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Assessment of Antimicrobial Activity of the Essential Oil from the Stem Powder of *Cissus populnea* and the Leaves of *Sesamum radiatum*, Herbal Medications for Male Infertility Factor

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Abstract: One of the causes of male infertility factor is venereal diseases. *Cissus populnea* and *Sesamum radiatum* are two tropical medicinal plants used to correct male infertility factor. The aim of this study was to extract the essential oil from the stem powder of *C. populnea* and the leaves of *S. radiatum* and screen them for antimicrobial activity. The essential oil from the two plants were obtained by hydro-distillation and were both found to inhibit the growth of Gram-negative and Gram-positive bacteria including Citrobacter sp., *Escherichia coli*, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus albus* and *Staphylococcus aureus*. The essential oil from *C. populnea* was in addition found to be active against *Enterococcus faecalis*, *Staphylococcus aureus* ATCC 25923 while the essential oil from *S. radiatum* was found to be active against *Serratia marcescens*. The essential oil samples from the two plants were both inactive against *Candida albicans*. The results confirm the antimicrobial properties of the essential oil from the two plants and hence, these plants may correct male infertility factor arising from bacterial infection.

Key words: *Cissus populnea*, *Sesamum radiatum*, essential oil, antibacterial activity, medicinal plants, male infertility factor

INTRODUCTION

Cissus populnea Guill and Per belongs to the Vitaceae family. It is used extensively in medicinal preparations in West Africa. In Benin Republic, it is used as a diuretic and in Ghana it is used as a post-harvest ethnobotanical protectant (Belmain *et al*., 2000). The aqueous extract of the stem bark is used as a fertility enhancer in males in South Western Nigeria (Ojekale *et al*., 2006). The stem bark has been shown to contain tannins, flavonoids, saponins and steroids (Ojekale *et al*., 2006). Extracts from the root of the plant have been used for the management of skin diseases, boils, infected wounds (Kone *et al*., 2004) and for treating urinary tract infections (Ojekale *et al*., 2006) thus suggesting antibacterial activity. Anthraquinone derivatives, steroidal and cardiac glycosides have been reported present in the root extract while alkaloids and tannins were found to be absent (Moody *et al*., 2003).

Sesamum radiatum Schum. and Thonn belongs to the Pedaliaceae family (Hutchinson and Dalziel, 1954). The decoction of the leaves is used for the treatment of catarrh, eye pains, bruises and erupted skins (Bankole *et al*., 2007). The decoction of combined roots and leaves has been reported to exhibit anti-viral and anti-fungal activity

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(Gill, 1992). The aqueous extract of the leaves has been reported to be rich in phenols, flavonoids, lignans and sterols (Shittu *et al*., 2006). The phenols would be expected to exhibit antimicrobial activity (Konan *et al*., 2008).

The two plants investigated are used in treating male infertility factor in South-Western Nigeria. It has been reported that venereal diseases could be responsible for male infertility factor (Greendale *et al*., 1993). It is thus possible that these plants may exhibit antimicrobial activity which may cure venereal diseases and thus correct male infertility factor arising from bacterial infection. This is the reason for this investigation of the antimicrobial activity of the essential oils from these plants.

MATERIALS AND METHODS

Collection of Plant Materials

Several batches of the fresh stem of *C. populnea* and fresh leaves of *S. radiatum* were obtained from Mushin market in Lagos between the months of October 2005 and May 2007. They were identified and authenticated by Mr. T.K. Odewo and Mr. Seun Osiyemi both of the Forestry Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen for *C. populnea* with the number FHI 108222 was deposited at the Herbarium in FRIN on 27th May, 2008 while the voucher specimen for *S. radiatum* with number FHI 107823 was deposited at the Herbarium in FRIN on 12th September, 2007.

