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Ateeq Rahman
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia

Arnold Magurausche
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia

Celine Mukakalisa
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia

David Nanhapo
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia

Daniel Likius
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia

Veikko Uahengo
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia

Correspondence:
Ateeq Rahman
Department of Chemistry and Biochemistry, University of Namibia, Pionierspark, Post Bag 13301, Windhoek, Namibia
Email: ateeqr786[at]yahoo.com

Recent studies on phytochemical analysis, radical scavenging and anti-microbial activity of the leaves of *Gampocarpus fruticosus* from Namibia

Ateeq Rahman*, Arnold Magurausche, Celine Mukakalisa, David Nanhapo, Daniel Likius, Veikko Uahengo

ABSTRACT

Gampocarpus fruticosus is a wild plant that belongs to the *Asclepiadaceae* family and has many traditional uses. The plant has demonstrated a significant level of microbial and phytochemical activity; the drug-resistant strains of microbes and traditional uses which have emerged in the last decade will be eradicated by the compounds this indigenous plant carries. Phytochemical screening was executed followed by the evaluation of total flavonoid, phenol and tannin content using aluminium chloride method, folin-ciocalteu method and the potassium ferrocyanide methods respectively. Antimicrobial screening as well as MIC determination was done using the disk diffusion method. The antioxidant activity was determined using the DPPH Free Radical Scavenging Method. The quantitative phytochemical determinations were performed in triplicate and expressed as means (± standard deviation) and of the phytochemicals that were quantified, tannins were present in large amounts with 6.937±0.115 mg TA/g in the methanolic extract and 5.356±0.185 mg TA/g in the ethanolic extract. The MIC values for antimicrobial analysis were recorded as the minimum application of plant extracts that totally repressed the growth of the microorganism. The MIC value for the ethanolic extract on *Staphylococcus aureus* was 1 mg/ml and for *Escherichia coli* it was 6 mg/ml. For DPPH, the extract with the lowest IC_{50} was the one with the highest scavenging activity. The standard used was Vitamin C and it had an IC_{50} value of 0.37 mg/ml. The IC_{50} value of the methanolic extract and ethanolic extract were 0.25 mg/ml and 0.34 mg/mlrespectively. The main highlights of this article are their greater medicinal value of phytochemicals present, antimicrobialactivity and antioxidant activity that was exhibited by the leaf extract of *G.fruticosus* plant.

Keywords: *Gampocarpus fruticosus*, antioxidant, antimicrobial activity.

INTRODUCTION

Gampocarpus fruticosus locally known as *//horapob* in the Nama/Damara dialect of Namibia is an indigenous plant belonging to the Asclepiadaceae family. There are basically two common species of *Gampocarpus* in Africa and these are *G. physocarpa* and *G. fruticosus* [1]. The main distinction between the two species are that of *G. physocarpus* has larger and more rounded ornamental seed balls than *G. fruticosus* whose inflated seed balls have sharp pointed ends and covered in short stout hairs. *G. fruticosus* demonstrates an erect growth habit with multiple stems in terms of morphology and these stems are of 1 to 3 m in height [2-3]. The plant is found along roadsides and old cultivated lands. Asclepiadaceae is the family to which this plant belongs to and *Gampocarpus* consist of almost 22 species in tropical Africa and Peninsular Arabia [4-11]. Five subspecies are distinguished in *Gampocarpus fruticosus* most importantly provisional is that the hairiness of the plant, and the colour of the flower and the form of fruit. The sub species are namely *G. cancellatus* (Namibia and South Africa), *G. glaucophyllus* (Zimbabwe), *G. purpurascens* (Ethiopia), *G. stenophyllus* (Kenya) and *G. solstitialis* (Togo) [1]. The plant *G. fruticosus* is widely distributed in the Southern part of Africa stretching to the eastern parts of the continent. The traditional Uses of *Gampocarpus fruticosu* as medicines are used in the maintenance of health by prevention, diagnosis or treatment of physical and mental illness [4]. The reliance on traditional medicines has been of great significance since pre-colonial rule specifically amongst rural populations and this has been due to the general lack of access to public healthcare facilities in such areas [5]. Plants are endowed with various phytochemical compounds that have different medicinal activities that are traditionally used in the management of diseases [6-7]. The plant has quite a number of uses depending on its geographical location on the African continent. *Gampocarpus fruticosus* is actually of toxic nature to livestock and humans, and has caused deaths in cattle, sheep and poultry [8].

