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Full Length Research Paper

Evaluation of extracts of *Triclisia suboardata* Oliv and *Heinsia crinita* (Afz) G. Taylor for antimicrobial activity against some clinical bacterial isolates and fungi

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We report antimicrobial potential of extracts of roots of *Triclisia subcordata* and whole plant of *Heinsia crinita* used as components of various herbal portions in ethnomedine in South West Nigeria to treat acute urinogenital infections and infertility. Methanol and hexane extracts of each plant were obtained by maceration and tested for antimicrobial activity using agar diffusion and microbroth dilution techniques. The extracts were tested against strains of *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa* and fungi including four species of *Candida.* The study showed that extracts of *H. crinita* and *T. subcordata* exhibited profound antibacterial activity against the typed and clinical isolates obtained from patients with STD and meningitis. High antifungal activity, particularly against the *Candida* species was noted as *Candida* species are implicated in candidiasis and vaginal thrush. Generally, the methanol extract was more effective than the hexane extracts on the test micro-organisms. The study justifies the ethnopharmacological uses of these medicinal plants for treatment of microbial infections.

Key words: *Triclisia subcardata, Heinsia crinita,* antimicrobial activity, non-gonococcal urethritis, infertility.

INTRODUCTION

Infectious diseases account for approximately one-half of all deaths in tropical countries. In industrialized nations, despite advances in microbiology, incidence of epidemics due to drug resistant micro-organisms and emergence of hitherto unknown pathogenic microbes pose enormous public health concerns (Pinner et al., 1996). Historically plants have provided a good source of anti-infective agents (Erdemeier et al., 1996; Abo et al., 1999; Abo et al., 2000).

Even though there are many commercially available anti-bacterial agents, there are relatively fewer anti-fungal drugs. Drug resistance and incidence of chronic mixed infections caused by opportunistic organisms as with AIDS patients, have led to a continuous search for alternative antimicrobial agents of plants origin (Iwu et al., 1999) with emphasis on agents with antifungal properties in view of the fact that over 50% of all modern clinical drugs are natural products (Stuffness and Douros, 1982) and natural products play an important role in drug development programmes of pharmaceutical industries (Baker et al., 1995; Cordell, 1995).

Furthermore, WHO estimates that a sizeable majority of the world population presently use herbal medicine for various chemotherapeutic purposes and for some aspect of their primary health care (Ripa et al., 2009) and its is a major component in all indigenous peoples traditional medicine (Lino and Deogracious, 2006; Okwori et al., 2008). For these reasons, we have investigated *Heinsia crinita* (Afz) G. Taylor and *Triclisia subcordata* Oliv used as components of various herbal portions in ethnomedicine in South West Nigeria for acute microbial

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infections and infertility. There are reports indicating increasing incidence of STD and infertility in South West Nigeria in recent times (Obisesan and Adeyemo, 1980; Abo et al., 2000).

Infertility is considered to be secondary to reproductive tract infections, the risk of which is increased by multiple sexual partners and septic induced abortions. The acute stages of STD are the main cause of infertility (Oyebola, 1981; Obisesan and Adeyemo, 1988; Abo et al., 2000).

T. subcordata Oliv (Menispermaceae) is commonly called "Alugboran" in Yoruba language (Dalziel, 1937). It is a woody twiner with broad-veined and reticulate leaves, small flowers in short pedunculate clusters and appressed puberscent fruits. The stem is used as a rough fibre (tietie) (Irvine, 1961).

H. crinita (Afz) G. Taylor (Rubiaceae) is known as "Bush Apple" and it is called "Tonoposho" in Yoruba (Dalziel, 1937). It is a scrambling shrub with persistent and very conspicuous leafy calyx-lobes (Irvine, 1961; Keay, 1989), produces edible yellow or reddish fruits, sweet when ripe and pleasantly acid. The Efiks in Southern Nigeria use the leaves in vegetable soup and also for the treatment of hypertension and abscess (Ajibesin et al., 2008). The scented leaves are used for treatment of craw craw and head lice in children.

