Qualitative and quantitative analysis of phytochemical compounds in Namibian *Myrothamnus flabellifolius*

Ahmad Cheikhyoussef[1]*, Rowan Wayne Summers[2], Gladys Kahaka[2]

[1]Science, Technology and Innovation Division, Multidisciplinary Research Centre (MRC)
University of Namibia, P/Bag 13301, Windhoek, Namibia

[2]Chemistry and Biochemistry Department, Faculty of Science
University of Namibia, P/Bag 13301, Windhoek, Namibia

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Abstract

The phytochemical content and antioxidant activity of the Namibian resurrection plant, *Myrothamnus flabellifolius*, which is traditionally used in the management and treatment of various diseases was determined. Samples were collected from Remhoogte pass, 130 km south west of Rehoboth. Solvent extraction using ethanol, methanol, water on powdered extracts (leaves and twigs) were done by soxhlet extraction and maceration. Twelve classes of phytochemicals tested positively; flavonoids, anthocyanins, alkaloids, steroids, terpenoids, triterpenes, cardiac glycosides, saponins, phlobatanins, tannins, polyphenols and reducing sugars in the leaves extracts. However, anthraquinones, anthranoids, iridoids, leucoanthocyanins, proteins and amino acids tested negatively in all three solvent extracts from both leaves and twigs. The quantitative antioxidant activity analysis namely; the total phenolic content (TPC) and total flavonoid content (TFC) of the solvent extracts from *M. flabellifolius* were also determined. The TPC and TFC ranged from 372.42±0.21 to 375.14±0.21 mg gallic acid equivalent (GAE)/g and 1.43±0.03 to 3.49±0.15 mg equivalents for quercetin (QE)/g respectively. The high TPC in *M. flabellifolius* makes it suitable for potential application as a potent natural source of antioxidants and validates the ethnobotanical uses of this plant in the traditional healing system in Namibia.

Keywords: Antioxidant activity, Bioactive compounds, *Myrothamnus flabellifolius*,

*Corresponding author: Tel: +264 61 2063283; Fax: +264 61 2063684; Email: acheikhyoussef@unam.na, acheikhyoussef@gmail.com

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1 Introduction

Phytochemicals are non-nutritive compounds (secondary metabolites) found in plants. They work with nutrients and dietary fibre to protect against diseases and they contribute to flavour and colour (Molefe-Khamanga et al. 2012a). They can be classified into sub-groups according to their chemical structure, which include terpenoids (e.g. carotenoids), phytosterols, polyphenols (e.g. tannins, flavonoids, phenolic acids) and glucosinolates (Campos-Vega and Oomah, 2013). Phytochemicals have beneficial and lethal effects in the body, of which the beneficial functions include, promoting the function of the immune system, acting as antibacterial or antiviral agents, reducing inflammation, prevention of cancer and cardiovascular diseases (James et al. 2007). It is important to know the structure of phytochemical constituents, thus knowing the type of biological activity which might be exhibited by the plant (Agbafor and Nwachukwu, 2011). Phytochemicals are isolated and extracted through different techniques (Harbourne et al. 2013) are then screened for their biological activity according to Harborne (1998).

The antioxidative phytochemicals have received increasing attention for their potential role in preventing/reducing the risk of human diseases such as coronary heart disease (Johnson 2013). It is believed that if Antioxidative phytochemicals are incorporated into the diet, it could lead to beneficial physiological changes in human microflora (Gyawali and Ibrahim, 2012), which could contribute to their chemo-preventive effects (Almeida et al. 2011).

Myrothamnus flabellifolius Welw. (Myrothamnaceae), commonly known as resurrection bush, bush tea and wonderbos, is one of the largest representative of the Resurrection plants (Child 1960) and occurs in a vast area stretching from Namibia in the west, through Botswana, to Zimbabwe and the northern parts of South Africa in the east (Kruger 1998). The plant grows between rocky outcrops with very shallow soils (Schneider 1998) and for approximately half of the year it exists in a dehydrated quiescent state (Farrant and Kruger, 2001). Leaves extract of *M. flabellifolius* has been reported to be used by Nama people in Namibia as an aid in wound healing and to treat asthma and general chest ailments (Van Wyk et al. 1997). Moore et al. (2005) identified the main polyphenol constituent of *M. flabellifolius* leaves to be 3,4,5 tri-O-galloylquinic acid. In addition, smaller quantities of higher molecular mass gallic acid polyesters were also present, the result of multiple galloylation of the central 3,4,5 tri-O-galloylquinic acid core. These compounds are collectively present in very high concentrations; almost half the dry mass of hydrated leaves and approximately

three-quarters of the dry mass of desiccated leaves is due to the presence of these compounds (Moore et al. 2005).

