogbebor and Adekunle, 2005; Ndip *et al*., 2007). Phytochemical

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screening has revealed the presence of alkaloids, tannin, saponin, steroids, terpenoids flavonoids and cardiac glycosides (Edeoga *et al*., 2003; Mroczek *et al*., 2004).

Cuscuta australis, commonly known as dodder, is a parasitic plant and belongs to the Convolvulaceae family. In herbal medicine, the whole plant is used as a laxative, anthelmintic, astringent and for the management of sores, measles, kidney and liver diseases (Odugbemi, 2008). Six flavonoids including kaempferol, quercetin, astragalin and hyperoside have been isolated from the plant (Guo and Li, 1997).

In the study hereby reported, extracts of various polarities were obtained from the stem of *C. dependens* and the leaves of *E. coccinea* as well as the whole plant of *C. australis* and screened for antimicrobial activity. The hexane extract is largely non-polar, the ethyl acetate fraction medium polar while the butanol fraction is polar.

MATERIALS AND METHODS

Plant materials: Batches of *C. dependens* were obtained from Olokemeji Forest Reserve, Oyo State, Nigeria, while the other plants were obtained from Mushin Market, Lagos State between May and June 2007. *C. dependens* was identified by Mr. O. S. Shasanya of the Forestry Research Institute of Nigeria (FRIN), Ibadan, where a voucher, specimen No. 107965 was deposited at the Herbarium of the Botany Department of the Institute on 1st May 2008. *E. coccinea* and *C. australis* were identified by Mr. T. K. Odewo of FRIN and vouchers with numbers 107681 and 107682, respectively were deposited in the Herbarium on 7th November 2006.

Extraction of fractions

***Chasmantera dependens*:** 3.5 kg of the fresh stem was cut into small pieces and soaked in 1.5 L of distilled water. The mixture was refrigerated for 72 h with gentle and intermittent shaking and thereafter filtered. To the filtrate was added hexane equal to 20% of its volume and kept at room temperature for 2 h with intermittent shaking. The hexane layer was evaporated to give lipids and pigments (100 g). The lipids and pigments constitute the hexane fraction. To the aqueous layer was added sufficient 96% methanol to give a 50% solution. The mixture was shaken gently and intermittently for 72 h at room temperature after which the solvents were removed by evaporation at 45°C in a vacuum oven to give 24.7 g of the 50% methanol extract which was dissolved in 50 cm^3 of distilled water and sequentially extracted with 4×10 cm^3 hexane, 4×10 cm^3 ethyl acetate and 17×10 cm^3 butanol. The various fractions were evaporated to give 2.5 and 3.4 g ethyl acetate and butanol fractions respectively. No material was further extracted by hexane.

***Emilia coccinea*:** 0.75 kg of fresh leaves were extracted with 500 cm^3 distilled water from which 350 cm^3 of filtrate was obtained. The same procedure as described previously was used for defatting, giving 30 g of the lipids and pigments and thereafter obtaining the 50% methanol extract yielding 14.4 g dry material. After sequential extraction with hexane, ethyl acetate and butanol, no material was extracted by hexane while 3.1 g of ethyl acetate fraction and 1.0 g of butanol fraction were obtained.

***Cuscuta australis*:** An aqueous extract obtained from 346 g of the fresh whole plant yielded 35 g of the hexane fraction, 0.5 and 1.95 g of the ethyl acetate and butanol fractions respectively.

Preparations of solutions for antimicrobial screening: Saturated solutions of the hexane and ethyl acetate fractions in the corresponding solvents were prepared. While the two solvents did not exhibit anti-microbial activity, butanol exhibited significant antimicrobial activity hence the butanol fractions were dissolved in dimethylsulfoxide (DMSO).

For *C. dependens* the concentrations used for antimicrobial screening were 91 mg cm^{-3} of hexane fraction in hexane and 82 mg cm^{-3} of ethyl acetate fraction in ethyl acetate. The concentrations for *E. coccinea* were 200 mg cm^{-3} for hexane fraction and 125 mg cm^{-3} for ethyl acetate fraction.