Severe gastroenteritis is the main symptom of poisoning by this species and dense infestations may also reduce the productivity of pastures. In Namibia, a tea made using the root is drank daily to treat diabetes and the San people usually practice the latex hence it acts as projectile and poison ingredient [12]. Usually leaves are used to rub the body in order to treat skin cancer in Southern parts of Namibia implying that the plant has a vital medicinal significance. In Uganda, an extract from the roots is taken to treat backache and swellings of the neck [13]. Hence, in Botswana, the Bayei drink the extract of root to cure gonorrhoea and for the treatment of hepatitis fresh leaves are immersed in water and the liquid is drunk to induce vomiting. The inner bark of the plant yields a white fibre that is spun into cotton that is used for sewing clothes and for snaring birds as stated by Miller [14]. Whereas in Zambia, bitter latex is sprinkled over eggs in chicken poultry to prevent snakes and dogs from consuming chickens eggs. Hence, in Lesotho, the rootstock are cooked and consumed as vegetable and in Kenya the Masai people eat the fruit part of the plant [14]. Whereas the plant *G. fruticosus* it is traditionally used to treat various ailments such as coughing, diabetes, tumors, skin disease, scabies and itching. The root is used as snuff for headache, convulsions, veterinary medicine for dogs and chickens, treating stomach ailments and body pains. The root is also used to treat sexually transmitted diseases [7]. The therapeutic effectiveness of these traditional medicines have been attributed to the presence of various phytochemicals such as vitamins, terpenoids, phenolic acids, lignin, stilbene, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other secondary metabolites. From literature reports have demonstrated that many of these phytochemicals are useful as antioxidants, anti-inflammatory, anti-atherosclerotic, antitumor, anti-mutagenic, anti-carcinogenic, antibacterial, and antiviral agents [8].

RESULTS AND DISCUSSION

Phytochemical screen testing was evaluated for the leaves of *G. fruticosus* in this study and the phytochemicals that were screened are alkaloids, tannins, flavonoids, saponins, terpenoids, cardiac glycosides, phenols and quinines. Quantification was done for the phenolic content, flavonoid content and tannin content. In terms of the antimicrobial activity study, screening was done to determine whether the ethanolic and methanolic extracts have inhibition of growth activity on the strains. Minimum Inhibitory Concentration (MIC) was then determined for the extracts on the different strains. According to the World Health Organisation [4]. Hence, over and above 80% of the world's population in poor and underdeveloped countries usually depends on traditional plants with established medicines for their major healthcare requirements. In developing nations like Namibia, microbial infections are still directly responsible for merely half of all cases of death [9]. In the past decade there has been the development of drug-resistant strains of microbes and this has necessitated the development of new inexpensive drugs to treat related diseases causing microbes which will be of great significance. The main highlights of this article are: a) to determine the radical scavenging activity of the leaf extracts. d) To evaluate the anti-microbial activity of the leaf extracts. The Significance of this study will contribute to the phytochemical knowledge of this indigenous medicinal plant and scientifically validate its traditional use.

Antimicrobial activity of *Pergularia daemia, Secamone afzelii, and Leptadenia hastate* of the Asclepiadaceae family used in traditional medicine in South Togo

Pergularia daemia, *Secamone afzelii*, and *Leptadenia hastate* contain latex and they belongto the Asclepiadaceae family. This family consists of about 130 genera and 2000 species distributed all over the world of which some of them are tropical and subtropical shrubs or perennial herbs. According to Okusa [15] the emergence of multi-drug resistant phenotypes is a major public health problem in the treatment of bacterial infections. As a result of the alarming worldwide incidence of antibiotic resistance, an increase in the need for new compounds has resulted and medicinal plants represent a valuable source for these kinds of compounds [16]. Several plants from Togo are traditionally used against infectious diseases and among these plants is *Pergularia daemia*, *Secamone afzelii*, and *Leptadenia hastate*. Plant latex is a good source of various secondary metabolites, which shows growth inhibition effect against bacteria, fungi, viruses, tumours and cancer cell lines. These three latex plants have quite a number of traditional medicinal uses which vary from plant to another plant. *P. daemia* is usually used as anthelmintic, laxative, antipyretic and cough mixture, and is often used for the treatment of infantile diarrhoea and malaria [17-18]. The plant *Secamone afzelii* is used to treat stomach problems, diarrhoea, gonorrhoea, malaria, cough, catarrhal conditions and diabetes [19]. *Leptadenia hastata* is used in the management of onchocercosis, scabies, hypertension, catarrh, skin diseases, sexual potency, and wound-healing. The main aim of this particular literature research project was to evaluate antibacterial potentials of the aqueous ethanolic extracts of *P. daemia*, *S. afzelii*, and *L. hastata* against six selected human pathogenic bacteria. In previous studies the ethanolic extracts of *P. daemia* revealed the presence of medicinal bioactive components of tannins, saponins, terpenoids, alkaloids, flavonoids, phenols and steroids [20-21]. The antibacterial activities observed can be attributed due to the presence of these antioxidants and in agreement with the results, it was found in India that the methanolic extract of leaves of *P. daemia* was active against *E. coli, Pseudomonas aeruginosa*, and *S. aureus* [22]. In Ghana, it was demonstrated that the methanolic extract of aerial part of *S. afzelii* inhibited the growth of *S. aureus* and *E. coli* and was inactive against *P. Aeruginosa* [22]. The lowest MICs were determined in the study for *P. daemia* and in order to elucidate whether the observed antibacterial effects were bactericide or bacteriostatic, MBC/MIC ratios were calculated. Extracts with ratios greater than 1 were considered as bacteriostatic, while the extracts with ratios equal to 1 are bactericide [23]. Thus, the ethanolic extract of leaves of *P. daemia* had a bactericide effect against *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213 and *S. aureus*, and a bacteriostatic effect against *S. typhi* and *K. pneumoniae.* The extract of leaves of *S. afzelii* had a bactericide activity against *E. coli* ATCC 25922 and a bacteriostatic effect against *S. aureus* ATCC 29213 and *S. aureus.* A bacteriocidal activity was observed for the extract of *L. hastata* against the two strains of Staphylococcus tested and a bacteriostatic activity against *S. typhi* and *K. pneumoniae* [23-24]. The biological activities of medicinal plants vary widely based on the type of plant part, geographic location and solvent used in extraction. These biological activities can also depend on the condition of the plant parts reported by Tchacando *et al.* [21], traditional healers follow certain practices such as harvesting plant material early in the morning to prepare their herbal medicines. From the literature data its evident that the leaves of *P. daemia* should be taken early in the morning to optimize the antibacterial activity. Tchacando *et al.* [21] reported these tested not only the ethanolic extract but also the aqueous decoction of leaves of these three plants. The activities obtained for these extracts were consistent with the use of such plants in the treatment of bacterial infections. This research study provided useful data concerning the antibacterial activities of leaves extracts however the toxicological investigations are yet to be done to provide the medicinal safety uses of these species.

Phytochemical Screening and Antioxidant activity of ethanolic extract of *Boucerosiatruncato-coronata gravely muyar*

The Asclepiadaceae family has quite a number of species and the members of this familyshow great morphological diversity. The plant *Gampocarpus fruticosus* belongs to this family and the phytochemistry of plants belonging in this family are characterized by many pregnane glycosides while in recent studies, megastimane glycosides have also been isolated [24]. The main phytochemicals that are found in medicinal plants are saponins, flavonoids and polyphenols and they are known to be major bioactive compounds in Ayurvedic medicine [25]. The antioxidants that have been isolated from most medicinal plants have depicted quite a number of activities which include antibacterial, anti-carcinogenic and antiviral. The extract activity of *B. truncato-coronate* was tested for antioxidant activity by DPPH and the phytochemical studies were also done for this plant [25]. For the qualitative phytochemical analysis that was done on this plant, confirmation of the presence of various phytochemicals was done. It was confirmed that

secondary metabolites such as alkaloids, terpenoids, saponnins, flavonoids and phenols were present in *B. truncato-coronate*. In terms of the radical scavenging activity, both the in vivo and the *in vitro* extracts exhibited significant scavenging activity which actually increased with an increase in concentration of the extract. There are three main techniques that can be used to measure the antioxidant capacity measurement. They are divided into three main classes which are Spectroscopic technique, Electrochemical Techniques and the Chromatographic technique. DPPH technique is commonly used in the determination of antioxidant capacity in many fruit extracts and juices hence making it possible for DPPH technique to be used on medicinal plant extracts [26].

Phytochemistry of medicinal plants

Phytochemicals are classified as either primary or secondary constituents which is basically dependent on their main character in the plant metabolism. Major components are usually, sugars, amino acids, proteins, purines and pyrimidines of nucleic acids and chlorophyll's hence, the secondary components are the remaining antioxidants such as alkaloids, terpenes, phenolics flavonoids, lignans, plantsteroids, curcumines, saponins, and glucosides [27-30]. Phenolic compounds are the largest grouping of phytochemicals and extremely dispersed in the plant kingdom and there are three main sets of dietary phenolics of vital importance are phenolic acids, flavonoids, and polyphenols [31-32]. Flavonoids have major role in successful medical treatments because of their broad biological and pharmacological activities.

They exhibit various biological properties such as antimicrobial, cytotoxicity, anti-inflammatory and antitumor activities. Well known properties of almost every group of flavonoids are to act as powerful antioxidants and they protect the human body from forming free radicals and reactive oxygen species [33-35]. Tannins are additional class of phytochemicals with various groups of high molecular weight polyphenolic compounds with the ability to form reversible and irreversible complexes with proteins, polysaccharides, alkaloids, nucleic acids and minerals [36]. There are several health benefits have been familiar for the intake of tannins and some epidemiological relations with the decreased frequency of chronic diseases have been recognized [37-38]. They are also used as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals [39]. Tannins have various applications in industries as caustics for cationic dyes and inks. In the food industry tannins are used to clarify wine, beer, and fruit juices. The other industrial uses of tannins include textile dyes industries [40-41]. Alkaloids have many pharmacological activities including antihypertensive effect, antiarrhythmic effect, antimalarial activity and anticancer actions. Few alkaloids have intoxicant properties such as caffeine, nicotine, and morphine which are used as analgesics and quinine as anti-malarial drug [42]. Whereas these classes of natural lipids are found in every class of living things and they are considered as the largest group of natural products [43]. The physiological role of saponins in plants is not yet fully researched and however there are a number of publications reporting their identification in plants and their multiple effects in animal cells and on fungi and bacteria [44]. The most important bio-active constituents from plants are such as Cardenolides, alkaloids, tannins, flavonoids and phenolic compounds [41-44].

Cardenolides are the type of cardiac-active steroids find in plants, they have a long history of therapeutic relevance for treatment of heart failure and arrhythmia, and however, they are produced almost exclusively by plants and serve to protect them from herbivory. The recent research revealed that natural products developed as promising agents in various diseases including cancer, cystic fibrosis, spin bulbar muscular atrophy and other polyglutamine-related diseases [41-44].

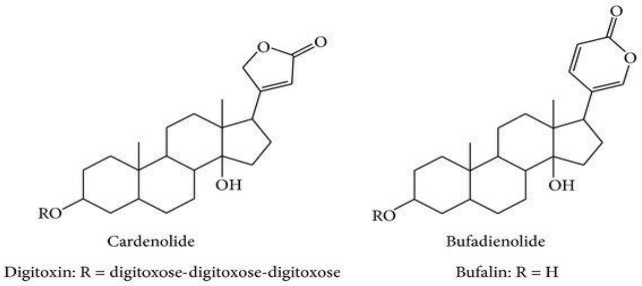
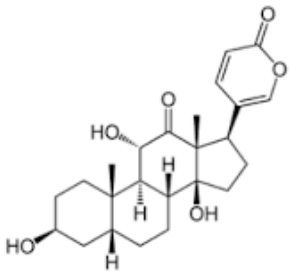
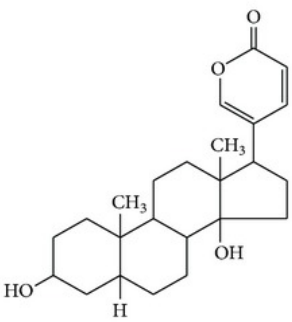


Figure 1: Structure of cardenolides

Biological activity performed on the selected Cardiac glycosides compounds revealed that the presence of different functional groups on compound structures is responsible for activities. Furthermore, these compounds were also test for antitumor activity on the human cells against rodent cells and human cancer cells against human nonmalignant cells [1, 2, 3].

Table 1: Cardiac glycosides and their biological activity

Compounds	Structure	Biological activity	Refer.
Arenobufagin		Carcinogenesis: Liver cancer	63
Bufalin		*in vitro* antitumor effects against various malignancies: Hepatocellular and lung carcinoma	65

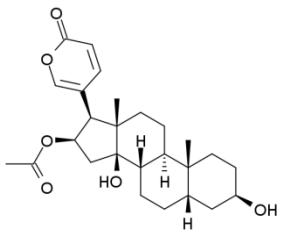
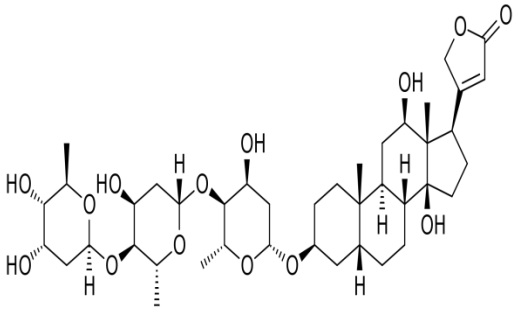
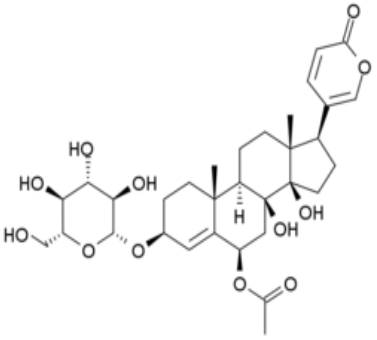
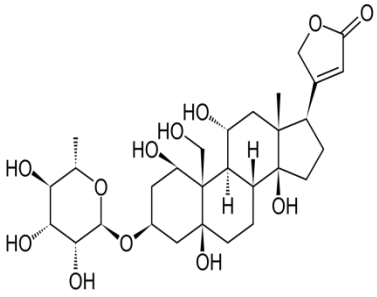
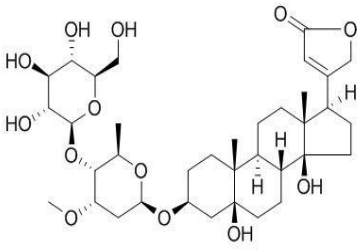
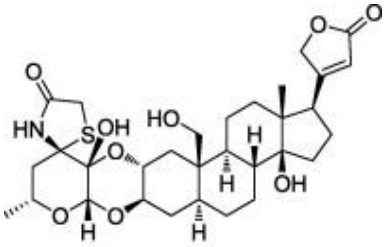
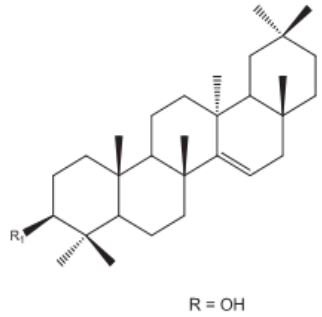
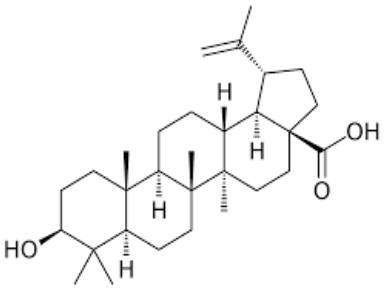
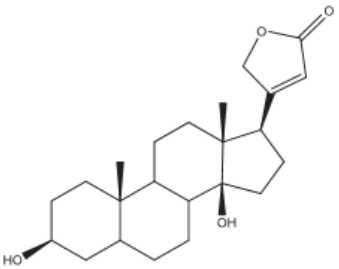
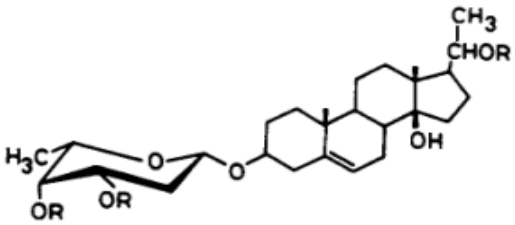
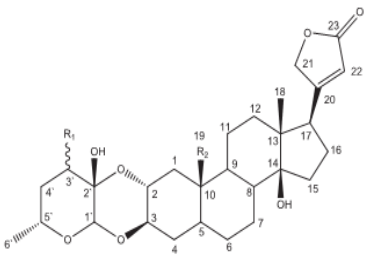
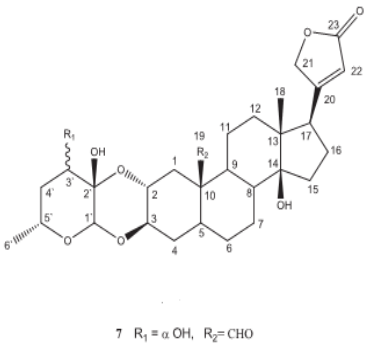
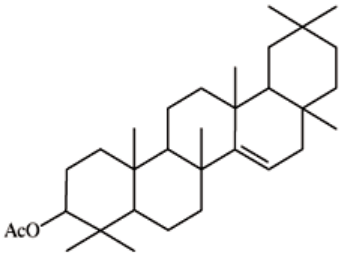
Bufotalin		Induces apoptosis *in vitro* in human hepatocellular carcinoma Hep 3B cells as well as caspases and apoptosis inducing factor (AIF)	65
Digoxin		It used as intrafetally or amniotically during abortions and for heart failure	64
Lanatoside C		Used in the treatment of congestive heart failure and cardiac arrhythmia	66
Ouabain		Its used intravenously and orally in angina pectoris and myocardial infarction as well as for treatment of congestive heart failure	62
Periplocin		Antitumor effect by inhibiting the Ki67 expression in tumor samples	67
2″-oxovorusharin	 UNBS1450	Induces apoptotic cell death	68

Table 2: Summary of Cardenolide compounds found in *Gampocarpus fruticosus*

Compound	Chemical structure	Chemical formula	Activity	Reference
13α-methyl, 27-norolean-14-en, 3β-ol (3β-taraxerol)	 R = OH	$C_{30}H_{50}O$	Anti-asthmatic activity	Komarnytsky *et al.*, 2013 Wang S *et al.*, 2009
Betulinic acid		$C_{32}H_{48}O_3$	antiretroviral, antimalarial, and anti-inflammatory anticancer	Komarnytsky *et al.*, 2013
Cardenolide aglycone uzarigenin	 4	$C_{23}H_{34}O_4$	Inhibit growth in cancer cell	Komarnytsky *et al.*, 2013 Wang S *et al.*, 2009
Pregnane glycoside	 1 R=H 2 R=Ac	$C_{54}H_{86}O_{18}$	Anorectic activity in animals	Komarnytsky *et al.*, 2013
Cardenolide glycosides gomphoside	 6 R_1 = β OH, R_2 = CH_3	$C_{29}H_{42}O_8$	Heart failure	Komarnytsky *et al.*, 2013

Calotropin	 7 R_1 = α OH, R_2= CHO	$C_{29}H_{40}O_9$	Calotropin poisoning can cause respiratory and cardiac failure	Komarnytsky *et al.*, 2013 Wang S *et al.*, 2009
3β-taraxerol acetate		$C_{32}H_{52}O_2$	Anticancer, induce apoptosis	Komarnytsky *et al.*, 2013

RESEARCH METHODOLOGY

Sample Collection

The plant samples that were used in this research study were collected in Windhoek, Khomas Region of Namibia and in particular the areas surrounding the University of Namibia. A Research/Plant Collection Permit was obtained from the Ministry of Environment and Tourism and plants were collected using the procedures outlined by the Guide for Collecting Plants from the National Botanical Research Institute (Namibia). However *G. fruticosus* is not on the endangered species list.

Sample preparation

The plant samples were air dried for 15 days in the shade. Separation of the leaves from the stems was completed and the weight of the leaves was taken before grinding. The grinding was done using a mortar and pestle until a fine powder was obtained.

Plant Extraction

Procedure for Plant Extraction: The plant extraction was executed according to the method used by Packirisamy *et al.* [45]. A mass 25 g of ground plant material was added into two separate 250 mL Erlenmeyer flasks and 125 mL of ethanol and methanol each was added into the two separate flasks containing the ground plant material. The mixtures were then shaken for 30 minutes using an orbital shaker. When shaking was completed the mixtures were allowed to stand in the refrigerator for 24 hrs so as to allow the mixture to settle.

Screening of the Phytochemicals in *G. fruticosus*

The phytochemical screen testing was carried out according to the procedure previouslyreported by Packirisamy *et al.* [45].

1. Test for Alkaloids

1.1 ***Preparation of Dragendorf's reagent:*** Bismuth nitrate (0.5 g) was added into the beaker and then 10ml of distilled water was added into the same solution. Concentrated HCl (10 ml) was added and then the solution was stirred. Potassium iodide (4 g) was added in another beaker and I ml water until the KI had completely dissolved. The two solutions were mixed and a dark orange solution was observed indicating that the reagent was ready for use.

2. Procedure for testing Alkaloids: Plant extract (0.2 g) was mildly warmed with 2% sulphuric acid for 2 mins. The solution was filtered and a few drops of 8% Dragendoff's reagent were added and the formation of orange red precipitate indicated the presence of alkaloids.

3. Test for Saponins: Plant extract (0.5 ml) was added into two separate test tubes. One plant extract was ethanolic and the other one was methanolic. Into each test tube, 10 ml distilled water was added. The contents were thoroughly shaken. Frothing and bubbling indicated the presence of saponins.

4. Test for Flavonoids: The extract (0.2 g) was dissolved in 10% NaOH and then in 1% HCl was added. Then a yellow solution that turns colourless indicated the presence of flavonoids.

5. Cardiac glycosides: Approximately 5 g of the plant extract was soaked in 2ml of glacial acetic acid containing one drop of ferric chloride solution. The solution was added with 1 ml of concentrated sulphuric acid and a brown ring of boundary showed deoxy-sugar which is a class of cardenolides.

6. Test for Terpenoids: Nearly 0.2 g of extracts was mixed with 2 ml of chloroform and 3 ml of 2% sulphuric acid was cautiously added to form a layer. A reddish brown coloration of the border indicated the presence of terpenoids.

7. Test for Balsams: Two droplets of alcoholic ferric chloride solution were added to 5 ml of each extract and a green colour indicated the presence of balsam as shown in fig 10 below.

Quantification of Phytochemicals

1. Determination of total flavonoids: The determination of total flavonoids was conducted as previous reported by Kumaran *et al.* (46). Plant extract (100 µl) methanol (10 mg/ml) was mixed with 100µl of 20 % aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5ml. The absorption at 415 nm was observed after 40 minutes. Blank samples were prepared from 100 ml of plant extracts and a drop of acetic acid, and then diluted with 5ml methanol. The absorption of standard quercitin solution (0.5 mg CE/ml) in methanol was then measured under the same conditions and all the tests were repeated in triplicates.

2. Determination of total phenolic compounds: Determination of total phenolics was conducted as previously reported by Hagerman *et*

al. (47). A mass of 50 g of the sample was dissolved in 100 ml of triple distilled water. A volume of 1 ml of this solution was then transferred to a test tube. Folin-Ciocalteu reagent (0.5ml) and 1.5 ml 20% of Na_2CO_3 solutions were added and the volume was made up to 8 ml with TDW. Vigorous shaking was executed on magnetic stirrer and allowed to stand for 2 hours after which the absorbance was taken at 765 nm. An estimate of the total phenolic content was then determined using a standard calibration curve obtained from various diluted concentrations of gallic acid which were 0, 0.01, 0.02, 0.03, 0.04 & 0.05 mg GAE/g.

3. Determintation of total tannin content: Determination of total tannin content was completed conferring to the method reported by Gracelin *et al.* (48). A mass of 50 g of the sample was weighed into a 50 ml plastic bottle and 50 ml distilled water was added and stirred on magnetic stirrer for 1 h. It was then filtered in 50 ml volumetric flask up to the mark and then 5 ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.I N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Antimicrobial activity

1. Preparation of Nutrient Broth: A mass of 13 g nutrient broth powder was dissolved in 1 l of distilled water. Heating was done to dissolve the medium. The mixture was distributed into the final container and then sterilized by autoclaving at 121 ˚C for 15 mins.

2. Preparation of Nutrient Agar: Nutrient agar powder (28 g) was suspended in 1 l of distilled water. The solution was boiled using a water bath to dissolve the powder completely. Sterilization of the solution was done by autoclaving at 121 ˚C for 15 mins.

3. Antimicrobial screening Procedure: The screening procedure was done as reported by Purity Kaaria *et al.* [49]. Culture plates were prepared using the nutrient agar solution and the plates were left for 5 hours so that the nutrient agar could properly solidify. The bacterial strains that were used to do screening were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Candida albicans*. Each microbial strain was cultured onto nutrient agar and incubated at 30°C for 18 to 24 hr. A 100µl of each strain was placed on different Nutrient agar plates; spread evenly using a glass spreader then allowed to stand for 1 hr before the extract was introduced. About 10µl of plant extracts (1 mg/ 1ml) were placed onto a disc and placed on to the plate, allowed to stand for 30 min then the plates were incubated for 24 hrs at 37ºC. The plant extract that inhibited the growth of bacterial strains after 24 hr were recorded as positive and were investigated further whereas bacterial strains in plates were no inhibition took place at all were recorded as negative and were not investigated further.

4. Minimum Inhibitory Concentration: The determination of the MIC was done as reported by Purity Kaaria *et al.* (49). The filter paper discs taken were approximately 6 mm in diameter were soaked with 10 µl of the plant extract with different concentrations and placed in agar plates as mentioned above. Each disc was pushed down to ensure complete contact with the agar surface and distributed evenly so that they were no closer than 24 mm from each other, centre to centre. The agar plates were then incubated at 37 degrees and after 24 hrs of incubation, each plate was examined. The least concentration that showed inhibition on the different strains was the minimum inhibitory concentration (MIC).

Antioxidant activity

Free radical scavenging activity testing was completed as previously reported by K. Kalimuthi (50) however with minor modifications.

1. Procedure: A volume of 100 µl DMSO was added into wells respective wells. A volume of 100 µl plant sample was added into the wells which were then followed by dilutions that were done by pipetting 100 µl from each of the wells. A volume of 100 µl DPPH was added into the wells and hence the absorbance was measured at 517 nm. The formula that was used for calculating the % Scavenging activity was as follows

% Scavenging =

(Absorbance of Sample)/ (Absorbance of DPPH) x 100

The formula used for % inhibition is as follows

% Inhibition= 100 - % Scavenging

The results that were obtained were expressed as IC_{50}.

Data analysis procedure

The quantitative phytochemical determinations were performed in triplicates and expressed as means (± standard deviation). The MIC values for antimicrobial analysis were recorded as the least concentration of plant extracts that has completely reserved for the growth of the microorganism.

The amount of the total flavonoid in leaf was 4.16±0.01 CEmg/g in the ethanolic plant extract and 4.239±0.012 CEmg/g in the methanolic plant extract. The considerable amount of the flavonoid present in the leaf could be the main reason for its antioxidant activity. The leaf of *G. fruticosus* has little amounts of phenolic compounds in both the ethanolic plant extract and the methanolic plant extract. The ethanolic and methanolic plant extracts have 0.732±0.001 GAEmg/g and 0.961±0.001 GAEmg/g of phenolic content respectively. The tannin content in the methanolic plant extract was observed to be 6.937±0.115 TAmg/g and 5.356±0.185

TAmg/g in the ethanolic plant extract. The standard in this study was Vitamin C and it had an IC_{50} value of 0.37mg/ml. The ethanolic extract showed a DPPH radical scavenging activity with an IC_{50} value of 0.34mg/ml as compared with the methanolic extract which had an IC_{50} value is 0.24mg/ml. The strains were under study, *Staphlococcus aureus* had the least MIC value in the ethanolic extract which was 1 mg/ml and it is the only strain that had an MIC value from the methanolic extract. Methanol and Ethanol were the preferred solvents in this study due to the fact that these two solvents have the ability to dissolve both polar and non-polar molecules. Methanol has a polarity index of 5.1 [46-52]. There are quite a number of methods that can be used for extraction such as maceration, infusion, percolation and decoction according to the review paper that was reported by Azwanida [53]. In this particular study, maceration was the preferred method of extraction it is widely used in medicinal plant research [54]. The results obtained from the screening of phytochemicals indicated the presence of saponins, flavonoids, alkaloids, tannins, cardiac glycosides, phlobotanins, resins and balsams in the plant extracts. Only terpenoids tested negative for screening and of the phytochemicals that were detected in the plant, and only three of them were subjected to quantification and these are tanins, flavonoids and the phenols. The reason why only three of these phytochemicals were quantified is because of their significance in terms of contributing to antioxidant and antimicrobial activities in several literature review papers. Cardiac glycosides are an important class of naturally occurring compounds in plants and they have both beneficial and toxic properties [54]. Based on the amounts administered, they can be either deemed toxic or beneficial and in terms of their importance, they are used in the treatment of various cardiovascular conditions such as cardiac failure [55]. The San people of Namibia use the latex as an arrow poison ingredient [10] and this is attributed to the presence of cardiac glycosides in the plant *G. fruticosus*. The fact that cardiac glycosides possess cytotoxicity at a certain extent; it might be that these compounds are of great significance in terms of cancer treatment. Alkaloids are a class of nitrogen containing compounds and the bioactive compounds from plants containing alkaloids plays a major role in emerging as anti-tumor drug in human being. The increase of the inhibition of tumor growth

with the increasing concentration of plant extract had been documented in several studies [56]. They are having abenzopyrone which is used for antioxidant activity or free radical scavenging and also have a cardio-protective role [58]. By preventing the estrogen producing enzyme, flavonoid suppresses the progression of cancer and a good example is the inhibition of estrogen synthetase which binds with estrogen to its receptor. Phenolic compounds have ability to combat cancer and also prevent heart ailments to an appreciable degree and sometimes act as anti-inflammatory agents [59]. They are highly potent vasodilators and due to the presence of hydroxyl group that phenols contain possesses potential scavenging activity [60]. Hence, the detection and quantification of total phenolic compounds present in the leaf of *G. fruticosus* contributes in the field of herbal remedy as a potent antioxidant in future. Tannins are phenolic compounds that are of high molecular weight and are used as antiseptic and this activity is attributed to the presence of the phenolic group. Hence, it is related with antiviral activity. According to Rajan [61], in the presence of very high quantity of tannin, there are numerous viruses like polio virus, herpes simplex viruses have been found to be deactivated. The leaf of *G. fruticosus* comprises of very high level of tannin content in both the ethanolic and methanolic plant extracts. The IC_{50} is a measure of the effectiveness of a particular substance in terms of inhibiting a specific biological or biochemical function. Two extracts were used to determine the IC_{50} values of the leaf extract in this study and these were the methanolic plant extract and the ethanolic plant extract. The antioxidant activity was determined by the DPPH radical scavenging activity test. The antioxidant on interference with DPPH both transfers electron or hydrogen atom to DPPH and hence neutralizes its free radical character and converts it to 1-1,diphenyl-2- picryl hydrazine and the degree of discoloration indicates the scavenging activity of the drug. Whereas the reduction capacity of DPPH radical activity is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The decrease in absorbance of DPPH radical is caused by the antioxidants is attributed due to the reaction between antioxidant molecules and radical reaction process which results in the scavenging of the radical by hydrogen donation. Finally, it is visually noticeable as a change in color from purple to yellow. This implies that both the methanolic and the ethanolic extracts have a good antioxidant activity since their IC_{50} values did not deviate that much from the IC_{50} value of the standard used. However, between the two extracts, the methanolic extract had the lowest IC_{50} which means that it has the highest radical scavenging activity. The screening that was executed for the antimicrobial activity of the ethanolic plant extract and the methanolic plant extract exhibited that the ethanolic extract had activity against all the strains in this study however the methanolic extract only had activity on *S. aureus*. The Minimum Inhibitory Concentration (MIC) of the different concentrations of the methanolic and ethanolic plant extracts was determined. The difference in the MIC values of the two extracts on the different strains is attributed by the presence of the various phytochemicals such as phenols, saponins and flavonoids in different quantities in the extracts

CONCLUSION

In conclusion the article highlights the leaves of the plant *G. fruticosus* have quite a number of phytochemicals that are present and these are alkaloids, quinones, saponins, cardiac glycosides, flavonoids, tannins, phlobotanins, resins and balsams. Of the phytochemicals that were quantified, a considerable amount of tannin content in both the ethanolic and methanolic plant extracts was determined. In terms of the antimicrobial activity, Minimum inhibitory concentration was determined for each other extracts against the four strains. *Staphylococus aureus* had the least MIC values for both the extracts and regardless of the fact that terpenoids that are considered to be of great significance in terms of antioxidant activity tested negative in the leaf extract of the plant, antioxidant activity was however determined. At an overall point of perspective, it can be concluded that the leaf extract of *G. fruticosus* is of great medicinal significance due to the antimicrobial and antioxidant activity that was exhibited by the experimental work done and all these activities are attributed to the presence of the numerous phytochemical constituents that are found in the leaf extracts of *G. fruticosus.*

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