Our interest in these two medicinal plants arose because we observed that herbalists in South West Nigeria used them in ethnomedicine for the treatment of acute urinogenital infections and infertility complicated with chronic microbial infections particularly STD. Literature is very scanty on both plants. Anti- ulcer activity of methanol extract of *T. subcordata* in rats (Asuzu and Anaga, 1995) and a comparative pharmacognostic and antimicrobial studies of leaves of *H. crinita* have been reported (Ajibesin et al., 2002). There is no report on the antimicrobial activity of *T. subcordata,* neither is there any comprehensive study of the antifungal properties of *H. crinita*. We report the antimicrobial activities of whole plant of *H. crinita* and root of *T. subcordata* on typed strains and clinical isolates of various pathogenic bacteria and fungi.

MATERIALS AND METHODS

Plant material

Whole plant of *H. crinita* (Afz) G. Taylor (Rubiaceae) and roots of *T. subcordata* Oliv (Menispermaceae) were collected in Ibadan in July 2007 and authenticated by Mr. K. Odewo at the Forest Herbarium, Forestry Research Institute of Nigeria, Ibadan where herbarium specimens (Voucher numbers FHI 34134 and FHI 103433 respectively) are kept. The plant materials were oven-dried below 60°C and powdered for analysis.

Extraction

100 g of powdered sample (whole plant or root) of each plant was separately macerated in 600 ml methanol (MeOH) and 600 ml hexane for five days to obtain the MeOH and hexane extracts of each plant used for the analysis. Each extract was filtered and the solvent evaporated under reduced pressure in a rotary evaporator and weighed. Serial dilutions of each dried extract were prepared in 50% MeOH to give test concentrations of 200, 100, 50, 25 and 12.5 mg/ml.

Phytochemical screening

Screening for presence of secondary metabolites were performed following standard micro-chemical tests (Harborne and Harborne, 1998; Evans, 2002).

Test microbial strains

Six bacterial strains were isolated from hospital cases of STD or non-gonococcal urethritis and meningitis patients. The micro-organisms used were *Staphylococcus aureus* (strains *clin-1* and *clin-2*) isolated from urethral swap of STD patients; *S. aureus (clin-3)* isolated from urine sample of purulent male organ of STD patient; *Escherichia coli* (strains *clin-4* and *clin-5)* isolated from cerebrospinal fluid of meningitis patients while *E. coli (clin-6)* was isolated from high vaginal swab specimen and urine samples of two female urethritis patients who were also infertile. All clinical isolates were obtained from Department of Medical Microbiology, Department of Anatomy, Lagos University Teaching Hospital (LUTH), Idi-Araba, Lagos and the Department of Medical Microbiology and Parasitology, University College Hospital (UCH), Ibadan.

The typed bacteria strains were *Bacillus subtilis* ATCC 6051 and *Pseudomonas aeruginosa* NCTC 6750 obtained from the Department of Veterinary Microbiology, University of Ibadan. Test fungi used were *Candida albicans, Candida krucei, Candida glaberata, Candida tropicalis Aspergillus niger, Aspergillus flavus, Collectotricum gloesporoides, Trichoderma asperelum* and *Fusarium* species. These were obtained from the Departments of Pharmaceutical Microbiology, Veterinary Microbiology, University of Ibadan and the International Institute of Tropical Agriculture (I.I.T.A) Ibadan.

All strains were purified by three successive streaking and re-isolation on Mueller Hinton Agar or Saboraud dextrose agar. The purity and identity were confirmed by standard bacteriological methods (Cheesborough, 1984; Acheampong et al., 1988). Media: Nutrient broth No 2, pH 7.4; nutrient agar, pH 7.4; malt extract broth, pH 5.6; Mueller Hinton agar (MHA). All are products Oxoid Laboratories, UK.

Antimicrobial assays

The agar well diffusion method was adopted for screening for antibacterial activity (Reeves et al., 1979; Okeke et al., 2001). 0.1 ml of a 1 in 100 dilution of the overnight broth culture of each bacterium (10^6–10^7 viable cells [cfu]) per ml of culture medium, determined by McFarland Nephelometry (NCCLS, 1993), was used to seed sterile molten nutrient agar maintained at 45°C. The plates were allowed to solidify, 8 mm diameter wells were bored in the seeded plates. Concentrations of 12.5, 50, 100 and 200 mg/ml (dissolved in 50% MeOH) of each plant extract were added into appropriate wells and allowed to stand for two hours at room temperature to diffuse before incubating at 37°C for 24 h.