The concentrations used for *C. australis* were 400 mg cm^{-3} for hexane fraction and 100 mg cm^{-3} for ethyl acetate fraction.

The solutions of the butanol fractions in DMSO were 74 mg cm^{-3} for *C. dependens*, 74 mg cm^{-3} for *E. coccinea* and 57 mg cm^{-3} for *C. australis*. Ciprofloxacin antibiotic suspension 0.05% was used as control.

Test microorganisms: The test organisms used were *Candida albicans*, *Bacillus subtillis*, *Citrobacter* sp., *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella* sp., *Salmonella typhimurium*, *Shigella flexnerii*, *Staphylococcus albus*, *Staphylococcus aureus* and *Staphylococcus aureus* ATCC 25923. All the organisms were confirmed and obtained from the Research Laboratory at the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos, Nigeria.

Antimicrobial assay: This was carried out according to the method of Sharhidi-Bonjar (2004). The test organisms were subcultured on Blood Agar and Mac-Conkey Agar (Oxoid, UK). Suspensions of the microorganisms in sterile normal saline were adjusted to 0.5 McFarland standards

to give suspensions containing approximately 1×10^8 CFU cm^{-3}. The medium plates were labeled and each was uniformly seeded with a test organism using sterile swab rolled in the suspension and streaked on the surface of the plate. Wells of 5 mm in diameter placed about 2 cm apart were punched in the culture media with sterile cork borer. One hundred microliter of various concentrations of test extracts were dropped into each well (Sharhidi-Bonjar, 2004). Ciprofloxacin, water, hexane and ethyl acetate without test compounds were placed in wells on each plate as control. Each plate was kept in the refrigerator at 4°C for 1 h before incubating at 37°C for 24 h. Zones of inhibition around the wells were measured in millimeter and were used as positive bioactivity.

RESULTS

The results which are presented in Table 1 and 2 show that the ethyl acetate fraction of *C. dependens* is active against *C. albicans*, *B. subtillis*, *Citrobacter* sp., *E. coli*, ATCC 25922, *K. pneumoniae*, *P. mirabilis*, *S. albus* and *S. aureus*. The activity against *S. aureus* is comparable to that of the ciprofloxacin control. However, the butanol fraction exhibits activity against only two of the microorganisms, namely, *E. faecalis* and *S. aureus* ATCC 25923.

The ethyl acetate fraction of *E. coccinea* was found to exhibit activity of comparable magnitude as the ciprofloxacin control against *B. subtillis*, *Citrobacter* sp., *E. coli*, *E. coli* ATCC 25922, *K. pneumoniae*, *E. coli*, *P. mirabilis*, *S. albus*, *S. aureus* and *S. aureus* ATCC 25923. In addition it was found to be strongly active against *C. albicans*. It was inactive against *E. faecalis*, *P. aeruginosa* and *S. marcescens*. The butanol fraction was found to be active against all the microorganisms tested except *C. albicans*. The activity against *B. subtillis*, *E. faecalis* and *S. aureus ATCC* 25923 was found to be comparable to that of the ciprofloxacin control.

The ethyl acetate fraction of *C. australis* was found to exhibit activity against only three microorganisms, namely, *E. coli*, *K. pneumoniae* and *S. aureus*. The butanol fraction was found to exhibit activity against only three microorganisms, namely, *B. subtillis*, *E. faecalis* and *P. aeruginosa*.

DISCUSSION

The results in Table 1 show that all the ethyl acetate fractions from the three plants exhibited antimicrobial activity. *E. coccinea* appears to be the most potent of the three plants and exhibited about the same potency as 0.05% ciprofloxacin against *B. subtillis*, *Citrobacter* sp.,

Table 1: Antimicrobial activity of the ethyl acetate fractions of the stem exudate of *C. dependens*, leaves of *E. coccinea* and whole plant of *C. australis*

