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EVALUATION OF MUTAGENICITY, ANTIMUTAGENICITY, CYTOTOXICITY AND COMPOUND IDENTIFICATION OF *PHILENOPTERA VIOLACEA (KLOTZSCH) SCHRIRE* AND *XANTHOCERCIS ZAMBESIACA (BAKER) DUMAZ-LE-GRAND* METHANOLIC EXTRACTS *IN VITRO*

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Abstract

Philenoptera violacea (klotzsch) Schrire and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* methanolic *extracts* mutagenic and antimutagenic properties were determined by Ames test using *Salmonella typhimurium* strains TA98 and TA100 without S9. The cytotoxic effects of the extracts were tested by Sulforhodamine B assay on the WI38 cell line. Radioprotective effect of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* was determined using the clonogenic assay. Results revealed that both plants do not have mutagenic effects against all tester strains without the presence of metabolic activation and with no direct carcinogenic potential to human cells. Liquid chromatography-mass spectrometry analysis showed the presence of two unknown compounds from *Philenoptera violacea (klotzsch) Schrire.* Based on clonogenic cell survival, *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* can lead to over 50% reduction in cell death. We therefore conclude that both plant extracts can be considered safe as the absence of mutagenicity indicates lack of toxicity and this was also confirmed by the negative cytoxicity screening.

Key word: *Philenoptera violacea (klotzsch) Schrire*, *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand*, Ames test, Sulforhodamine B (SRB) assay, cytotoxicity

Introduction

Numerous medicinal plants are traditionally used in the treatment of a variety of diseases by African population. Among the plants used for therapeutic purposes are *Philenoptera violacea* (klotzsch) Schrire and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* species, which both belong to the Fabaceae family [1]. *Philenoptera violacea* (klotzsch) Schrire has traditionally been used to treat gastro-intestinal problems, powdered root-bark for colds and snakebite treatment, earache treatment, root infusions as hookworm remedy and most part of the plant to treat diarrhoea [2-3]. *Xanthocercis zambesiaca* has been traditionally used to treat diabetes mellitus and tuberculosis [4] and literature did support its antihyperglycemic activity [4] and anti-tuberculosis activity [5]. Although plants used medicinally are widely assumed to be safe by its users, the issue of quality control may be addressed. These plant extracts should consequently be examined carefully for their safety perspective and caution to their use should be taken.

Plant polyphenols are important components of human diet and a number of them are considered to possess chemopreventive and therapeutic properties against cancer [6]. A large number of plants containing antioxidant phytochemicals are reported to be radioprotective in various model systems [7]. Most plants with antioxidant properties have shown anticarcinogenic activities in several in vitro and in vivo assays [8-11]. They have also shown the capacity to modulate radiation-induced damage by means of various biological endpoints, by radiosensitizing tumor cells while radioprotecting non-cancerous cells [6]. This suggests that, besides the anticarcinogenic effect, another possible application of the antioxidant capacity of polyphenols from plants could be based on their radioprotective activity. Xanthocercis zambesiaca (Baker) Dumaz-le-Grand showed the ability to scavenge free radicals thus its radioprotective effect was evaluated on radiation-induced human prostate cancer cell lines at a dose of 2 Gy and *Philenoptera violacea (klotzsch) Schrire* showed less potential to scavenge free radicals [12], thus it was excluded in the determination of radioprotective effect. Therefore, the present study aimed at (1) investigating the mutagenicity, antimutagenicity, (2) investigating the cytotoxicity effects, (3) determination of the chemical composition of both plants extracts, and (4) evaluating radioprotective activity of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand*.

Methods

Plant material

The plant materials *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* were authenticated by scientists at the National Botanical Gardens in Bloemfontein South Africa (MAS002). The collected materials were dried at room temperature and pulverized by mechanical mills and weighed. It was then stored in a cool place until analysis.

Extraction method

The extraction was done using soaking method. Plant material (10g of the dried twig, leaves and flowers of each plant) was weighed, pulverized and soaked with methanol for 72 hours with occasional stirring. The extracts were filtered and new solvent was added again for more extraction until the solvent remains clear. The methanol solvent was removed completely by rotator evaporator.

Sterilization

Autoclavable materials such as agar and broth were aseptically sterilized in an autoclave at 121ºC for 15 minutes. Petri dishes, beakers, McCartney bottles, pipette, test tubes, filter papers and other metal apparatus such as spatula and forceps were sterilized using hot air oven at a temperature of 160ºC for 1 hour. The wire loops were sterilized by heating on the blue flame of the Bunsen burner until red-hot and allowed to cool and 70% alcohol was used to swab/clean the work bench area to prevent contamination. The process was carried out aseptically.

