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Anti-mycobacterial and GC-MS Studies of *Irvingia gabonensis* Baill Ex. Lanen Stem Extracts

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Abstract-

Irvingia gabonensis baill ex. lanen (Bush mango) is an ethno-medicinal plant that has been used for traditional therapeutic purposes. With the increasing rate of drug resistance to various diseases in the society today, there is the need for alternative sources of drugs for the treatment of such disease. The Bush mango plant presents a potential candidate for such drugs discovery. Extracts from the plant stem were derived by cold maceration separately in methanol (MeOH) and Dichloromethane (DCM) for a period of 7 days. Phyto-constituents were also identified in extracts of stems by using hyphenated mass spectrometer and chromatographic technique, the Gas Chromatography - Mass Spectrometer (GC/MS) while the functional group of such phyto-constituents were identified with the aid of Nicolet 5700 Fourier Transform–Infra-Red spectrometer. Anti-tubercular screening was performed on extracts against clinically isolated drug-susceptible strains (DS-MTB-1 - DS-MTB-5), drug resistant strains (DR-MTB-1, DR-MTB-2) and a standard tubercular strain, H37Rv. This was controlled with drug standards, rifampicin and levofloxacin. Extracts revealed the presence of phyto-constituents such as saponins, tannin, alkaloids and phenol. It was observed that both extracts recorded high % alkaloid content at 10.37 ± 0.02 . Also, identified by FTIR as the principal part of the extracts are hydrocarbon groups such as carboxylic acid (1042), while the presence of the volatile components such as 9-Oxabicyclo [6.1.0] nonane ($C_8H_{14}O$) and 1-Chlorobutatriene (C_4H_3C) were reported by GC-MS. Extracts exhibited significant anti-tubercular activity against all organisms. Therefore, this study promotes the use of *Irvingia gabonensis* baill ex. lanen for phyto-therapeutics purposes.

Key words: Anti-tubercular; bush mango; GC-MS; infrared spectroscopy; phyto-constituents; rifampicin.

1. Introduction

You may ask why we need new TB drugs, when there are several drugs out there in the market. There are three main reasons why we need new TB drugs: there is complication in the use of the current TB drug regimens; the issue of resistance TB drug use; and the problematic interaction of current TB drugs with antiretroviral drugs taken by HIV patients [1].

So, what are we looking for in the new drugs? According to Ginsburg:



- Simplified but economical multi-drug treatments for drug sensitive TB;
- More effective with less harmfulness regimens for drug resistant TB;
- And few drug interactions for co-infection patients with HIV [1].

However, Tuberculosis (TB) is an infectious disease/ailment caused mainly by *Mycobacterium tuberculosis* that distresses the bronchitis (air passage) of all ages of individuals. According to the World Health Organisation, roughly 2 billion people died of tuberculosis from 1993 – 2016 [2]. In the year 2017, the World Health Organisation recounted that additional 10.4 million persons, (above the usual) of which 6.2 million were men, 3.2 million women and 1 million children developed TB while 1.7 million persons died during the cause of treatment, giving a figure of 4, 400 deaths daily in the year 2016 [2].

Although, majority of the peoples of the developing countries of the world still rely on traditional medicine derived mainly from medicinal plants. This is primarily because medicinal plants remain important sources for finding new active drugs or new therapeutic agents. More so, Phyto-medicines according to Mann et al., have shown promising future in the treatment of intractable infectious diseases such as tuberculosis (TB) [3]. The plant of study *Irvingia gabonensis* (Bush Mango) has been used to treat chronic cough traditionally. With these observations in mind, it was decided to extract and characterise the secondary metabolites from the stem of *Irvingia gabonensis* baill ex. lanen then determine their anti-mycobacterial potential. Extracts from plant possess bioactivities as hepatoprotective and antioxidant activities, analgesic effects, reduced intestinal motility in test animals, and can reduce plasma glucose and lipid level.

Therefore, with the failure of the Directly Observed Therapy, Shot Course Strategy (DOTS), the prevalence of multi drugs resistance tuberculosis (MDRTB) and the emergence of extensively drug resistant tuberculosis (XDR-TB) it has become necessary to look for alternative sources of drugs and the screening of herbal plants with anti-mycobacterial effect therefore needs to be paid serious attention.

2. Materials and Methods

The plant, *Irvingia gabonensis* baill ex. lanen grow wild in the covenant university's premises. The Fresh stem were collected in the early hours of the day between 6 am and 7 am on the 14th of May 2015 at 27 ± 2 °C temperature (Latitude 6.674265 6^o, 40 min., 27.36 sec., North; Longitude 3.157948 3^o, 9 min., 28.61 sec., East). The stem was separated from the leaves, cleaned and air-dried. The plant was identified by a botanist from the department of biological sciences, Covenant University Ota Nigeria and authenticated at Forestry Research Institute of Nigeria (FRIN) Ibadan. This was stored with a voucher number FHI: 110018.

2.1 Extraction

The extraction of *Irvingia gabonensis* baill ex. lanen stem was carried out using conventional method of extraction via cold maceration. The stem obtained were air-dried at room temperature and ground into powder with a mechanical grinder to enhance homogeneity and especially increase the surface area of sample with the solvent system. The powder (500g) was extracted in methanol (MEOH) and Dichloromethane (DCM) for seven (7) days before filtering. Extracts were filtered out and concentrated with a rotary evaporator as MeOH and DCM extract respectively, then stored at 2 °C for further use.