Microorganisms	*C. dependens*	*E. coccinea*	*C. australis*	*Ciprofloxacin*
Candida albicans	3+	3+	-	-
Bacillus subtillis	2+	3+	-	3+
Citrobacter sp.	2+	3+	-	3+
Enterococcus faecalis	-	-	-	2+
Escherichia coli	2+	3+	2+	3+
Escherichia coli ATCC 25922	2+	3+	-	3+
Klebsiella pneumoniae	2+	3+	2+	3+
Proteus mirabilis	2+	3+	-	3+
Pseudomonas aeruginosa	NT	-	-	-
Serratia marcescens	NT	-	-	-
Staphylococcus albus	2+	3+	-	3+
Staphylococcus aureus	3+	3+	2+	3+
Staphylococcus aureus ATCC 25923	-	3+	-	3+

-: Negative result (no inhibition zone observed), 1+: 5-9 mm zone of inhibition, 2+: 10-19 mm zone of inhibition, 3+: >20 mm zone of inhibition, NT: Not Tested

Table 2: Antimicrobial activity of the butanol fractions of the stem exudate of *C. dependens*, leaves of *E. coccinea* and whole plant of *C. australis* dissolved in DMSO

Microorganisms	*C. dependens*	*E. coccinea*	*C. australis*	*Ciprofloxacin*
Candida albicans	-	-	-	-
Bacillus subtillis	-	3+	2+	3+
Enterococcus faecalis	2+	3+	2+	3+
Escherichia coli	-	2+	-	3+
Escherichia coli ATCC 25922	-	2+	-	3+
Klebsiella pneumoniae	-	2+	-	3+
Pseudomonas aeruginosa	-	2+	2+	2+
Salmonella sp.	-	2+	-	2+
Salmonella typhimurium	-	2+	-	3+
Shigella flexnerii	-	2+	-	3+
Staphylococcus albus	-	2+	-	3+
Staphylococcus aureus	-	2+	-	3+
Staphylococcus aureus ATCC 25923	2+	3+	-	3+

-: Negative result (No inhibition zone observed); 1+: 5-9 mm zone of inhibition, 2+: 10-19 mm zone of inhibition, 3+: >20 mm zone of inhibition

E. coli ATCC 25922, *K. pneumoniae*, *E. coli*, *P. mirabilis*, *S. albus*, *S. aureus* and *S. aureus* ATCC 25923. In addition it was active against *C. albicans* for which ciprofloxacin showed no inhibition. In a study on the *in-vitro* anti-*Helicobacter pylori* activity of extracts of selected medicinal plants from North West Cameroon, the methanolic extract of the dried whole plant of *E. coccinea* at 200 mg cm^{-3} was found to be weakly active against the isolates (Ndip *et al*., 2007). In another study on the antimicrobial activity of the leaf extract of *E. coccinea*, it was observed that at 5 mg cm^{-3} concentration, the aqueous extract did not have any antimicrobial activity on the microorganisms tested, but the methanolic extract was most active against *E. coli*. There was no activity against *C. albicans* (Teke *et al*., 2007). The report of a study on the fungicidal effects on conidial germination and mycelia growth of *Corynespora cassiicola* showed that the aqueous leaf extract of *E. coccinea* exhibited 25% inhibition while *Ocimum basilicum* exhibited 100% (Ogbebor and Adekunle, 2005). The solutions of the fractions of *E. coccinea* used for the antimicrobial screening in our study were 125 mg cm^{-3} for the ethyl acetate fraction and 74 mg cm^{-3} for the butanol fraction. The study by Ndip *et al*. (2007) was on whole plant and the 200 mg cm^{-3} solution of the methanolic extract was only weakly active against the isolates. The comparison between present results and those of Ndip *et al*. (2007) is limited by their use of whole plant, that is, leaves and stem, while our report is on the leaf extract. The moderate fungicidal effect of the aqueous leaf extract observed by Ogbebor and Adekunle (2005), is in agreement with the potent activity of the ethyl acetate extract of *E. coccinea* on *C. albicans*.