Gentamycin (10 g/ml) dissolved in 50% MeOH was used as positive control while 50% MeOH was used as negative control (no inhibition). All experiments were performed in triplicate. Antifungal tests were performed in a similar manner but seeded with appropriate fungi hyphae in saboraud dextrose agar, except that *C.*

Table 1. Antibacterial activity of methanol and hexane extracts of *H. crinite.*

Bacterial strains	Extract*	Concentrations (mg/ml)					
		200	100	50	25	12.5	+GT
Staphylococcus aureus (*clin-1*)	A	27	19	16	13	11	31
	B	17	14	12	10	-	32
Staphyllococcus aureus (*clin-2*)	A	25	21	18	14	10	32
	B	17	14	12	10	4	30
Staphyllococcus aureus (*clin-3*)	A	24	19	17	13	11	29
	B	20	15	12	10	3	29
Eschericha coli (*clin-4*)	A	17	14	12	10	-	30
	B	14	12	10	-	-	31
Eschericha coli (*clin-5*)	A	20	17	13	11	-	31
	B	15	12	10	-	-	31
Eschericha coli (*clin-6*)	A	18	14	12	10	-	32
	B	18	12	8	-	-	30
Bacillus subtilis (ATCC 6051)	A	19	15	14	10	-	33
	B	16	13	12	10	-	29
Pseudomonas aeruginosa (NCTC 6750)	A	20	17	14	12	-	29
	B	14	11	10	-	-	25

Figures are mean diameter of zones of inhibition in mm; n=3; *A (MeOH extract; B [hexane extract]); - = no inhibition; 50% MeOH (solvent, no inhibition); +GT (Gentamycin); MIC [A on *S. aureus* (*clin-1 clin-3*) = 6.26 mg/ml, *S. aureus* (*clin-2*) = 12.5 mg/ml]; B on *S. aureus* (*clin-1*) = 25 mg/ml, *S. aureus* (*clin-2 clin-3*) = 12.5 mg/ml].

albicans was inoculated in malt extract broth (Abo et al., 1998, 1999). All plates were subsequently incubated at room temperature for 96 h. Griseofulvin (10 g/ml) dissolved in 50% MeOH was used as reference antifungal drug. The mean diameter of zones of inhibition (mm) were measured as basis for activity. The minimum inhibitory concentration (MIC) was determined by macrobroth dilution technique as previously reported (Sahm and Washington, 1990; Okoli and Iroegbu, 2003; Abo and Olugbuyiro, 2004) . The tube with the lowest dilution with no detectable growth was considered as the MIC (El-Mahmood et al., 2008).

RESULTS

The antibacterial profile of extracts of *H. crinita* is shown in Table 1. The figures in the table are calculated mean zones of inhibition in mm. Both the MeOH (A) and hexane (B) extracts of *H. crinita* are significantly bactericidal on all clinical isolates of *s. aureaus* and *E. coli* up to concentration 50 mg/ml. However, the hexane extract lost antibacterial activity on *P. aeruginosa* and the strains of *E. coli* (*clin-4, clin-5 and clin-6*) and was practically inactive at 12.5 mg/ml on all test bacteria. The MIC for the MeOH extract on *S. aureus* (*clin-1* and *clin-3*) was 6.25 mg/ml while it was 12.5 mg/ml on *S. aureus* (*clin-2*). The MIC for the hexane extract on the strains of *S. aureus* range from 12.5 to 25 mg/ml showing that the clinical strains of *S. aureus* are highly susceptible to inhibition by MeOH extract of *H. crinita*. Generally, this extracts show more antibacterial activity than the hexane extract on the test organisms (Table 1). The MeOH extract of *H. crinita* exhibited comparable antifungal activity, in some cases, more active than griseofulvin at the concentration range 50 to 200 mg/ml (Table 2).