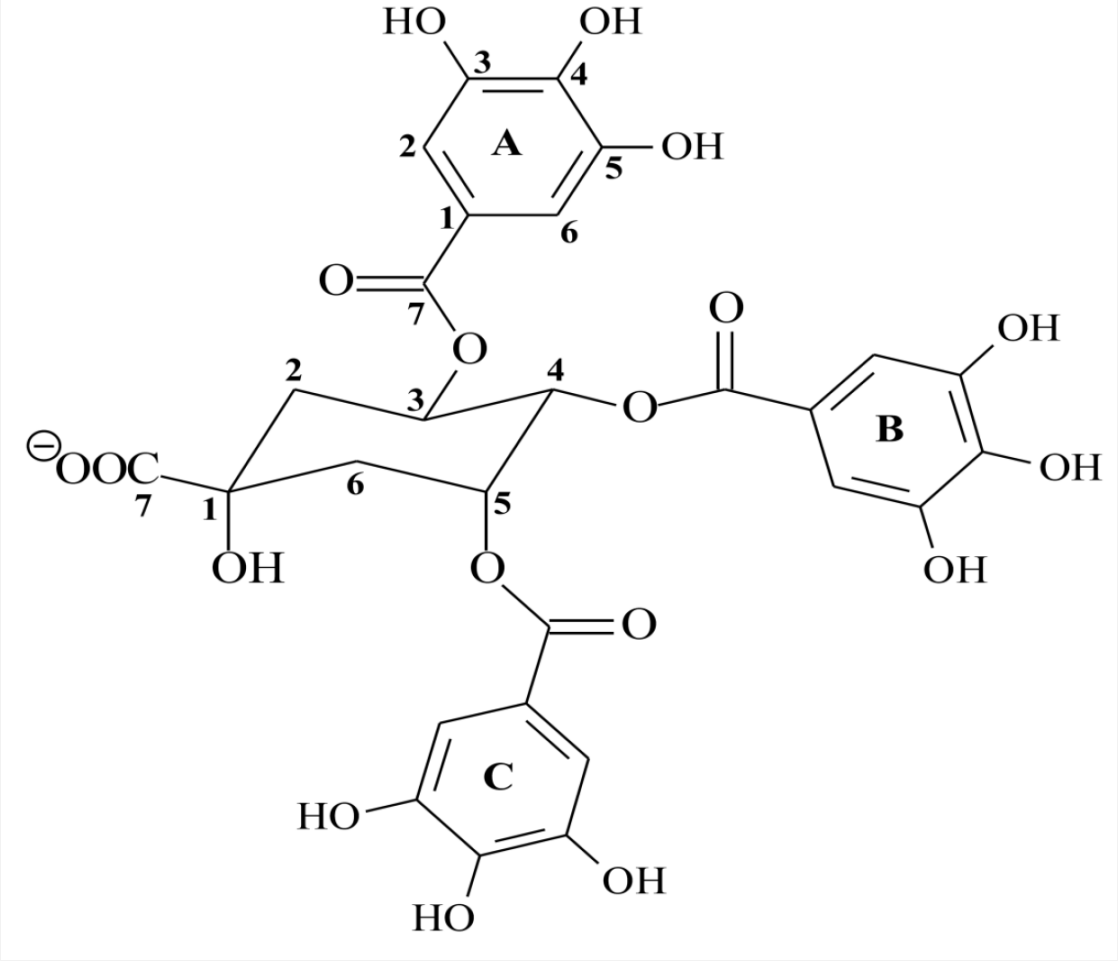

Figure 1: Structure of 3,4,5 tri-O-galloylquinic acid from *M. flabellifolius* (Moore et al. 2005)

Furthermore, Shona healers have used the plant to treat epilepsy, madness and coughs (Molefe-Khamanga et al. 2012a). Viljoen et al. (2000) reveals some chemistry literature which states that eighty-five compounds were identified in the hydro-distilled essential oil representing 87.79% of the total composition of *M. flabellifolius*. Molefe-Khamanga et al (2012a) has reported the existence of alkaloids, flavanoids, gums, glycoside, saponins, reducing sugar, amino acids, phenolics, tannins and steroids, however, the anthraquinones and triterpenoids were not detected. They have also reported that the total ash of the plant powder was found to be 3.03% w/w, water soluble ash was 55.9% w/w and sulphate soluble ash was 3.58% w/w. Molefe-Khamanga et al (2012b) reported that *M. flabellifolius* belongs

to the naturally caffeine free teas as it was confirmed by high performance liquid chromatography (HPLC) and thin layer chromatography (TLC) whereby no caffeine was detected from extracts solvent from *M. flabellifolius* solvent extracts samples.