The ethyl acetate fraction of *C. dependens* exhibited activity against *C. albicans*, *B. subtilis*, *Citrobacter* sp., *E. coli* ATCC 25922, *K. pneumoniae*, *E. coli*, *P. mirabilis*, *S. albus* and *S. aureus*. The activity against *S. aureus* was about the same as those exhibited by *E. coccinea* and the ciprofloxacin control. The activity against *C. albicans* was the same as that exhibited by *E. coccinea*. Its activity against the other microorganisms was less than that of *E. coccinea*.

C. australis appears to be the weakest of the three, but the ethyl acetate fraction exhibited significant antimicrobial activity against *E. coli*, *K. pneumoniae* and *S. aureus*.

The results in Table 2 show that the butanol fraction of *E. coccinea* also exhibited potent antibacterial activity of about the same magnitude as ciprofloxacin against *B. subtilis*, *E. faecalis* and *S. aureus* ATCC 2593. The activity against *E. coli*, *E. coli* ATCC 25922, *K. pneumoniae*, *E.coli*, *P. aeruginosa*, *Salmonella* sp., *S. typhimurium*, *Shigella flexnerii*, *S. aureus* and *S. albus* were of lower magnitude than that of ciprofloxacin. It is significant that both the ethyl acetate and butanol fractions from the leaves of *E. coccinea* show potent antimicrobial activity with the ethyl acetate fraction exhibiting greater potency. The results support the use of the leaf extract in the management of veneral diseases, abscesses and eye diseases (Odugbemi, 2006; Ogunlesi *et al*., 2008).

The antimicrobial activity of both the ethyl acetate and butanol fractions of the leaf extract of *E. coccinea* can be used to support some of the ethnomedicinal uses of the plant. In the Cameroon, the leaves are chewed to treat diarhoea, stomach-ache, bowel and bladder disorders (Teke *et al*., 2007). The major causative organisms of diarrhoea in human include *C. albicans*, *E. coli*, *S. typhimurium*, *S. flexnerii* and *S. aureus* (Robert *et al*., 200l; Jouret-Mourin and Geboes, 2002). The results in this study show that *C. albicans* is inhibited by the ethyl acetate fraction of *E. coccinea* leaf extract, *E. coli* is inhibited strongly by the ethyl acetate fraction and moderately by the butanol fraction. *S. typhimurium* and *S. flexnerii* are inhibited by the butanol fraction and *S. aureus* is inhibited by both fractions. Hence the results show that the leaf extract of *E. coccinea* should function as an anti-diarrhoeal phytomedicine. Teke *et al*. (2007) reported that the methanol extract of *E. coccinea* leaves showed antimicrobial activities on some gastrointestinal microorganisms thus providing scientific support for the anti-diarrhoeal activity. The results in this report could be used to infer that the ethyl acetate fraction of *C. dependens* would also exhibit some measure of anti-diarrhoeal activity though not as potent as *E. coccinea*, because it also exhibits bioactivity against *C. albicans*, *E. coli* and *S. aureus*.

The butanol fraction from the stem of *C. dependens* was found to be active against only two of the microorganisms namely *E. faecalis* and *S. aureus* ATCC 25923. The butanol fraction of *C. australis* exhibited activity against only three of the microorganisms tested; these are *B. subtilis*, *E. faecalis* and *P. aeruginosa*. The results lend support to its use in the management of bacterial infections in sores and some eye diseases (Odugbemi, 2008; Ogunlesi *et al*., 2008).

S. aureus has been isolated from wound and eye infections (Adelowotan *et al*., 2008) and the ethyl acetate fractions from the plants were found to be active against a strain of this microorganism hence this may constitute scientific support for the use of the plants in treating eye infections and the use of *C. australis* for the management of sores and *E. coccinea* for the management of abscesses of the breast.

CONCLUSION

The ethyl acetate fraction from the stem of *C. dependens* and both the ethyl acetate and butanol fractions from the leaves of *E. coccinea* were found to exhibit strong antimicrobial activity against several microorganisms while the butanol fractions of *C. dependens* and *C. australis* and the ethyl acetate fraction of *C. australis* exhibited activity against only few microorganisms. The results lend support to the uses of the various plants as antibacterial agents in herbal medicine.

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