At the high concentrations tested, both extracts (A and B) showed high antifungal activity. However, at concentration of 12.5 mg/ml, the MeOH extract was only active on *C. albicans, C. tropicalis, C. gloesporoides, T. asperelum* and *Fusarium spp.* The MIC for the MeOH extract on *C. albicans* and *C. tropicalis* was 6.25 and 25 mg/ml for *T. asperelum* and *C. globerata.* The MIC for hexane extract on *C. albicans, C. tropicalis, C. kurcei* and *C. glaberata* was 25 mg/ml showing very high antifungal activity against the four *Candida* species tested. This activity on the *Candida* spp is significant as this fungus is also implicated in atrophic candidiasis, vaginal thrush and aflatoxin production.

Table 3 shows the antibacterial profile of root extracts

Table 2. Antifungal activity of methanol and hexame extracts of *H. crinite.*

Fungi	Extract *	Concentrations (mg/ml)					
		200	100	50	25	12.5	+GSF
Candida albicans	A	23	18	15	13	11	24
	B	16	14	12	10	-	20
Candida krucei	A	21	18	12	10	-	20
	B	14	12	14	11	-	21
Candida glaberata	A	21	17	13	10	-	22
	B	17	14	12	11	-	20
Candida tropicalis	A	27	22	16	12	10	23
	B	19	16	14	11	-	20
Aspergillus niger	A	24	19	14	12	-	25
	B	19	15	12	-	-	20
Aspergillus flavus	A	22	16	14	-	-	25
	B	14	12	16	13	-	23
Colletotrichum gloesporoides	A	25	18	15	12	10	18
	B	18	14	12	10	-	16
Trichoderma asperelum	A	22	20	16	13	11	16
	B	20	16	13	10	-	14
Fusarium species	A	17	14	12	10	10	14
	B	17	14	12	10	-	12

Figures are mean diameter of zones of inhibition in mm; n=3; *A (MeOH extract; B [hexane extract]); - = no inhibition; +GSF (Griseofulvin); MIC [A on *C. albicans C. tropicalis* = 6.25 mg/ml; *C. gloesporoides T. asperelum* = 25 mg/ml]; B on *C. albicans, C. krucei, C. glaberata C. tropicalis* = 12.5 mg/ml].

of *T. subcordata* on the test microbes. The root extract exhibited good antibacterial activity at concentration range 50 to 200 mg/ml. However, antibacterial activity as recorded only on the strains of *S. aureus* and *E. coli*. There was no inhibition of test organisms at 12.5 mg/ml. The MIC recorded for the strains of *S. aureus* and *E. coli* was in range 6.25 to 25 mg/ml. Generally, griseofulvin exhibited more antifungal activity than both extracts of *T. subcordata.*

Table 4 shows that high antifungal activity was recorded for extracts of *T. subcordata* on all test fungi at concentration 50 mg/ml. It also shows that at concentration 25 mg/ml, antifungal activity was observed for all *Candida* species (MIC for MeOH extract on *C. albicans, C. krucei and C. tropicalis* and *Aspergillus niger* was 12.5 mg/ml. No inhibition was observed for *Fusarium spp, C. gloesporoides* and *Aspergillus flavus*. As with previous results, the MeOH extract of *T. As. Flavus subcordata* is more active than its hexane extract on the test fungi.

The results shown in Tables 2 and 4 indicate that extracts of *H. crinita* exhibited better antifungal properties than extracts of *T. subcordata.* Alkaloids, tannins and saponins were detected in extracts of *T. subcordata* whereas cardenolides, tannins and saponins were present in extracts of *H. crinita* (Table 5) . The role of these natural products on the antimicrobial properties of the two medicinal plants is presently unclear and will be subject for further investigations.

SUMMARY AND CONCLUDING REMARKS

This study indicates that the extracts from *H. crinita* and *T. subcordata* exhibited profound antibacterial activity against clinical strains of *S. aureus* and *E. coli* isolated from patients with STD or non- gonococcal urethritis, and were suspected to be possible cause of infertility in some

Table 3. Antibacterial activity of extracts of roots of *T. subcordata*.