This study was therefore aimed to evaluate the phytochemical contents and antioxidant activity of three solvent extracts of two plant parts of *M. flabellifolius*.

2 Materials and methods

2.1 Plant material

M. flabellifolius samples were collected from Remhoogte pass, 130 km south west of Rehoboth in Khomas region. The samples were transferred to the biotechnology laboratory of the Department of Chemistry and Biochemistry, University of Namibia (UNAM). Leave and twigs samples were washed in distilled water and dried at 40°C over night. The dried plant materials were ground into powder in an electric blender and stored in aseptic plastic bags at 4°C for further analysis.

2.2 Qualitative Analysis of Phytochemicals

2.2.1 Preparation of Ethanol and Methanol Extracts

About 5g of each fine powder was subjected to soxhlet extraction for 1 hour at 70°C according to Nikhal et al (2010) and macerated for 48 hours at room temperature (Ncube, 2008). Three solvents ethanol, methanol and water were prepared for the qualitative phytochemical screening and quantitative antioxidant activity determination. All extracts were filtered using Whatman^{TM} syringe filters 0.45 μm NYL W/GMF (Whatman GmbH, Dassel, Germany) and concentrated using a rotary evaporator (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). The concentrated extracts were stored at 4°C.

2.2.2 Phytochemical Screening

The ethanolic, methanolic and aqueous extracts were subjected to various qualitative phytochemical tests to detect alkaloids, leucoanthocyanins, polyphenols and reducing sugars (Obasi et al. 2010), anthocyanins, proteins and amino acids (Audu et al. 2007), anthranoids,

iridoids and triterpenes (Roopashree et al. 2008), anthraquinones, saponins and steroids (Hettiarachi 2006), cardiac glycosides and terpenoids (Ayoola et al. 2008), flavonoids (Aiyegoro et al. 2009), tannins and phlobatanins (Egwaikhide and Gimba 2007) respectively.

2.3 Quantitative Analysis of Phytochemical Classes

2.3.1 Total Phenolic Content (TPC)

Folin-Ciocalteu method (Singleton & Rossi, 1965) was used to determine the total phenolic content in the three plant extracts. An amount of 100μl of Folin-Ciocalteu was added to each extract (500μl) and incubated for 15 minutes at room temperature in the dark. A saturated sodium carbonate (2500μl) was added and incubated for a further 30 minutes at room temperature before reading the absorbance at 760nm using a spectrophotometer (GeneSyn 20, Thermo, USA), and gallic acid was used in the construction of the standard curve. Estimation of the phenolic compounds was carried out in triplicates. The results are mean values and expressed as mg of gallic acid equivalent (GAE)/g of dry extract (McDonald et al. 2001).

2.3.2 Total Flavonoid Contents (TFC)

The aluminium chloride colorimetric method as described by Chang et al (2002) with some modifications was used for this analysis. Each extract (1 ml) in methanol was separately mixed with 1 ml of methanol, 0.5 ml of 1.2% aluminium chloride, 0.5 ml of 0.12 M potassium acetate and 2.8 ml of distilled water. Mixtures remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm using a spectrophotometer (GeneSyn 20, Thermo, USA). Total flavonoid content of the extract was expressed as milligram equivalents to quercetin per gram (mg QE/g) dry weight of the extract (Ordonez et al. 2006).

2.4 Statistical Analysis

All analytical determinations and measurements were performed in triplicates. Values of different parameters are expressed as the mean ± standard deviation.

3 Results and Discussion

3.1 Phytochemical Screening

Phytochemicals are bioactive, non-nutrient, naturally occurring compounds in plants (Okarter et al. 2009). The various phytochemical compounds detected in *M. flabellifolius* plant extracts (Table 1) are known to have health benefits, physiological activities and medicinal importance (Jaberian et al. 2013). Flavonoids, anthocyanins, alkaloids, steroids, terpenoids, triterpenes, cardiac glycosides, saponins, phlobatanins, tannins, polyphenols and reducing sugars tested positive in the leaves extracts (Table 1).