Hydro-Distillation of Samples

The plant parts were cut into small pieces and air-dried at room temperature in a dust-free environment for 3 weeks. The dried materials were powdered and the essential oil from each plant was extracted by hydro-distillation in batches of 100 g mixed with 3 L of water. The essential oil was extracted into hexane and the solution obtained was concentrated by evaporation of the hexane. The volume of the essential oil was 0.3 cm^3 and weighed 350 mg.

Preparation of Test Organisms

The test microorganisms used were collected from the stock cultures of the Medical Microbiology and Parasitology Department of the College of Medicine, University of Lagos, Idi-Araba, Surulere, Lagos. They included *Candida albicans* (a yeast) and Gram-negative and Gram-positive bacteria, namely Citrobacter sp., *Enterococcus faecalis*, *Escherichia coli*, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Staphylococcus albus*, *Staphylococcus aureus* and *Staphylococcus aureus* ATCC 25923.

The essential oil samples from the two plants were screened for antimicrobial activities using the agar disc diffusion method (Dosso and Kette, 1995). The test organisms were subcultured on Blood Agar and Mac-Conkey Agar (Oxoid, UK). Suspensions of the microorganisms were prepared in sterile normal saline and adjusted to 0.5 McFarland standards. The resulting suspension contained approximately 1×10^8 cfu cm^{-3}. Each medium plate was uniformly seeded with a sensitive strain of each microorganism. The sample of each essential oil was dissolved in hexane to give a concentration of 260 μg cm^{-3} and sterile paper discs, 6 mm diameter, were soaked in each solution. The discs were transferred to the inoculated plates immediately. After refrigeration at 4°C for 1 h, the plates were incubated at 37°C for 24 h. The 0.05% ciprofloxacin in hexane and neat hexane were used as controls. Zones of inhibition around the discs were measured in millimeter and used as an assessment of antimicrobial activity.

Table 1: The results of antimicrobial screening of the samples of essential oil obtained from the stem of *Cissus populnea* and the leaves of *Sesamum radiatum*

Microorganisms	Ciprofloxacin	Essential oil from *Cissus populnea*	Essential oil from *Sesamum radiatum*
Candida albicans	0	0	0
Citrobacter sp.	3+	2+	2+
Entrococcus faecalis	3+	2+	0
Escherichia coli	3+	3+	2+
Escherichia coli ATCC 25922	3+	2+	2+
Klebsiella pneumoniae	3+	2+	2+
Proteus mirabilis	3+	2+	2+
Pseudomonas aeruginosa	3+	3+	2+
Serratia marcescens	3+	0	2+
Staphylococcus albus	3+	2+	2+
Staphylococcus aureus	3+	3+	2+
Staphylococcus aureus ATCC 25923	3+	2+	0

1+: 5-9 mm zone of inhibition; 2+: 10-19 mm zone of inhibition; 3+: > 20 mm zone of inhibition

RESULTS

The results of antimicrobial screening of the samples of essential oil obtained from the stem of *C. populnea* and the leaves of *S. radiatum* are shown in Table 1. Hexane did not inhibit the growth of any of the microorganisms. The essential oil obtained from the stem of *C. populnea* inhibited the growth of ten microorganisms. It strongly inhibited *E. coli*, *P. aeruginosa* and *S. aureus*, exhibiting the same extent of inhibitory zone of 3+ as the control, 0.05% ciprofloxacin. There was significant but lower activity with inhibitory zone of 2+ against Citrobacter sp., *E. faecalis*, *E. coli* ATCC 25922, *K. pneumoniae*, *P. mirabilis*, *S. albus* and *S. aureus* ATCC 25923. There was no activity against *C. albicans* and *S. marcescens*.

The essential oil from the leaves of *S. radiatum* inhibited the growth of nine microorganisms, with no activity against *C. albicans*, *E. faecalis* and *S. aureus* ATCC 25923. The activity against the other microorganisms namely Citrobacter sp., both strains of *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. marcescens*, *S. albus* and *S. aureus* all showed inhibitory zone of 2+. The essential oil from *S. radiatum* was consistently less potent than the ciprofloxacin control.