Preparation of culture media and organisms

All culture media were prepared according to manufacturers' instructions, agar was dissolved by boiling with the microwave and autoclaved at 121ºC for 15 minutes. The bacterial strains used for mutagenicity testing are the histidine-requiring *Salmonella typhimurium* tester strains TA98 (detects frameshift mutagens) and TA100 (detects mutagens that cause base-pair substitution) without metabolic activation.

Mutagenic and antimutagenic screening

The sample residues were dissolved in DMSO to a known concentration (5000, 500 and 50 μg/ml) prior to biological activity testing. The potential mutagenic effects of the investigated extracts were detected using the Ames test. The Ames assay was performed with *Salmonella typhimurium* strains TA98 and TA100 using the plate incorporation procedure

described by Maron and Ames [13]. Briefly, One hundred microliters of bacterial stock was incubated in 20 ml of Oxoid Nutrient for 16 h at 37ºC on an orbital shaker. The overnight culture (0.1 ml) was added to 2ml top agar (containing traces of biotin and histidine) together with 0.1 ml test solution (test extract, solvent control or positive control) and 0.5 ml phosphate buffer (for exposure without metabolic activation). The top agar mixture was poured over the surface of the agar plate and incubated for 48 h at 37ºC. After incubation, the number of revertant colonies (mutants) was counted. All cultures were made in triplicate (except the solvent control where up to five replicates were made) for each assay. The positive control used was 4-nitroquinoline-1-oxide (4-NQO) at a concentration of 2 µg/ml. Results were expressed as mean ± standard deviation and are based on number of induced revertant colonies.

Cytotoxicity screening

The cytotoxicity screening was analysed using SRB assay which was performed at CSIR; Pretoria, South Africa in accordance with the protocol of the Drug Evaluation Branch, NCI, and the assay has been adopted for this screen. The WI-38 cell line - normal Human Fetal Lung Fibroblast from European Collection of Cell Cultures (ECACC) was routinely maintained as a monolayer cell culture at 37ºC, 5% CO_2, 95% air and 100% relative humidity in Eagle's minimal essential medium (EMEM) containing 10% fetal bovine serum, 2 mM L-glutamine and 50µg/ml gentamicin. For screening experiment, the cells were inoculated in a 96-well microtiter plates at plating densities of 10 000 cells/well and were incubated for 24 h. After 24 h the cells were treated with the experimental drugs which were previously dissolved in DMSO and diluted in medium to produce 5 concentrations. The plates were incubated for 48 h after addition of the compounds. Viable cells were fixed to the bottom of each well with cold 50% trichloroacetic acid, washed, dried and dyed by SRB. Unbound dye was removed and protein-bound dye was extracted with 10mM Tris base for optical density determination at the wavelength 540 nm using a multiwell spectrophotometer. The IC50 was then calculated. Data analysis was performed using GraphPad Prism software 50% of cell growth inhibition (IC50) and was determined by non-linear regression. Cells without drug addition served as control, the blank contained complete medium without cells and Etoposide was used as a standard.

Identification of active compounds with LC-MS

LC–MS/MS analysis for the identification of active compounds from *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* methanol crude extracts was carried out using the Waters Synapt G2 instrument. This is an Agilent 1100 LC system consisting of degasser, binary pump, auto sampler and column heater. The column outlet was coupled to an Agilent MSD Ion Trap XCT mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out on a personal computer with Acquity binary solvent manager instrument system. For the chromatographic separation, Waters UPLC on a Waters BEH C18, 2.1x100 mm column was used. The column was held at 95% solvent A (0.1% formic acid in water) and 5% solvent B (0.1% formic acid in acetonitrile) for 1 min, followed by an 11 min step gradient from 5% B to 100% B, then it was kept for 4 min with 100% B; finally, the elution was achieved with a linear gradient from 100% B to 5% B for 2 min. The flow rate was 0.4 ml/min and the injection volume was 0.01 ml. The following parameters were used throughout all MS experiments: for electrospray ionization with negative ion polarity the capillary voltage was set to 3 kV, the drying temperature to 350^0C and cone voltage of 15 V, the maximum nebulizer pressure to 15000 psi and the seal wash was 5 min. The total run time was 15 minutes, the scan speed was 26 000 m/z/s (ultra-scan mode) and the lock mass was Leucine enkaphelin. The phenolics were identified using a combination of HPLC with diode array detection and liquid chromatography with atmospheric pressure chemical ionization mass spectrometry (ESI-LC/MS/MS) on the basis of their ultraviolet spectra, mass spectra and by comparison of the spectra with those of available authentic standards.