Table 1: Phytochemical Screening of Ethanol, Methanol and Water Extracts of *Myrothamnus flabellifolius*.

Phytochemical	Leave extracts			Twig extracts		
Classes	Water	Methanol	Ethanol	Water	Ethanol	Methanol
Flavonoids	+*	+	+	-*	-	-
Steroids	-	+	-	-	-	-
Terpenoids	+	-	+	-	+	+
Triterpenes	-	-	+	-	-	-
Cardiac Glycosides	-	+	-	+	+	+
Anthraquinone	-	-	-	-	-	-
Saponins	+	-	-	-	-	+
Phlobatannins	+	-	-	-	-	-
Tannins	-	-	+	+	-	-
Alkaloids	-	+	+	+	+	+
Polyphenols	-	+	-	-	-	-
Iridoids	-	-	-	-	-	-
Anthocyanins	-	+	+	-	-	-
Leucoanthocyanins	-	-	-	-	-	-
Reducing Sugars	-	+	+	-	-	-
Proteins & Amino acids	-	-	-	-	-	-
Anthranoids	-	-	-	-	-	-

*+ present, - absent

These results are in agreement with Pizzi and Cameron (1986) who proposed that the *M. flabellifolius* leaves possess high tannin content. It is also in agreement with Molefe-Khamanga et al (2012a) who reported on the presence of alkaloids, flavonoids, gums, glycoside, saponins, reducing sugar, amino acids, phenolics, tannins and steroids in the South African *M. flabellifolius* methanol and water extracts. However, anthraquinones, anthranoids, iridoids, leucoanthocyanins, proteins and amino acids tested negatively in all three

solvent extracts from both leaves and twigs (Table 1). This result is in agreement with Molefe-Khamanga et al (2012a,b) who reported that anthraquinones and triterpenoids and caffeine were not observed in both water and methanol extracts of the South African *M. flabellifolius*. The above results suggested that there are similarities and differences between *M. flabellifolius* grown in Namibia and South Africa. This is in agreement with Moore et al (2005) and Gechev et al (2014) who indicated that there are differences and similarities in the chemical profiles of the two species, as well as variation between populations from different regions.

Water leaf extract was tested positive for flavonoids; however, anthocyanin's which is a water soluble flavonoid was tested negative, this can be explained as that the leaves contains other types of water soluble flavonoids. The same case was observed for the alkaloids, whereby the leaves of this plant contain water insoluble alkaloids but the twigs contain water soluble alkaloids. The presence of alkaloids was easily detected by ethanolic and methanolic extracts (Table 1). The presence of reducing sugars suggests that glucose, maltose, fructose or lactose is present in the leaves extracts of *M. flabellifolius* (Table 1). Phytochemical testing revealed that both plant parts extracts contain saponins and tannins but these compounds are extracted with different solvents regarding each extract. In addition, the leaves of this plant contain water insoluble tannins and water soluble phlobatanins (Table 1). Extraction of water soluble flavonoids and phenolics are amongst the reasons for the choice of this solvent (Das et al. 2010).

Water can also be used to extract phytochemicals anthocyanins, tannins, saponins and terpenoids. Generally, traditional healers used water during treatment preparations of many diseases by *M. flabellifolius* (Moore et al. 2007). Ethanol can be used to extract tannins, polyphenols, flovonols, terpenoids, sterols and alkaloids (Tiwari et al. 2011). The presence of higher amounts of polyphenols in ethanolic extracts as compared to aqueous extracts is solely the reason for the higher antioxidative potency of ethanolic extracts as compared to aqueous extracts. Methanol as a solvent has priority for extraction of plants for evaluating their antioxidant activity. Methanol is used to extract anthocyanins, terpenoids, tannins, saponins, flavones and polyphenols alkaloids (Tiwari et al. 2011). The testing for tannins and polyphenols was encouraged by the fact that an extract of *M. flabellifolius* leaves has been reported to be used by indigenous people in Namibia as an aid in wound healing and to treat asthma and general chest ailments.

In line with this, gallotannins are known for their anti-burn properties (Onayade et al. 1996) and galloylquinic acids have been identified as possessing high activity against bronchial hyper-reactivity and allergic reactions (Neszmelyi et al. 1993). Moore et al (2007) and Lohr et al (2011) reported on the wide uses of *M. flabellifolius* in traditional African medicine to treat chest complaints (smoke of burning leaves), and wounds (ointments for topical application), and to treat cough, influenza, mastitis, backaches, kidney disorders, hemorrhoids, abdominal pains, scurvy, halitosis and gingivitis (tea or decoction).