In comparison, the essential oil obtained from *C. populnea* inhibited all the Gram-positive microorganisms tested namely *E. faecalis* and the three staphylococcus strains while the essential oil from the leaves of *S. radiatum* inhibited the growth of two Gram-positive bacteria tested namely *S. albus* and *S. aureus*, showing no inhibitory action against *S. aureus* ATCC 25923 and *E. faecalis*. The essential oil from *C. populnea* exhibited higher activity against *E. coli*, *P. aeruginosa*, both strains of *S. aureus* and *E. faecalis* but no activity against *S. marcescens* compared to the sample from *S. radiatum*.

DISCUSSION

The results show that the samples of the essential oil from the two plants have great potential as antimicrobials hence might be able to correct male infertility factor arising from infection by the bacterial species. Extracts obtained from *C. populnea* have been reported to be useful for treating skin diseases, boils (Kone *et al*., 2004) and urinary tract infections (Ojekale *et al*., 2006).

In a screening of 50 medicinal plants used in North Cote-d'Ivoire as traditional remedies for bacterial diseases, *C. populnea* was among the ten plant species which exhibited

promising level of activity against bacteria including strains resistant to aminosides, macrolides, penicillin M, lincosamide and streptrogramin B (Kone *et al*., 2004). The ethanolic root extract of *C. populnea* was used in the study. The bacteria used in the study included Gram-positive strains, *S. aureus*, *E. faecalis*, *B. subtilis* and *Streptococcus pyogenes* as well as Gram-negative strains such as *E. coli* and *P. aeruginosa*. The root extract was not active against *E. coli* and *P. aeruginosa* but was found to be active against a methicillin-resistant hospital strain of *S. aureus* and some aminoside-resistant hospital strains of *E. faecalis*. In our study, the essential oil of *C. populnea* was found to be significantly active against *E. faecalis*, the three staphylococcus strains and even against *E. coli* and *P. aeruginosa* which were not inhibited by the root extract (Kone *et al*., 2004).

In a study on the antimicrobial activity of ethanolic and aqueous extracts of the dried leaves of *S. radiatum* against five microorganisms, namely *S. pneumoniae*, *C. albicans*, *S. aureus*, *E. coli* and *P. aeruginosa*, it was observed that the ethanolic extract exhibited mild inhibitory effects on *S. pneumoniae* and *C. albicans* while the aqueous extract of the same concentration did not show any inhibitory effects (Shittu *et al*., 2006). The essential oil in our study did not exhibit activity against *C. albicans* but was active against *S. aureus*, *E. coli* and *P. aeruginosa*.

The aqueous extract (Shittu *et al*., 2006) was obtained by boiling the aqueous suspension of the powdered leaves for 3 h and thus some of the constituents of essential oil in the leaves would have been extracted and thus would be expected to show some inhibitory effects against some of the microorganisms tested as was observed in the study being presented in our report. However, the aqueous extract was lyophilized and this process might have resulted in extensive loss of the essential oil constituents. This may explain the inactivity of the aqueous extract.

Several medicinal plants which are used for the management of male infertility factor in Nigeria are also used as antimicrobials. Such plants include *Abrus precatorius*, *Adenia venata*, *Agelenopsis chevaleri*, *Aloe barteri*, *Areca catechu*, *Craterispermum laurinum*, *Circuligo pilosa*, *Datura stramonum*, *Ritchiea brachypoda*, *Saba florida*, *Securidaca longepedunculata*, *Tabernaemontana pachysiphen*, *Tribulus terrestris* and *Withania somnifera* several of which are used for treating venereal diseases and urinary tract infections (Odugbemi, 2008a). *Mucuna pruriens* which is also used as a male fertility enhancer is used as a phytomedicine for genito-urinary diseases (Odugbemi, 2008b). Some phytomedicines classified as aphrodisiacs are alleged to be antimicrobials in herbal preparations. In a review on Infections and male infertility, Megory *et al*. (1987) stated that bacteria could affect semen quality and cause changes which could impair fertility function. Infections could cause inflammation of the accessory gland or total damage to the gonads and adrexa (Megory *et al*., 1987). Greendale *et al*. (1993) reported that venereal diseases could reduce fertility in male subjects. Thus the use of antimicrobials is relevant in the management of male infertility.

The essential oil from *Syzygium aromaticum* (L.) Merr. and Perry, a herbal aphrodisiac has been reported to exhibit significant antimicrobial activity against a collection of 25 different genera of test bacteria and 20 different isolates of *Listeria monocytogenes* (Deans *et al*., 2006). It was also reported to exhibit high levels of inhibition against three fungal strains of a plant pathogen (Deans *et al*., 2006). *Terminalia catappa* another herbal aphrodisiac has also been reported to show promising antibacterial properties. In the antimicrobial screening of nine Peruvian medicinal plants, the leaves of *Terminalia catappa* and aerial parts of *Phyllantus amarus* showed the most promising antibacterial properties

inhibiting all the bacterial strains tested with minimum inhibitory concentrations ranging from 0.25 to 16 mg cm^{-3} (Kloucek *et al*., 2005). The microorganisms used were *Bacillus cereus* ATCC 11778, *B. subtilis* ATCC 6633, *Bacteroides fragilis* ATCC 25285, *E. faecalis* ATCC 2912, *E. coli* 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *Streptococcus pyogenes* ATCC 19615. The ethanolic extracts of *T. catappa* demonstrated activity in the following decreasing order: *S. epidermidis* ATCC 12228 > *S. aureus* 25923 > *B. cereus* > *B. subtilis* = *P. aeruginosa* > *E. coli* 25922 = *E. faecalis* > *S. pyogenes* ATCC 19615 = *B. fragilis*. In our study the essential oils from the two plants were active against the two strains of *E. coli* one of which was strain ATCC 25922 as well as *P. aeruginosa*. In addition, the essential oil from *C. populnea* was active against *E. faecalis* and *S. aureus*. Thus these results are similar to those on *T. catappa*. High antifungal but no antibacterial activity of methanol and methylene chloride extracts from *T. catappa* aerial part was observed (Goun *et al*., 2003). However, Pawar and Pal (2002) detected appreciable antimicrobial activity of the chloroform and methanol extracts of the roots of *T. catappa* against *E. coli* and *S. aureus*.

Tribulus terrestris, a herbal aphrodisiac, analgesic, diuretic, anti-hypertensive agent also used for the management of urinary disorders, impotence and gonorrhea was investigated for antibacterial activity (Kianbakht and Jahaniani, 2003). The methanolic extracts of Iranian plant parts, fruits, stems, leaves and roots, showed considerable activity against all the bacteria used in the study, namely *S. aureus*, *E. faecalis*, *E. coli* and *P. aeruginosa*. However, the ethanolic extracts of Yemini plant did not exhibit antibacterial activity against any of the reference bacteria (Ali *et al*., 2001). However, some researchers reported that all plant parts of the Turkish *T. terrestris* showed activity against several reference bacteria and some researchers and noted that the ethanolic extracts of the fruits and leaves of Indian *T. terrestris* were active against *E. coli* and *S. aureus* (Kianbakht and Jahaniani, 2003).

The reports on the studies on *T. terrestris* support antibacterial activity in plant parts (except in the Yemeni plant) and hence its usefulness in the management of urinary tract infection especially the activity against *E. coli*, a microorganism which has been implicated in urinary tract infection (Tena *et al*., 2008).

The report of our study on eleven bacterial samples and the bioactivity of the essential oils from the two plants on several of these microorganisms show that these essential oils are broad-spectrum antibacterial agents which may be useful in inhibiting the growth of some microorganisms which can be causative agents of infertility in male subjects.

CONCLUSION

The essential oil from the stem of *C. populnea* and the leaves of *S. radiatum* have been shown to demonstrate significant antibacterial activity against several bacterial strains including both Gram-positive and Gram-negative microorganisms.

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