Clonogenic assay for Radioprotective effect

Human prostate cancer cells (DU145) were obtained from Professor P Bouic (Synexa Life Sciences, Montague Gardens, South Africa), and grown in Minimum Essential Medium (Sigma-Aldrich, Germany) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, UK), penicillin (100 U/ml) and streptomycin (100 µg/ml) (Lonza, Belgium). Cultures in exponential growth were trypsinised to give single-cell suspensions, plated in triplicate (400–1500 cells per flask) into 25 cm^2 tissue culture flasks (Greiner Bio-One, Frickenhausen, Germany) and incubated for 3 hours to allow the cells to attach. To confirm the free radical scavenging features of extract *Xanthocercis zambesiaca (Baker)*

Dumaz-le-Grand, cell cultures were irradiated to 2 Gy in the presence of the extract at a concentration of 0.1 mg/ml, and were subsequently incubated at 37°C in a humidified atmosphere (95% air and 5% CO_2) for 10 days to allow for colony formation. The cells were irradiated using a ^{60}Co γ-irradiation source at a dose rate of 0.827 Gy/min. Colonies were fixed in glacial acetic acid:methanol:water (1:1:8, by volume), stained with 0.01% amido black in fixative, counted, and the cell surviving fraction [SF2 (with *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* extract)] was determined. Another batch of cell cultures was irradiated to 2 Gy in the absence of extract and the corresponding surviving fraction [SF2 (no *Xanthocercis zambesiaca* extract)] was obtained. To evaluate the radioprotective capacity of the extract, an RPF was derived as:

$$RPF = \frac{SF_2 \text{ (with } \textit{Xanthocercis zambesiaca}\text{)}}{SF_2 \text{ (without } \textit{Xanthocercis zambesiaca}\text{)}}$$

Statistical analysis

All analyses were run in triplicate and the results expressed as mean ± standard deviation (SD). Fifty percent of cell growth inhibition (IC50) was determined by non-linear regression. Data analysis was performed using Graph Pad Prism software.

Results

Mutagenic and anti-mutagenic screening results

(Table1)

Cytotoxicity screening results

(Figure 1)

Identification of active compounds with LC-MS

(Figure 2, Figure 3, Table 2, Table 3)

Radioprotective effect results

Based on the clonogenic assay, the cell surviving fractions for DU145 at 2 Gy with and without *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* were found to be 0.23 ± 0.13 and 0.15 ± 0.10, respectively. The corresponding RPF value emerged as 1.53 ± 0.57, indicating that an extract of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* can lead to over 50% reduction in cell death. This confirms the free radical scavenging features of the extract and suggests that it might be useful as a radioprotector.

Discussion

Mutagenicity and antimutagenicity activity

People have been relying on traditional medicine since ancient times but as some natural plants are toxic and can be carcinogenic through a process of mutation, there is a need to determine the safety of these traditional medicines. Traditional medicine can be described as unsafe until proven to be safe. People can use traditional medicine for years without knowing the toxicity or damage the medicine is doing to them. Compounds from secondary plant metabolism can be beneficial to human beings, but, some are toxic and makes mutagenicity effect [17] when used by human population. Gómez-Arroyo et al., demonstrated a high correlation between mutagenicity and carcinogenicity [18]. The Ames mutagenicity test is a short term bacterial reverse mutation assay specifically designed to detect a wide range of chemical materials that can produce genetic damage that leads to gene mutations [19]. The Ames test can play a central role in a program of prevention to identify mutagenic chemicals and to aid in the development of non-mutagenic products to prevent future human exposure [20].

The Ames assay is commonly used to detect mutagenic and antimutagenic activities and is a widely accepted method for identifying different chemicals and drugs that can cause bacterial reverse mutations. These gene mutations act as hot spots for mutagens that cause DNA damage via different mechanisms. When the *Salmonella* tester strains are grown on a minimal media agar plate containing a trace of histidine, only those bacteria that revert to histidine independence are able to form colonies [21]. In this study, we investigated the mutagenic and antimutagenic activities of *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* by the *S. typhimurium*/microsome assay, without addition of an extrinsic metabolic activation system [22].

The Ames test is a well-known bacterial mutagenicity test [21, 23]. In this test, reverse His− →His+ mutations is visualised by plating *Salmonella typhimurium* bacteria in a histidine-poor growth mutants that are able to form medium. In this medium only, His+ will able to form visible colonies. Different bacterial strains are used to identify different types of mutations. In this study, bacterial strains used for mutagenicity testing are the histidine-requiring *Salmonella typhimurium* tester strains TA98 and TA100 without metabolic activation. Strain TA98 gives an indication of frame-shift mutations, while strain TA100 indicates base-pair substitution. The colonies were counted to determine the mutagenic and antimutagenic activity of these two plant extracts. For a substance to be considered mutagenic in the Ames test, the number

of revertant colonies on the plates containing the test compounds should be more than twice the number of colonies produced on the solvent control plates. In addition, a dose-response should be evident for the various concentrations of the mutagen tested. Results obtained from the mutagenicity test of *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant are expressed as mean ± S.E.M (Table 1) and are based on number of induced revertant colonies.