Bacterial strains	Extract*	Concentrations (mg/ml)					
		200	100	50	25	12.5	+GT
Staphylococcus aureus (c*lin-1*)	A	20	17	14	12	10	32
	B	18	16	15	13	12	33
Staphyllococcus aureus (*clin-2*)	A	19	16	14	12	10	33
	B	17	15	14	13	-	30
Staphyllococcus aureus (*clin-3*)	A	23	17	15	12	10	32
	B	21	18	16	14	-	31
Eschericha coli (*clin-4*)	A	17	14	12	10	-	30
	B	15	13	11	10	-	29
*Eschericha coli(*clin-5)	A	17	14	12	10	-	34
	B	14	13	11	-	-	34
Eschericha coli (*clin-6*)	A	16	14	12	10	-	33
	B	14	12	11	-	-	31
Bacillus subtilis (ATCC 6051)	A	15	13	11	-	-	30
	B	13	11	10	-	-	30
Pseudomonas aeruginosa (NCTC 6750)	A	15	12	10	-	-	32
	B	16	15	12	-	-	29

Figures are mean diameter of zones of inhibition in mm; n=3; *A (MeOH extract; B [hexane extract]); - = no inhibition; +GT (Gentamycin); MIC [A and B on all strains of *S. aureus and E. coli* between range 6.25 – 25 mg/ml].

Table 4. Antifungal activity of extracts of roots of T. subcordata.

Fungi	Extract*	Concentrations (mg/ml)					
		200	100	50	25	12.5	GSF^{+}
Candida albicans	A	19	16	14	12	6	22
	B	16	15	13	10	-	22
Candida krucei	A	19	16	12	10	8	20
	B	17	14	12	10	-	21
Candida glaberata	A	21	16	13	10	-	18
	B	20	18	16	12	-	20
Candida tropicalis	A	19	16	14	12	-	18
	B	17	15	13	10	-	19
Aspergillus niger	A	18	15	12	-	-	21
	B	16	14	12	10	-	21
Aspergillus flavus	A	16	14	12	-	-	19
	B	14	12	10	-	-	16

Table 4. (Continued).

Colletotrichum gloesporoides	A	17	14	12	-	-	16
	B	15	13	11	-	-	17
Trichoderma asperelum	A	15	13	10	-	-	14
	B	13	10	10	-	-	13
Fusarium species	A	15	12	10	-	-	12
	B	12	11	10	-	-	14

Figures are mean diameter of zones of inhibition in mm; n=3; *A (MeOH extract; B [hexane extract]); - = no inhibition; GSF+ (Griseofulvin); MIC for A on *C. albicans, C. krucei and C. tropicalis* = 12.5 mg/ml.

Table 5. Summary of classes of natural products in root of *T. subcordata* and whole plant of *H. crinite.*

Plant species	Alkaloid	Tannin	Saponin	Cardenolide	Anthraquinones
*Triclisia subcordata (*root)	+++	+	+	-	-
Heinsia crinita (whole plant)	-	+	++	+	+

+++ = strongly positive; ++ = positive; + = trace - = absent.

of the patients. Antibacterial and antifungal activity of the medicinal plant methanol extracts was 100 g ml^{-1} (Mahesh and Satish, 2008) and 5 mg ml^{-1} (Erturk, 2006) whereas the current work the effectiveness was 12.5 to 200 mg ml^{-1}. Both medicinal plants exhibited good antifungal properties. The high activity against the four test *Candida* species was notable since *Candida* spp are implicated, along with other organisms, in candidiasis and vaginal thrush. Acute microbial infections may lead to secondary infertility of patients.

The use of plants as medicine is an art as old as mankind and still represents a very important phenomenon in traditional medicine, an established part of the culture of inhabitants of developing countries (Hamburger and Hostettmann, 1991; De Feo and Senatore, 1993, Duraipandiyan et al., 2006*;* Firas et al., 2008). Indeed, herbal medicines have served as sources of medicines for prevention and treatment of diseases (De Feo and Senatore, 1993; Li et al., 2004; Ashidi et al., 2005; Abo et al., 2008).

In conclusion, this study shows that *H. crinita* and *T. subcordata* are effective against the test pathogens and it justifies the ethnopharmacological uses of both plants in the treatment of microbial infections. Further investigations will be conducted on these medicinal plants to ascertain the active antimicrobial constituents since they may have potential use in medicine.

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