Epidemiological studies suggest that the consumption of polyphenolic flavonoid is effective in lowering the risk of coronary heart diseases (Rice-Evans and Miller, 1995), thus *M. flabellifolius* could be useful in treating coronary heart disease, which support the ethno medicinal claim of the use of the plant in the management of a stroke. Terpenoids rich plants are widely used in herbal medicine (Hayashi et al. 1993). Flavonoids, tannins, alkaloids and saponins are generally more soluble in polar solvents (El Hajaji et al. 2011). Tannins are reported to exhibit antiviral, antibacterial, anti-tumor activities (Chung et al. 1998). It was also reported that certain tannins are able to inhibit HIV replication selectivity and are also used as a diuretic (Heslem, 1989). Saponins are considered by some to be as natural antibiotics and are produced by plants to stop bacterial and fungal attacks (Okwu and Emenike, 2006). Therefore, the detection of saponins in the extracts of *M. flabellifolius* could contribute to their antimicrobial properties. Saponins are used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins; also it is used in hyperchloles-trolaemia, hyperglycaemia, anti-cancer, anti-inflammatory and anti-fungal properties (Aiyelaagbe and Osamudiamen, 2009).

Table 2: Total Phenolic and Flavonoid Contents of Ethanol, Methanol and Water Extracts of *Myrothamnus flabellifolius*.

Plant Part	Solvent	TPC (mgGAE/g)	TF (mgQE/g)
Leaves	Ethanol	373.88±0.21	3.49±0.15
	Methanol	374.44±0.21	3.48±0.15
	Water	372.42±0.21	3.29±0.21
Twigs	Ethanol	374.56±0.19	1.43±0.03
	Methanol	373.39±0.20	3.22±0.06
	Water	375.14±0.21	3.35±0.14

The values are mean±standard deviation mean ($n = 3$);
GAE: Gallic acid equivalent, QE: Quercetin equivalent.

3.2 Quantification of the Phytochemical Compounds

TPC of the twigs' aqueous extract (Table 2) showed the highest values (375.14±0.2) mg GAE/g; meanwhile the leaves' aqueous extract (372.42±0.2) mg GAE/g was the lowest. The phenolic contents of *M. flabellifolius* (Table 2) may contribute directly to its antioxidant activity. Naturally occurring phenolic compounds, such as phenolic acids, flavonoids, curcuminoids, lignans, tannins, stilbenes, quinones and others have been reported to be significantly associated with the antioxidant activity of plant and food extracts (Huang et al. 2010). This is mainly due to their redox properties, allowing them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers, and metal chelators (Gupta and Prakash, 2009). TFC in *M. flabellifolius* extracts was determined based on

the formation of flavonoid-aluminium complexes and absorbance measured at spectrophotometrically at 415 nm. The total flavonoid of *M. flabellifolius* extracts (Table 2) varied from 1.43 ±0.03 to 3.49 ±0.15 mg QE/g dry extract. Total flavonoid in leaves extracts was relatively higher than twigs extracts (Table 2). It is well-known that flavonoid contain hydroxyl functional groups which are responsible for antioxidant effect in the plants (Jaberian et al. 2013). Aiyelaagbe & Osamudiamen (2009) reported that flavonoids compounds from in Mangifera indica leaves extracts showed anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. They have been referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergies, virus and carcinogens (Aiyelaagbe and Osamudiamen, 2009). The TPC and TFC can be considered as important indicators for the antioxidative capacity of phytochemicals from *M. flabellifolius* that is intended to be a natural source of antioxidants.

4 Conclusion

The qualitative phytochemical screening of *M. flabellifolius* extracts revealed the presence of flavonoids, anthocyanins, alkaloids, steroids, terpenoids, triterpenes, cardiac glycosides, saponins, phlobatanins, tannins, polyphenols and reducing sugars in both tissues. Meanwhile, anthraquinones, anthranoids, iridoids, leucoanthocyanins, proteins and amino acids were absent in all extracts. Phytochemical screening of *M. flabellifolius* revealed differences in the constituents of the leaves and twigs extracts. TPC and TFC determination revealed high concentration of TPC in twigs extracts and high concentration of TFC in leaves extracts of *M. flabellifolius*. In this study, the phytochemical constituents of *M. flabellifolius* are presented as possible rationale for the traditional uses of this plant in the Namibian traditional healing system to successfully treat different disorders and ailments.

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