Substances are considered active or mutagenic if the number of induced revertant colonies is twice the revertant colonies of the negative control [13]. From our results (Table 1), the numbers of revertant colonies on the plates containing the test compounds were not more than twice the number of colonies produced on the solvent control plates. Thus, neither *Philenoptera violacea (klotzsch) Schrire,* nor *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* were mutagenic in the Salmonella tester strain TA 100 and TA98. Both plants extracts showed positive antimutagenic activity as they repressed the growth of strain TA98 which gives an indication of frame-shift mutations and of strain TA100 which indicates base-pair substitution. Phenolics are used as antimutagenic and anti-inflammatory agents due to their strong antioxidant properties [24]. Based on research findings by Ntsoelinyane et al., the antimutagenicity activity of *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* extracts can be due to the presence of phenolic compounds in these plant extracts [12].

Cytotoxicity activity

The SRB assay is used to measure drug-induced cytotoxicity and cell proliferation. Its principle is based on the ability of the protein dye sulforhodamine B (Acid Red 52) to bind electrostatically in a pH-dependent manner to protein basic amino acid residues of trichloroacetic acid-fixed cells. Under mild acidic conditions it binds to the fixed cellular protein, while under mild basic conditions it can be extracted from cells and solubilized for measurement. The results of five dose screening were reported as 50% of cell growth inhibition (IC50). The biological activities were separated into 4 categories: Low Hazard (IC50 >100 µg/ml), weak Hazard (30 µg/ml < IC50 <100 µg/ml), moderate Hazard (5 µg/ml < IC50 <30 µg/ml) and High Hazard (IC50 <5 µg/ml). According to criterion CSIR cytotoxicity criteria, *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant extracts can be considered as Low Hazard as the parameter IC50 for WI-38 cell line was higher than 100 µg/ml. While Etoposite can be considered as High Hazard as the parameter IC50 for WI-38 cell line was less than 5 µg/ml. From these results (Figure 1), we can conclude that *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant extracts do not have cytotoxic activity on normal cells.

Identification of compounds

There are two classes of compounds from plants, namely primary and secondary metabolites. Primary metabolites are required for the sustenance of the plant, while secondary metabolites are not a necessity for the plant's survival [25]. The latter are mostly compounds that are active for treatment and prevention of diseases in humans and animals and are thus termed bioactive compounds. Bioactive compounds are generally unique to individual plant species and are thus species-specific, but some may be found in several or many plant species of a genus, in several related genera, or even families [26]. The crucial factor for definitive accomplishment of isolating bioactive plant constituents is the selection of the "right" plant and the relevant part of the plant, which contains the active compounds. As distillate, certain secondary metabolites in specific organs and variation in bioactivity are often encountered in different parts of the same plant [27]. In this study, LCMS techniques have been employed to investigate the phenolic content of *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* methanol plant extracts. This was aimed at identifying only compounds present in these plant extracts, but not to isolate and re-analyze the activity of the isolated active compounds. Fragmentation of the compounds by the mass spectrum was performed to obtain the molecular chemical formula of the compounds that formed significant peaks. The relative retention times and mass spectra of the extract components were compared with those of authentic samples and with mass spectra from a data library. As shown in Figure 2, LC-MS analysis of the *Philenoptera violacea (klotzsch) Schrire* methanol extract resulted in the identification of nine compounds, with more than 90% similarity with the standard mass spectra in the library. The LC-MS analysis of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* extract shown in Figure 3 resulted in the identification of seven compounds, also with more than 90% similarity with the standard mass spectra in

the library. In short, for *Philenoptera violacea (klotzsch) Schrire* LC-MS analysis, the first five components with the greatest peak area to be eluted were at 2.24 min till 3.32 min (peak 1 to peak 5), which was in the more hydrophilic region (short retention time). The other four main components eluted at 6.47 min (peak 6), 7.81 min (peak 7), 6.87 min (peak 8) and 8.04 min (peak 9) in the more hydrophobic region (longer retention time). Then, for *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* LC-MS analysis, the first three components were eluted in the more hydrophilic region, with the greatest peak area at 2.75 min till 3.69 (peak 1 to peak 3) and the other four main components eluted in the more hydrophobic region at 4.93 min till 6.84 min (peak 4 to peak 7). The deprotonated molecule mass (m/z) measured by the MS in ESI negative mode was used to determine the molecular formula of the compounds extracted. The tentative names of these identified compounds were determined using the molecular formula and the molecule mass, using the literature [15]. Previous investigations of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* revealed the presence of 7,8-dioxy-isoflavonoids, 2-benzylbenzo[*b*]furan-3(2H)-ones [28], along with the isolation of novel isoflavones, 7,8,3'-trihydroxy-4'-methoxyisoflavone and α,3,4,4'-tetrahydroxy-2'-methoxydihydrochalone [29]. However, these were isolated from the heartwood of the plant and it is known that different parts of the plant may contain dissimilar compounds. *Xanthocercis zambesiaca* leaf extract was demonstrated to have five compounds, namely fagomine, 3-*epi*-fagomine, 3,4-di-*epi*-fagomine, 3-*O*-β-d-glucopyranosylfagomine and 4-*O*-β-d-glucopyranosylfagomine [30]. The two compounds mentioned last were identified as new natural products. Fagomine is a good inhibitor of isomaltase and certain α- and β-galactosidases, whereas 3-*epi*-fagomine is a more potent inhibitor of isomaltase and β-galactosidases than fagomine, but does not inhibit α-galactosidase. Compound 3,4-di-*epi*-fagomine exhibited no significant inhibition against the glycosidases used [30]. *Xanthocercis zambesiaca* was also identified as having diarylpropanes such as diarylalkanes and/or diarylalkanols, which have the capability of inhibiting binuclear enzyme function, particularly tyrosinase, which prevents melanin overproduction. When one considers that a single plant may contain up to thousands of phytoconstituents, the possibilities of making new discoveries become self-evident. Our phytochemical investigation of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* and *Philenoptera violacea (klotzsch) Schrire* (mixture of leaves, twigs and flowers) has resulted in the identification of other compounds of which the presence has not been recorded before from these specific parts of the plants.

The following compounds were identified from this study (Table 3): 4-Acetoxy-3,5-dimethoxybenzoic acid, 2″-O- *β –D-Apriofuranosyl, 6*″-O-α-L-*rhamnopyranosyl*, Quercetin-3-*O*-[α-L-rhamnopyranosyl(1→6)]-*β –D*-galactofuranoside, 3-O- *β –D-Galactopyranosyloxy-3' .4' .5.7-tetrahydroxyflavone. Quercetin 3-galactoside, 7-O-[α-L-Rhamnopyranosyl-(1→6)- β-D-glucopyranoside] or 6,8-Bis(C-β-glucosyl)-apigenin, 3-O-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside], 20-O-α-L-rhamnopyranoside* and 2 unknown compounds from *Philenoptera violacea (klotzsch) Schrire,* and 7 compounds: *Apigenun-6-C-α-L-arabinopyranosyl-8-C-β –L-arabinopyranoside*, *Metaplexigenin-3-O- β-D-oleandropyranosyl-(1→4)- β-D-digitoxopyranosyl –(1→4)- β-D-digitoxopyranoside*, *Arvenin I, 3-O- β-D-Glucuronopyranoside: (Cloversaponin II), Apigenin-4'-O- β –D-xylofuranodyl(-(1→4)-O- β-D-glucopyranoside and 6-Me ether, 7-O- β –D-galactopyranoside: (Chamaejasmoside)* from *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand*. There was no literature on the identification of these compounds from species belonging to the Fabacea family. Therefore, this is the first report of the presence of these compounds from methanol extract (leaf, twig and flower mixture) from *Philenoptera violacea (klotzsch) Schrire* and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* belonging to the Fabacea family. *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* was found to contain compounds with a molecule structure named 'pyranoside' and 'pyranosyl' in abundance. Pyranoside has been reported to possess neuroprotective properties [31-32] and pyranosyl has been reported as an iron-chelating agent. Furthermore, control of iron in the body by chelating drugs may have a significant beneficial effect on tissue damage caused by free radicals [33]. Therefore, the presence of this molecule in *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* may support its activity against free radicals, as demonstrated by Ntsoelinyane et al., [12]. *Philenoptera violacea (klotzsch) Schrire* has also been found to contain pyranoside pyranosyl and two unknown compounds. These compounds where identified as 4-Acetoxy-3,5-dimethoxybenzoic acid, 2″-O- *β –D-Apriofuranosyl, 6*″-O-α-L-*rhamnopyranosyl*, Quercetin-3-*O*-[α-L-rhamnopyranosyl(1→6)]-*β -D*-galactofuranoside, 3-O- *β –D-Galactopyranosyloxy-3' .4' .5.7-*

tetrahydroxyflavone, Quercetin 3-galactoside, 7-O-[α-L-Rhamnopyranosyl-(1→6)- β-D-glucopyranoside] or 6,8-Bis(C-β-glucosyl)-apigenin, 3-O-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside], 20-O-α-L-rhamnopyranoside and two unknown compounds. In addition to that, flavonoids were present in abundance. Flavonoids are classified into various classes [34], such as flavonols (quercetin), flavones (apigenin), flavanones (hesperetin, naringenin), flavonoid glycosides (astragalin, rutin), flavonolignans (silibinin), flavans (catechin, epicatechin) to name a few. Flavones are reported as possible antioxidant, anti-proliferative, anti-tumor, anti-microbial, estrogenic, acetyl cholinesterase, anti-inflammatory agents and are also used to treat cancer, cardiovascular disease and neurodegenerative disorders [35-36]. Despite the presence of abundant flavones in this plant extract, tests for its anticancer, antioxidant and antimicrobial activity yielded negative results. Therefore there was no correlation with the identified active compounds and pharmacological activities of this plant and this was not expected. This might be due to the concentration of these compounds; they are present, but not in large quantities. To elucidate this, each part of this plant must be analyzed separately and a quantitative analysis to determine the amount of active flavonoids present must be done in the future.

Radioprotective effect

Traditionally, radioprotectors are defined as agents that are administered before radiation exposure, whereas therapeutic agents are administered after exposure. Many naturally occurring antioxidants exhibit a long window of protection, including post-irradiation protection against lethality and mutagenesis [37]. To prove the radioprotective capacity of antioxidants present in *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant extracts, we applied the extract to radiated cancer cells. *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant extract studied in this project showed the capability of offering 50% protective effect but was less toxic than synthetic radioprotectors, such as phosphorothioates. The potential application of this plant as a protective agent shows promise - either prophylactic benefits for anticipated exposure in emergency situations or therapeutic benefits after radiation accidents/incidents. We have previously reported the free radical scavenging activity of *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* [12]. In continuation, the present *in vitro* studies were undertaken to elucidate its precise role in the modulation of radiation-induced damage in human prostate cancer cell line. DU145 cells were treated with radiation using a ^{60}Co γ-irradiation source at a dose rate of 0.827 Gy/min in order to determine radiation-induced damage. Radiation-induced DNA clustered lesions and apoptosis are the major likely mechanisms implicated in clonogenic cell death. Radiation exposure (2 Gy) triggered a loss of clonogenicity in DU145 cells. Cell growth is hindered by alteration of membrane permeability, damage to DNA and proteins responsible for cell cycle progression [38]. Cell count studies and cell surviving fraction values were determined, and the RPF value obtained indicated that *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant extract can lead to over 50% reduction in cell death. Nuclear and radiological accidents can result in moderate to severe radiation injuries and many casualties. The harmful impact of γ-radiation on biological systems is well researched and documented. Aglycones, which are compounds remaining after the glycosyl group on a glycoside is replaced by a hydrogen atom, were proven to have high radioprotective capacity. The observed radioprotective effect of this compound was explained by Konopacka et al., to result from its antioxidant properties [39]. From identified compounds, aglycones were found in abundance in *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand,* therefore its radioprotective activity compared relatively well to aglycones' radioprotective capacity. The results obtained suggested that the free radical scavenging activity agents present in this plant extract may be used as compounds with promising radioprotective potential. However, these results could be influenced by the increase in cell number and competition among growing cells for the available, but limited supply of nutrients and growth factors. Such argumentation in cell growth or proliferation rate against radiation damage could be the result of several factors, such as inhibition of free radical generation, repair of damaged DNA, protection of cell membrane and an increase in the producing ability of cells. Furthermore, the predominant mechanism of radiation injury, both in tumors and in normal tissues, is by induction of apoptosis or clonogenic cell death by free radical-mediated DNA damage [40]. Thus, screening potent free radical scavengers as radioprotectors is one of the important strategies of drug development.

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Table 1: No. of his+ revertants in *Salmonella typhimurium* strains TA98 and TA100 produced by *Philenoptera violacea* (klotzsch) Schrire and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* plant extract.

Test Sample	TA98			TA100		
	Number of colonies (revertants)			Number of colonies (revertants)		
	Concentration (µg/ml)			Concentration (µg/ml)		
	5000	500	50	5000	500	50
Philenoptera violacea (klotzsch) Schrire	34.3±4.9	43.5±3.5	33.7±4.0	211.7±21.6	143.7±33.8	152.0±10.4
Xanthocercis zambesiaca (Baker) Dumaz-le- Grand	36.3±4.0	36.7±6.8	40.0±2.6	158.3±8.1	159.7±12.5	160.3±1.5
4NQO (1)	157.3.±22.0			355.0±20.8		
Solvent control	26.6±2.7			155.6±8.3		

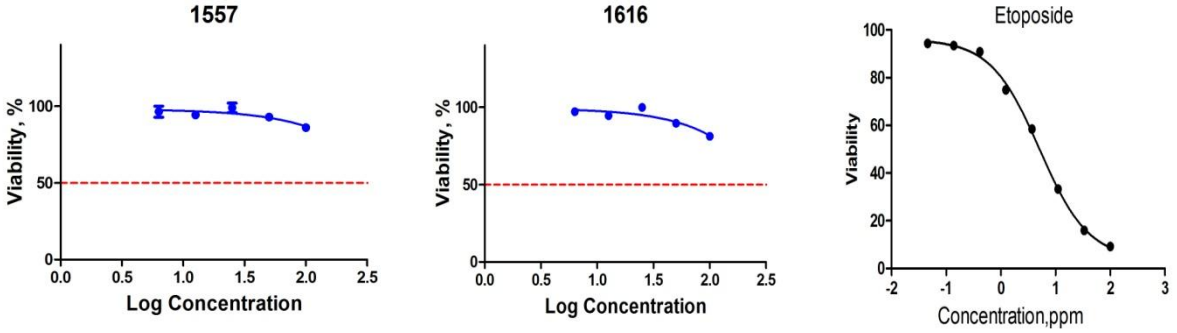

Figure 1: IC50 of methanol extract of *Philenoptera violacea* (klotzsch) Schrire (<100 µg/ml) and *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* (<100 µg/ml) on WI-38 cell line against Etoposite (4.1 µg/ml).

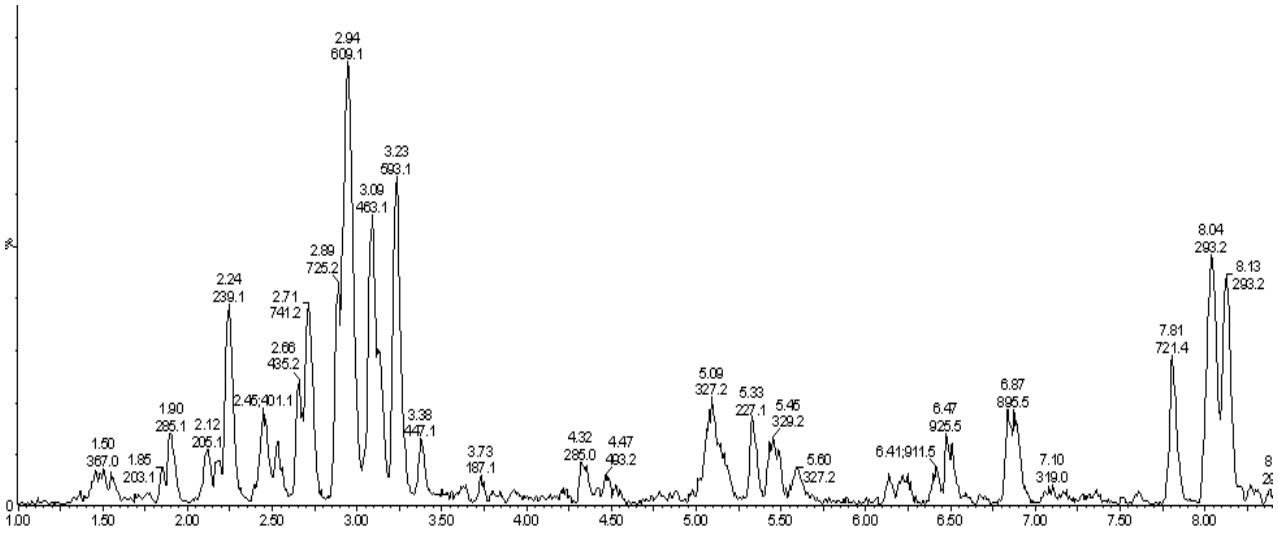

Figure 2: LC/MS chromatograms of *Philenoptera violacea (klotzsch) Schrire* extract (ESI negative). Nine phenolic components were identified.

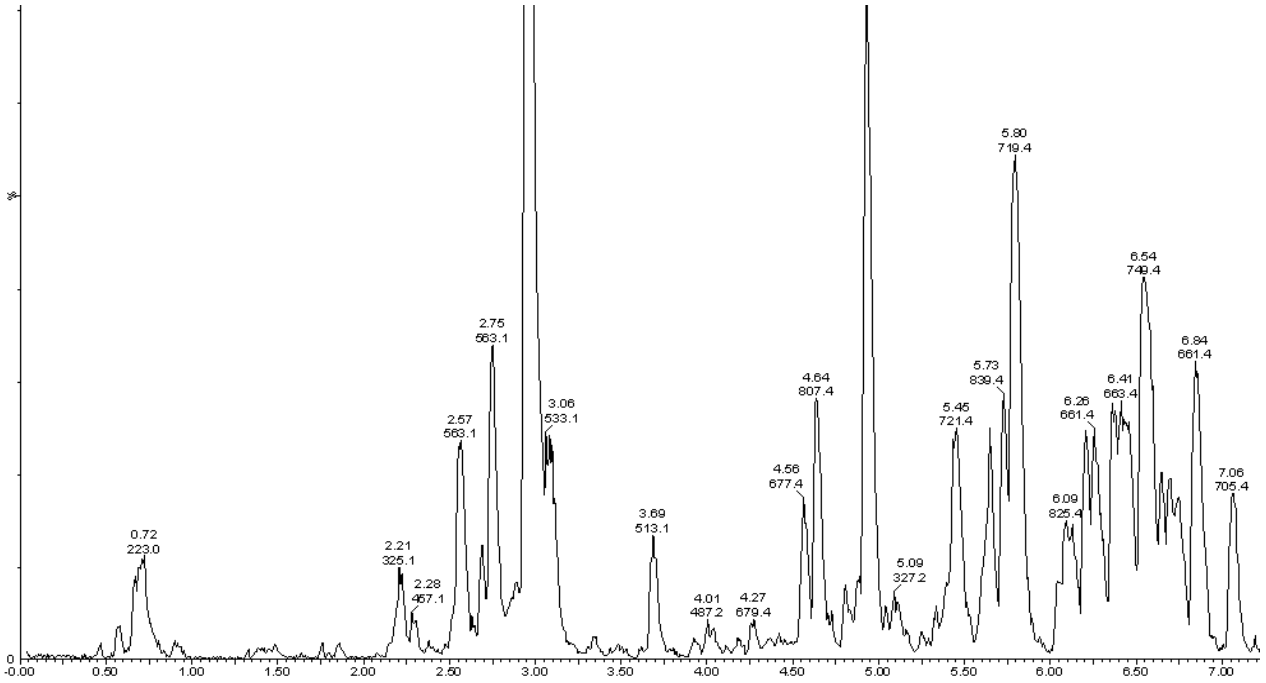

Figure 3: LC/MS chromatograms of *Xanthorcercis zambesiaca* (ESI negative). Seven phenolic components were identified.

Peak	(m/z)	Rt (min)	Tentative ID	References
1	239.05	2.24	*4-Acetoxy-3,5-dimethoxybenzoic acid*	[14]
2	741.18	2.71	*2"-O- β –D-Apriofuranosyl, 6"-O-α-L-rhamnopyranosyl*	[15]
3	609.14	2.94	*Quercetin-3-O-[α-L-rhamnopyranosyl(1→6)]-β -D-galactofuranoside*	[16]
4	463.08	3.09	*3-O- β –D-Galactopyranosyloxy-3' .4' .5.7-tetrahydroxyflavone. Quercetin 3-galactoside*	[15]
5	593.14	3.32	*7-O-[α-L-Rhamnopyranosyl-(1→6)-β-D-glucopyranoside]* *or 6,8-Bis(C-β-glucosyl)-apigenin*	[16]
6	925.51	6.47	*3-O-[α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside], 20-O-α-L-rhamnopyranoside*	[16]
7	895.50	6.87	*Unknown*	*
8	721.36	7.81	*2-oxepanone; 2-propenoic acid [3-hydroxy-2-[[3-hydroxy-2,2 bis(hydroxymethyl)propoxy]methyl]-2-hydroxymethyl)propyl] ester; 2-propenoic acid 1-[2-[2-(1-oxoprop-2 enoxy)propoxy]propoxy]propan-2-yl ester*	[15]
9	293.21	8.04	*Unknown*	*

Table 2: Identification of phenolic compounds of *Phileneptera violacea (klotzsch) Schrire* methanol extracts using their mass unit and retention times. Identification was aided by comparison with previous literature reports. **(m/z) = Molecular mass, Rt = retention time, * = no literature**

Table 3: Identification of phenolic compounds in *Xanthocercis zambesiaca (Baker) Dumaz-le-Grand* methanol extract using their mass unit and retention times. Identification was aided by comparison with previous literature reports. **(m/z) = Molecular mass, Rt = retention time**

Peak	(m/z)	Rt (min)	Tentative ID	References
1	563.14	2.75	*Apigenin-4'-O- β –D-xylofuranodyl(-(1→4)-O- β-D-glucopyranoside and 6-Me ether*	[16]
2	533.1	2.29	*Apigenun-6-C-α-L-arabinopyranosyl-8-C- β -L-arabinopyranoside*,	[16]
3	513.1	3.69	*7-O- β –D-galactopyranoside: (Chamaejasmoside)*	[15]
4	825.4	4.93	*Metaplexigenin-3-O- β-D-oleandropyranosyl-(1→4)- β-D-digitoxopyranosyl -(1→4)- β-D-digitoxopyranoside*	[15]
5	719.3	5.80	*23,24-Dihydro, 25-Ac, 2-O-b-D-glucopyranoside:*	[15]
6	749.37	6.54	*5E,8Z,11Z,14Z)-eicosa-5,8,11,14-tetraenoic acid [1-acetyloxy-3-[[(3R,4R,5S)-3,4,5-triacetyloxy-6-(acetyloxymethyl)-2-oxanyl]oxy]propan-2-yl] ester*	[14]
7	661.35	6.84	*3-O- β-D-Glucuronopyranoside: (Cloversaponin II)*	[15]