2.2 Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical screening to identify the presence of saponins, tannin, alkaloids, phlobatannin, flavonoids, triterpenoids, cardiac glycoside, oxalate, quinone, phenol and coumarins as described in literatures [4] [5] [6].

2.3 Quantitative Phytochemical Screening

2.3.1 Alkaloid Determination

The mixture of 2.0 g of each of the extracts and 100 mL of 10% acetic acid in alcohol was poured into a 250 mL beaker and covered with a glass petri-dish. This remained at a room temperature for 4 hours. The solution was then sifted and concentrated by evaporating over a water bath to one-quarter of its original volume. Dropwise addition of concentrated ammonium hydroxide was until there was complete precipitation of alkaloid. A weighed filter paper was used to recover the alkaloid precipitate by filtration and washed with 1% ammonium hydroxide and dried in an oven at 60 °C for 30 minutes, cooled in a desiccator and reweighed [7]. The difference in weight is the crude alkaloid present. The procedure was repeated twice while the average weight of alkaloids was determined from the differences and reported in percentage weight of the sample analysed as shown here:

$$\% \text{ Alkaloid} = \frac{Wt_2 - Wt_1}{Wt} \times 100 \quad \dots \dots \dots \text{Eq. 1}$$

Where;

Wt = Weight of Sample

Wt₁ = Weight of Empty Filter Paper

Wt₂ = Weight of Filter Paper + Alkaloid Precipitate.

2.3.2 Tannin Determination

0.5 g of the extract was added alongside with 50.0 mL of distilled water into plastic bottle and shaken together for a period of one (1) hour via a mechanical shaker. Then the extract was sieved into a 50.0 mL volumetric flask with the filter folded into a glass funnel. The filtrate was then made up to mark. 5.0 mL of the filtrate was pipetted into a 'test tube' and added to it was 3.0 mL of 0.1M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance of the mixture was read in a spectrophotometer at 720 nm wavelength within 10 minutes. A blank sample was also prepared which was read at the same wavelength for the same period of time. 100 ppm of already prepared tannin acid was used as standard [6]. This experiment was repeated twice and an average was taken as the tannin content was calculated as shown below.

$$\% \text{ Tannin} = \frac{\text{Sample absorbance} - \{\text{Blank absorbance} \times \text{Conc. of Standard}\}}{\text{Standard absorbance} - \text{Blank absorbance}} \times 100 \quad \dots \text{Eq. 2}$$

2.3.3 Flavonoid Determination

10.0 g of the dried extract was extracted repeatedly with 100.0 mL of 80 % aqueous methanol at room temperature. This was then filtered with a filter paper (Whatman No 42 125 mm). The filtrate obtained was transferred into a properly washed, dried and weighed crucible and then evaporated to dryness over a water bath, and weighed to a constant weight. [8]. This experiment was done twice and the average was determined.

The quantity of flavonoid was determined using this formula:

$$\% \text{ Flavonoid} = \frac{Wt_2 - Wt_1}{Wt} \times 100 \quad \dots \dots \dots \text{Eq. 3}$$

Where;

Wt = Weight of Sample

= Weight of Empty Filter Paper

Wt₁ = Weight of Filter Paper + Flavonoid Precipitate

Wt₂

2.3.4 Saponin Determination

200.0 mL of 20% ethanol was poured in a beaker after which 20.0 g of the sample extract was added. The suspension was heated in hot water bath at about 55 °C. and it was continuously stirred for 4 hours. The mixture was filtered and the residue re-extracted again with 200.0 mL of 20 % ethanol. “The combined extracts were further reduced to 40.0 mL over a water bath at 50 °C. The concentrate was transferred into a 250.0 mL separating funnel and 20.0 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60.0 mL of n-butanol. The combined n-butanol extracts were washed twice with 10 mL of 5 % aqueous sodium chloride. The resultant solution was heated over a water bath” [9]. The remaining solution, after evaporation, was dried in an oven at 60°C using a pre-weighed evaporating dish and reweighed as the saponin content. The difference in weight is the quantity of saponin present [10]. This experiment was repeated twice and an average was determined. The saponin content was calculated in percentage:

$$\% \text{ Saponin} = \frac{Wt_2 - Wt_1}{Wt} \times 100 \quad \dots \dots \dots \text{Eq. 4}$$

Where; Wt = Weight of Sample

= Weight of Empty Filter Paper

Wt₁ = Weight of Filter Paper + Saponin Precipitate

Wt₂

2.3.5 Phenol Determination

According to Dewanto, 2.0 g from the extracts was defatted with 100.0 mL of diethyl ether using a Soxhlet apparatus for 2 hours [11]. The fat-free extract was boiled for 15 minutes with 50.0mL of ether in order to extract the phenolic component. 5.0 mL of the extract was pipetted into a 50 mL volumetric flask and 10.0 mL of the distilled water was added. 2.0 mL of ammonium hydroxide and 5.0 mL concentrated amyl alcohol were also added to the mixture in the flask. The solution mixture was then made up to mark and left to react for 30 minutes for colour development. The absorbance of the coloured solution was read at 505 nm wavelengths using a UV spectrophotometer. The standard solution of phenol was prepared using the same treatment. A blank was prepared by adding the reagents in the same proportion without phenol and the value of phenol in the sample was calculated. This procedure was repeated for the second time and an average was determined. The phenol content was calculated in percentage thus: