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Preliminary Qualitative Analysis for Cancer Chemopreventive Agents in *Irvingia gabonensis* Baill, *Alstonia boonei* De Wild. and *Bridelia ferruginea* Bth. Stem Bark from South West, Nigeria

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Abstract: Presently, researchers have been given remarkable attention to complementary and alternative medicine to deal with cancer treatment from medicinal plants mainly due to its fewer side effects and ease availability. The leaves of four plant parts commonly used in Nigeria namely *Irvingia gabonensis* (Yoruba name: *Oro*), *Alstonia boonei* (Yoruba name: *Ahun*) and *Bridelia ferruginea* (Yoruba name: *Ira odan*) stem bark were each screened for the presence of known potential chemopreventive agents using paper chromatography, thin layer chromatography and various chemical tests. All four plants showed the presence of phenolic compounds, alkaloids, flavonoids, phytosterols, tannins, saponins and glycosides. All the plants showed traces of chlorophyll.

Key words: *Irvingia gabonensis* · *Alstonia boonei* · *Bridelia ferruginea* · Chemopreventive Agents

INTRODUCTION

Cancer is a dreaded disease which is best characterized by abnormal cell division andiscaused by mutation of genes involved in the control of cell division. Cancer grows outof normal cells in the body. Normal cells multiply when the body needs them and die when the body doesn't need them [1]. Chemoprevention is one of the strategies employed in cancer control and has evolved into one of the most exciting and promising area in cancer research [2]. Chemoprevention involves use of natural and synthetic compound to inhibit, reduce or reverse carcinogenesis [3]. A large number of potential chemopreventive agents have been identified from epidemiological surveys, experimental, pre-clinical and clinical observation and structural homology with known chemopreventive agents [4, 5]. Chemopreventive agents are compounds that prevent development of cancer. Their preventive effects are attributed to Jemal *et al*. [6] intervening in interaction of the carcinogen with cellular DNA, altering intracellular signalling pathways as results of stopping progression of an initiated cell through pre-neoplastic changes into a malignant cell [7], inhibiting angiogenesis [8], inducing cell cycle arrest [9] and triggering apoptosis [10]. It is believed that the apoptosis induced by chemopreventive agents may not only inhibit the carcinogenesis induced by carcinogens, but also may suppress the growth of tumor and enhance the cytotoxic effects of antitumor drug on tumor, which plays a pivotal role in the antitumor therapies [11]. Dietary constituents especially from plant foods have been found to protect against the occurrence of cancer [12]. Some of these naturally occurring compounds are phytochemicals. Use of medicinal plants in chemoprevention is considered as an ideal treatment with good efficacy and few side effects compared with allopathic medicine [8]. Mounting evidences have shown that dietary intake of phytochemicals, an important group of chemopreventive agents, reduces the risk of cancer [9] and has antitumor potential against cancer [10]. The mechanisms underlying chemoprevention involves antioxidant, anti-inflammatory, immune-enhancing and anti-hormone effects. This preliminary study is intended to identify possible potential chemopreventive agents in these commonly used Nigerian plant parts namely *Irvingia gabonensis*, *Alstonia boonei*, and *Bridelia ferruginea* stem bark as a prelude to conducting more extensive studies on them.

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MATERIALS AND METHODS

Plant Materials: The stem bark of four locally commonly used plant parts namely *Irvingia gabonensis*, *Alstonia boonei* and *Bridelia ferrugineas*tem bark were bought from the local market atAdo-Ekiti metropolis in Ekiti State, Nigeria.

Preparation of Plant Materials: The stem bark of the four plant samples were picked to remove infected ones and air dried at room temperature. The dried stem bark were then ground to a coarse powder and stored in amber bags at room temperature.

Phytochemical Screening: Phytochemical screening for potential chemopreventive agents was done according to various screening methods [13, 14].

Test for Phenolic Substances: Plant material (5 g) was stirred with50 mL of 2M HCl and heated in a waterbath at 100°C for 30 min, cooled and filtered. The filtrate was extracted into 30mL of ether and the extract was concentrated to dryness. Residue was dissolved infew drops of chloroform and chromatographed one dimensionally on silica gel plate (20 cm x 20 cm) in acetone-chloroform (1:9). Phenolic substancesshow a variety of colours including blue, gray, red and pink.

Test for Flavonoids: Plant material (5g) was hydrolysed by heating in 2M HCl at 100°C for 30 min, cooled and filtrate was extracted in 30 mL ethyl acetate and the extract was concentrated to dryness. Residue was dissolved in few drops in Forestal-butanol-acetic acid-water (BAW) and phenol-water (phOH-H_2O)solvents. Flavones and glycosyl flavones give varying yellow colours while biflavonyls give brown colours. Also, 1 mL of dilute ammonia solution was added to about 2 mL of the ethyl acetate extract and shaken. A yellow colour at the lower ammonia layer is a positive indication for flavonoids.

Test for Tannins: Plant tissue (1g) was extracted in 50 mL of distilled water and filtered. The filtrate was concentrated and chromatographed on silica gel in chloroform-acetone-formic acid (75:16.5:8.5). Also, 1 g of plant tissue was boiled in 10 mL of 45% ethanol for 3 min, cooled and filtered. Filtrate was divided into three portions. To one portion was added few drops of lead subacetate and observed for formation of gelatinous precipitate. To another portion was added 1 mL of bro-mine water and observed for pale brown portion precipitates. The last portion was diluted with distilled water and few drops of ferric chloride added and observed for a transient greenish to black colour.

Test for Saponins: Plant tissue (5 g) was extracted with 25 mL methanol for 5 min at 50°C in a water bath. Filtrate was evaporated and mixed with 2.5 mL water and extracted with 15 mL of butanol and the butanol layer was then chromatographed on silica gel in chloroform-methanol-water. Also 1 g of plant tissue was boiled with 10 mL of distilled water for 5 min and decanted while still hot. 2 mL of the filtrate was diluted with 8 mL water and vigorously shaken and observed for a stable froth. To another 2 mL of filtrate was added 4 drops of olive oil, shaken and observed for formation of emulsion.

Test for Alkaloids: Plant tissue (5 g) was mixed with 10 mL of 10% ammonia solution and extracted with 25 mL ethanol at 60°C for 15 min. The extract was concentrated and chromatographed on silica gel in ethyl acetate-methanol-water (100:13.5:10). Blue or yellow fluorescence and brown ororange spots with Dragendorff's reagent are positive. Also 1 g of plant tissue was boiled with 5 mL of 2% Hcl and filtered. The filtrate was divided into four portions each of which is added 2 drops of Mayer's reagent, Dragendorff's reagent, Wagner's reagent and picric acid solution to observe cream coloured, orange coloured, red-dish-brown and yellow precipitate respectively.

Test for Glycosides: Plant tissue (5 g) was mixed with 50 mL of water and the solution heated for 5 min in a water bath, filtered and the filtrate was divided into two portions. Fehling solutions (1 mL) were added to 10 mL of the filtrate until alkaline and heated for 3 min. A brick red precipitate is positive. For cyanogenic glycosides, 5 g of tissue was mixed with 20 mL of water and heated in water bath with sodium picrate paper. A change from yellow to orange is positive. For cardiac glycosides, 5 g was extracted with 29 mL of water and filtered. The filtrate was evaporated and residue dissolved in 10 mL glacial acid with 3 drops of ferric chloride.

Test for Anthocyanadins and Chalcones: Plant material (5 g) was hydrolysed in HCl for 30 min at 100°C. Hydroxylase wasfiltered and the filtrate was washed twicewith ethyl acetate. The aqueous layer was heated at 80°C to remove all the ethylacetate and extracted into amyl alcohol. The extract was concentrated todryness. Residue was dissolved in few drops of methanolic-HCl and

chromatographed on silica gel and paper in BAW and water. Anthocyanidins give red, purple and magenta colours while chalcones give red andbrown colours.

Test for Phytosterols: Plants tissue (5 g) was defatted three times with ether and extracted into hot methanol and concentrated to a small volume. Sample was chromatographed on silica gel in chloroform and detected with 50 % H_2SO_4. Brown and violet colours are positive indicators.

Test for Chlorophyll: All procedures are carried out in the dark. 2 g of fresh plant tissue was ground and extracted with 80% acetone and $CaCO_3$ (To prevent formation of pheophytin) until all the colour was released from the tissue. The extract was made up to100 mL with acetone and stored in an amber bottle in a refrigerator. The absorbance was read at 645 nm, 652 nm and 663 nm and the following calculation was made:

$$\text{Total Chlorophyll} = \frac{100 \times A652nmor278 \times A652nm}{36}$$

RESULTS

A summary of the screening results of the four local plants is presented in Table 1. The four plants showed the presence of phenolic substances. All the samples indicatedthe presence of flavonoids. All the plant samples showed the presence of phytosterol and chlorophyll. Tannins were found to be present in all the samples, with *B. ferruginea* having higher levels than *I. gabonensis* and *A. boonei*. All the samples showed the presence of saponins with *B. ferruginea* having higher levels than *I. gabonensis* and *A. boonei*. All the samples indicated the presence of alkaloids with *B. ferruginea* been the least. Generally, glycosides were found to be present in all the samples with *B. ferruginea* showing very slight traces. All the samples were positive for cyanogenic glycoside while all were seen to contain cardiac glycosides. Anthracene glycosides were absent in all the samples. All the samples showed presence of anthocyanidins or chalcones. However, traces of chlorophyll were found in all the samples.

Table 1: Result of qualitative phytochemical screening for potential chemopreventive agents in *Irvingia gabonensis*, *Alstonia boonei* and *Bridelia ferruginea* Stem bark.

Potential chemopreventive agents	*Irvingia gabonensis*	*Alstonia boonei*	*Bridelia ferruginea*
Phenolic compounds	+++	+++	+++
Alkaloids	+++	+++	+
Tannins	++	++	+++
Saponins	++	++	+++
Glycosides	++	++	+
Flavonoids	++	++	++
Phytosterols	+	+	+
Anthocyanidins/chalcones	+	+	+
Chlorophyll	+	+	+

+ indicates presence; - indicates absence

DISCUSSION

Phytochemicals are biologically active non-nutritive chemical compounds that occurnaturally in plants. They are found as a substance responsible for the health-promoting properties of varieties of natural and functional foods due to their ability to alter cell communication and DNA repair and influence cell processes that cause development of cancer and other diseases [15]. The concept of chemoprevention hasassumeda global significance as a result of its acceptance in the management,prevention and treatment of a wide range of life threatening diseases such as cancer, diabetes and coronary diseases and in the maintenance of good health [16]. The benefits to public health whichthe lowering of the incidence of cancer can generate are invaluable. Anumber of chemopreventive agents identified in plants have considerably contributed to the concept of cancer prevention and control [17]. Theplants investigated namely *Irvingia gabonensis*, *Alstonia boonei* and *Bridelia ferruginea* are common tropical plants found in South Western Nigeria. The preliminary screening of these plants using simple chemical tests, paper chromatography showed that they contain some known potential cancer chemopreventive agents such as flavonoids, tannins, chalcones, anthocyanidins, phytosterol, chlorophyll, saponins, glycosides and alkaloids. The presence of these bioactive phytochemicals has been reported in several African plants [18]. Phytosterolswhich are terpenoid compounds have been reported to inhibit certain cancers in animals [19]. Flavoniods, anthocyanadins and chalcones are ubiquitous phytochemicals with pronounced bioactivity. Some of these compounds such as apigenin and licochalcone have showed chemopreventive properties against several cancers [20-22]. Flavonoids have antioxidant activities and health promoting factors such as anti-allergic, anti-cancer, anti-oxidant, anti-inflammatory, anti-thrombotic, vasoprotective, tumour inhibitory and anti-viral effects. These factors

have been associated with the influence of flavonoids on arachidonic acid metabolism. Saponins such as diosgenin have been found to have therapeutic and chemopreventive effects [23]. Tannins consumed in large quantities as fruits and vegetables on a daily basis such as ellagitannins are also effective against some cancers [24]. In addition, it has been documented that saponins have anti-tumour and anti-mutagenic activities and can lower the riskof human cancers, by preventing cancer cells from growing. Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability [25]. Plants produce saponins has potentials to fight infections by parasites and in humans saponins serves as immune system booster and protect againstviruses and bacteria. The non-sugar part of saponins has a direct antioxidant activity which may result in reduced risk of cancer and heart diseases [26]. The chemopreventive properties of alkaloids and glycosides have also been reported [27, 28]. Chlorophylin found in chlorophyll produces an anti-promoting effect on skin cancer in mice [29]. These chemopreventive agents are able to enhance host protective systems such as detoxification enzymes against carcinogens.

CONCLUSION

This findings show thatthese plants would have greater protection against specific carcinogenic compounds. Further investigation sinvolving specific characterization of these potential chemopreventive agents andtheir effects in animal models are being carried out.

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