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Phytochemical and Antituberculosis Activity of *Coffea brivipes* HIERN Extracts

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ABSTRACT

The present study seeks to evaluate the medicinal relevance of the *Coffea brivipes* extracts against some clinically identified strains of *Mycobacterium tuberculosis,* using standard methods of microbial sensitivity test and phytochemical analysis. The whole plant samples of *Coffea brivipes* were extracted with absolute ethanol. The ethanol extract of *Coffea brivipes* HIERN (*RUBIACEA*) was then macerated with hexane, chloroform and ethyl acetate successively to obtain hexane, chloroform and EtOAc soluble extracts. The extracts were screened for their phytochemical composition and anti-bacterial activities. The phytochemical results depicted the presence of carbohydrate, alkaloids, tanins, sterols, flavonoids, resins and phenols distributed in varying degrees. The antibacterial activity of the extracts against *Mycobacterium tuberculosis* (MTB 050 and 303) were carried out using the egg enriched Lowenstein-Jansen medium with isoniazid, dihydrostreptomycin, enthanbutol and rifampicin as standard control drugs. The extracts showed different anti-bacterial activities at 10^{-2} and 10^{-4} bacteria load. MTB 050 strains showed resistance to rifampicin at both innoculum. Hexane and ethylacetate extracts of *C. brivipes* at 5 mg mL^{-1} exhibited good anti-bacterial activities against this rifampicin resistance strain. The result suggest that the hexane and ethylacetate extracts of *Coffea brivipes* can serve as a good cut for the replacement of rifampicin as an anti-Tb drug.

Key words: *Coffea brivipes,* MTB 050, MTB 303, *Mycobacterium tuberculosis*, lowenstein-Jansen medium

INTRODUCTION

Coffea brevipes HIERN, a small tree of about 12 ft tall belong to Rubiaceae family. It is found in the under storey of dark forest of West Africa especially in Nigeria, Ivory Coast, Western Cameroon and Zaire. The fruit is pink in colour when ripe and it is borne on the stem (Burkill, 2000). This plant bear close affinity to *C. arabica* and *C. Canephora* var. *robusta* the most widely studied Coffea genus because of their importance in the production of coffee (Nikhila *et al*., 2008; Sureshkumar *et al*., 2010).

No recorded investigation as been carried out on phytochemical screening, isolation and medicinal usage of any parts of *C. brevipes*. However, some other members of the family that are related to *C. brevipes* have been explored to some extent especially in relation to their morphological studies (Kufa and Burkhardt, 2011).

Daglia *et al.* (2007), isolated purine alkaloids: caffine, theobromide and theophyline from *C. arabica*. The report indicated that the seed and pericarp of *C. arabica* fruits contained a considerable amount of caffine and small amount of theobromide. Theophylline was also reported in pericarp of the ripened fruit. Alzoreky and Nakahara (2001), reported the antioxidant activity of various extract of *C. arabica* using the ferrymyglobin/ABTS method. Most of the researches done on the phytochemical screening and medicinal activities of coffea family were focused on the fruits possibly because of its edibility and economy value. There is less or no attention on other parts of the plant like the leaves, stem-bark and root-bark. This makes them a novel area in natural product research.

The common isolated compounds from the Coffea species include: β-carotene, α-carotene (Simkin *et al.*, 2008), nitrogenous alkaloids; Caffeine, trigonelline (Koshiro *et al.*, 2006), hydroxycinnamic, *p*-coumaric, *o*-coumaric, 3,4-dimethoxycinnamic, caffeic, ferulic, sinapic and 4-methoxycinnamic acids (Andrade *et al.*, 1998).

The primary causative bacterium of tuberculosis is *Mycobacterium tuberclosis*. Common tuberculosis affects the lungs known as pulmonary tuberculosis. Tuberculosis can also affect the central nervous system, lymphatic, circulatory, genitourinary systems, bones, joints and even skin. Tuberculosis is a highly contagious disease that is usually transmitted through coughing and sneezing. The epidemiology reports shows that on annual basis, 8 million people become ill with tuberculosis while 2 million people die from the disease worldwide. Tuberculosis was also reported to be the leading cause of death among people with HIV/AIDS (WHO, 2006; Cox, 2004; CDC, 2003; Imam *et al.*, 2010). The emergence of a man-made multi-drugs resistance strain of *M. tuberculosis* (Nikalje and Mudassar, 2011) also called for development of a better drug(s) or cuts from natural products. Medicinal plants in many indigenous African communities especially in Nigeria, are readily and cheaply available alternative to synthetic drugs. They are of great values to phytochemist because of their medicinal properties.

The present study aimed at investigating the phytochemical constituents and pharmacological effect of *C. brivipes* extracts on clinically isolated *Mycobacterium tuberculosis*.

MATERIALS AND METHODS

Plant collection: The *C. brevipes* plant was collected on 20th January, 2009 from a dry farmland at Lalupon village in Ibadan, Oyo state, Nigeria. The plant was authenticated by Mr. Ugbogu O.A. and Mr. Soyewo L.T. of Plant Taxonomy Department, Forestry Research Institute Ibadan, Nigeria (FRIN). A voucher specimen FHI NO 108450 was deposited at FRIN herbarium.

Plant extraction: The plant was air dried and crushed with mechanical crusher. The grinded material was hermetically sealed in plastic for use. The plant sample (2000 g) was subjected to cold extraction. It was soaked in 99% ethanol for a week. The ethanol extract was then collected and concentrated using rotary evaporator at 35°C to give ethanol residue. The ethanol residue (150 g) was then macerated with n-hexane, chloroform and EtOAc successively. Each of the extracts were collected separately and concentrated to give hexane, chloroform and EtOAc residues respectively using rotary evaporator at 35°C.

Antimycobacterial susceptibility test: This was carried out at the TB Research Laboratory, The Zankli Medical Centre (ZMC), Abuja, Nigeria. The agar dilution method using the egg enriched Lowenstein-Jansen (LJ) medium was used as reported by the International Union Against

Tuberculosis LJ Medium, 2007 (Nwachukwu *et al.*, 2009). This was done in a safety cabinet *Lamilplus* 7. Isoniazid, ethanbutol, dihydrostreptomycin and rifampicin were used as standard control drugs at final drug concentration of 0.2, 2.0, 8.0 and 40.0 μg mL^{-1}, respectively in the LJ. The phytodrugs were screened at 5 mg mL^{-1}. The slopes were stored at 4°C prior to inoculation. Well characterized clinical isolates (MTB 050 and MTB 303) of *Mycobacterium tuberculosis* which are positive to NO_3 reduction, negative catalyst labile tests and shows the presence of serpentinous cords on Zn smear. The isolates obtained from the ZMC were diluted in sterile distilled water to 10^{-2} and 10^{-4}. This corresponds to 1.0 and 0.5 Mcfarland, respectively. 10 μL of each of the 10^{-2} and 10^{-4} innoculum concentrations were inoculated using a micropipette on separate standard drugs, phytodrugs and negative control LJ slant media and incubated for six weeks at 37°C. Inoculated media were checked after 3 days for contamination.

Phytochemical methods: Preliminary phytochemical tests for alkaloids, steroids, tannins, anthraquinone, resins, carbohydrate, glycosides, saponins, phenolics and flavonoids were carried out on the extracts. The methods were based on those reported by Trease and Evans (1989) and Sofowora (1993).

RESULTS AND DISCUSSION

Phytochemical test: The result of phytochemical screening revealed some differences in the constituents of the three extracts tested distributed in varying degrees (Table 1). Results obtained from the qualitative phytochemical tests carried out on the fractionated extracts revealed that the extracts contained a wide array of phytochemicals which include carbohydrate, tannins, flavonoids, alkaloids, sterols, resins and phenols. The absence of glycosides, saponins and anthraquinones was also observed. It is a fact that the phytochemical constituent can help one to speculate on the medicinal value of the stem bark. Flavonoids, Tannins, saponins and alkaloids have been reported to have pronounced physiological effect particularly on the nervous system. The presence of these phytochemicals in the *C. brivipes* extracts suggests that the plant is pharmacologically active, supporting the claim by traditional healers. The result supported the reported phytochemical components indicating presence of alkaloids and flavonoids in the Coffea genus (Simkin *et al.*, 2008; Koshiro *et al.*, 2006; Andrade *et al.*, 1998). Phytochemical results of each extracts also suggested that carbohydrate, alkaloids, tanins, sterols, flavonoids, resins and phenols can be isolated from *C. brivipes* plant.

Table 1: Phytochemical screening of the extract of *C. brivipes*

Phytochemicals	Hexane extract	Ethyl acetate extract	Chloroform extract
Carbohydrates	-	+	+
Alkaloids	-	+	+
Tannins	-	+	-
Glycosides	-	-	-
Saponins	-	-	-
Sterols	+	-	+
Flavonoids	-	+	+
Resins	+	+	+
Anthraqninones	-	-	-
Phenols	+	+	+

+: Present; -: Not present

Antimycobacterial susceptibility test: Table 2 shows that there was no growth of MTB 050 observed against Isoniazid (0.2 μg mL^{-1}), Dihydrostreptomycin (8.0 μg mL^{-1}), Ethanbutol (2.0 μg mL^{-1}), Hexane extract (5 mg mL^{-1}), Ethyl acetate extract (5 mg mL^{-1}) after 6-weeks of incubation. These results suggested that the drugs and extracts are active against the MTB 050 isolate. The MTB 050 was not susceptible (after 6-weeks of incubation) to rifampicin because bacterial colonies growth observed was in the range of 200-500 and 20-100 for 10^{-2} and 10^{-4} inoculums concentrations, respectively. The chloroform extract (5 mg mL^{-1}) showed partial activity against MTB 050 because at it was not active at high inoculums concentration.

The results of antimycobacterial susceptibility screening (Table 3) depicted the following: Hexane extract show activity at 5 mg mL^{-1} against both MTB 303 at 10^{-2} and 10^{-4} innoculum concentrations; chloroform fraction show activity at 5 mg mL^{-1} against MTB 303 at both innoculum (10^{-2} and 10^{-4}) while ethylacetate fraction show activity at 5 mg mL^{-1} against MTB 303 at low innoculum (10^{-4}).

Table 2: Antimycobacterial susceptibility result for MTB 050 of the extract of *C. brivipes*

		MTB 050 innoculum		
Sample	Concentration μg mL^{-1}	10^{-2}	10^{-4}	Remark
Sterility control	Not applicable	-	-	LJ not contaminated
Negative control	Not applicable	3+	1+	Viable MTB isolate
Isoniazid	0.2	-	-	Susceptible
Dihydrostreptomycin	8.0	-	-	Susceptible
Rifampicin	40.0	3+	1+	not susceptible
Ethanbutol	2.0	-	-	Susceptible
Hexane extract	5000	-	-	Susceptible
Ethyl acetate extract	5000	-	-	Susceptible
Chloroform extract	5000	+1	-	Not susceptible at high innoculum

-: No growth after 6 weeks incubation at 37°C, +: 1-19 colonies growth after 6 weeks incubation at 37°C, +1: 20-100 colonies growth after 6 weeks incubation at 37°C, +2: 100-200 colonies growth after 6 weeks incubation at 37°C, +3: 200-500 colonies growth after 6 weeks incubation at 37°C, 4+: >500 colonies growth (confluent growth) after 6weeks incubation at 37°C

Table 3: Antimycobacterial susceptibility result for MTB 303 of the extract of *C. brivipes*

		MTB 303 innoculum		
Sample	Concentration μg mL^{-1}	10^{-2}	10^{-4}	Remark
Sterility control	Not applicable	-	-	LJ not contaminated
Negative control	Not applicable	2+	1+	Viable MTB isolate
Isoniazid	0.2	-	-	susceptible
Dihydrostreptomycin	8.0	-	-	susceptible
Rifampicin	40.0	-	-	susceptible
Ethanbutol	2.0	-	-	susceptible
Hexane extract	5000	-	-	Susceptible
Ethyl acetate extract	5000	+1	-	Not Susceptible at high innoculum
Chloroform extract	5000	-	-	Susceptible

-: No growth after 6 weeks incubation at 37°C ,+: 1-19 colonies growth after 6 weeks incubation at 37°C, +1: 20-100 colonies growth after 6 weeks incubation at 37°C, +2: 100-200 colonies growth after 6 weeks incubation at 37°C, +3: 200-500 colonies growth after 6 weeks incubation at 37°C, 4+: >500 colonies growth (confluent growth) after 6weeks incubation at 37°C

MTB 050 strains show resistance to rifampicin activity at both innoculum whereas hexane and ethylacetate fractions exhibited good bacterialcidal activity at 5 mg mL^{-1} against this rifampicin resistance strain, which serve as a good replacement for rifampicin as an anti-Tb drug. Other tested standard drugs exhibited good bactericidal activity on the two strains at both innoculum except rifampicin that show no activity on MTB 050 strain at both innoculum.

The result of the phytochemical and antimycrobacterial studies suggested the presence of active anti-Tb agent(s) from *C. brivipes* plant, a contribution to the development of new pharmaceuticals which can serve as good replacement for the existing Tb-drugs. The presence of these compounds may have accounted for the bacterialcidal activity of the various fractions of *C. brivipes* extract against *Mycobacterium tuberculosis* strains. Phenolic compounds, alkaloid and saponins are known to exhibit antibacterial activity and antioxidant activity (Ayoola *et al*., 2008; Hwang *et al*., 2001; Claus *et al*., 1990).

CONCLUSION

The active phyto-compound(s) in this present study can be isolated from various fractions of *C. brivipes* plant paving way for the discovery of novel drugs to combat the rifampicin resistant mycobacterial strain as indicated. The advantage of the extract of *C. brivipes* plant over the rifampicin and the possible mode of action calls for further work.

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