A Pilot Study of the Phytochemical Composition of Ethanolic Extracts from Eight Samoan Medicinal Plants

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Abstract- The therapeutic effects of plants are attributed to their phytochemicals. Consequently, the preliminary screening of phytochemicals has become an invaluable step in the discovery of pharmaceutical drugs. There is currently no information available in the literature on the phytochemical composition of Samoan medicinal plants. This study presents for the first time the quantification of some phytochemicals present in eight commonly used Samoan medicinal plants – *Syzygium inophylloides*, *Clerodendrum inerme*, *Kalanchoe pinnata*, *Vernonia amygdalina*, *Homalanthus nutans*, *Annona muricata*, *Chamaesyce hirta*, and *Morinda citrifolia*. The phytochemicals analyzed by UV-Vis spectroscopy were alkaloids, phenols, saponins, tannins and flavanoids. Results showed that *H. nutans* and *A. muricata* contained the highest concentrations of total alkaloids, with concentrations of 35 and 30 mg/g, respectively. *V. amygdalina* and *M. citrifolia* expressed the highest concentrations of total phenols, with concentrations of 31 and 27 mg/g, respectively. All extracts contained no saponins and relatively low amounts (< 10 mg/g) of tannins and flavonoids. There is no clear cut correlation between the results obtained in this study and the traditional usage of the tested plants. However, results show that plants tested have the potential to treat additional diseases. Comparison of results to literature values suggests that an improvement on the extraction method might be required in future studies.

Index Terms- Samoa medicinal plants, phytochemicals, traditional medicines, bioactive natural products, UV-Vis spectroscopy

I. INTRODUCTION

There is virtually an inexhaustible array of molecular compounds produced by nature through animals, plants and microbes. Traditionally, these natural products offer a rich spring of compounds for drug discovery due to their biological activities. In the past two decades, screening efforts have shifted from natural products to combinatorial chemistry due to the incompatibility of the former with modern high-throughput screening methods.[1,2] However, natural products have remained the best source of drugs and drug leads owing to their structural and chemical diversity that is unmatched by any synthetic library.[2,3] This is evidenced by the recent revival of interest in natural products as lead compounds in key therapeutic areas.[4]

The majority of the world's population relies on traditional medicine for their primary health care needs, mainly utilizing plant extracts and their biologically active natural products, otherwise known as phytochemicals.[5] Traditional knowledge on medicinal plants is a beneficial preliminary screening strategy for identification of novel bioactive compounds.[6–8] This is supported by historical discoveries such as that of the natural product galegine, which became the basis for the development of metformin - the most commonly prescribed drug for type-2 diabetes.[8,9] Unfortunately, utilizing ethnopharmacological data as a tool in drug discovery is threatened by the continuing extinction of biodiversity and generational loss of traditional medicinal knowledge.[8]

Scientists from many countries including those from nearby Pacific islands such as Tahiti, Fiji, and New Caledonia are presently active in natural products research utilizing their traditional knowledge and biodiversity. At present, Samoa has a rich pharmacopoeia, with names for various internal and external disorders, and a host of appropriate remedies.[10–13] However, very little research has been published on phytochemicals from Samoan medicinal plants, and none in the past 20 years. The most important work was carried out by Gustafson and colleagues more than 25 years ago on the Samoan mamala, otherwise known as *Homalanthus nutans*, species.[14] The study by Gustafson revealed that the mamala exhibits strong anti-HIV properties, which has been attributed to the phorbol ester – prostratin. The traditional healers have been using a concoction from *H. nutans* for treatment of hepatitis with no awareness of the plant's strong anti-HIV properties. Prostratin that was discovered by Gustafson *et. al.* has reached Phase I of human clinical trials as a promising anti-HIV drug lead.[15] This is an important example of how critical understanding the phytochemical composition of a medicinal plant is to realizing its full therapeutic potential.

The objective of the present study was to carry out a pilot study on the phytochemical composition of eight commonly used Samoan medicinal plants to provide not only a scientific basis for their usage as traditional medicines but also insight into their full potential. This was achieved by quantitative analyses of five of the major classes of phytochemicals - alkaloids, phenols, saponins, tannins and flavanoids – present in ethanolic extracts of eight commonly used Samoan medicinal plants. The overall objective was to provide initial critical mass for future in depth studies by the National University of Samoa.

II. METHODOLOGY

A. Plant Selection

Based on interviews with traditional healers, past and present recipients of traditional medicine, and the general public, eight of the most commonly mentioned Samoan medicinal plants were selected for the study. Table I lists detailed information on each plant.

B. Preparation of Plant Extracts

In the interest of minimizing damage to plants, leaves and fruits were the only plant parts collected for this study. Plant parts were sent to the Ministry of Natural Resources and Environment for authentication by an experienced botanist. Fresh plant parts were washed thoroughly to remove foreign matter and oven-dried at 45 ºC for approximately 5 hours or until a stabilized collective weight was obtained. The dried materials were ground with a mortar and pestle to obtain a fine powder. The weight of dried powder was measured and macerated in 1 L of ethanol three times in a period of 48 hours. The mixture was filtered through a Whatmann No.1 filter paper. The collected filtrate was concentrated using a rotary evaporator (Buchi Rotavapor R-210), and subsequently subjected to preliminary phytochemical screening and quantitative analysis by UV-Vis spectroscopy.

C. Quantitative Analyses

The UV-Vis analysis was carried out at the Scientific Research Organisation of Samoa using previously published methods.[16–19]

Total Alkaloids

A 1 mL volume of plant extract was dissolved in 2N HCl and filtered. The pH of the phosphate buffer solution was adjusted to 4.7 using 0.1 N NaOH. A volume of 1 mL of this solution was transferred to a separating funnel and 5 mL of bromocresol green solution plus 5 mL of phosphate buffer was added. The mixture was shaken, and added chloroform to extract the complex formed by vigorous shaking. The extract was collected in a 10 mL volumetric flask, and diluted to volume with chloroform. The absorbance of the complex was measured at 470 nm. Atropine was used as the reference standard.

Total Phenols

A 1.0 mL volume of filtrate (1mg/mL) was mixed with 5 mL of Folin-Ciocalteu reagent (1:10 v/v distilled water) and 4 mL (75 g/L) of sodium carbonate solution. The mixture was left to stand for 30 min at 40 ºC for colour development. The absorbance was read at 765 nm against a blank using double beam UV-Visible spectrophotometer. Gallic acid was used for generating a calibration curve (mg/g).

Total Saponins

A 100 mL volume of octanol was added to 2 g of extract. The mixture was shaken for 5hr to ensure uniform mixing before filtering through a Whatman No. 1 filter paper. A 20 mL volume of 40 % saturated solution of $MgCO_3$ was added to neutralise the filtrate obtained. The mixture was filtered twice to obtain a clear colourless solution. To 1 mL of the clear solution, 2 mL of 5 % $FeCl_3$ solution was added and the volume was brought up to 50 mL with distilled water. The mixture was allowed to stand for 30 min for developing a blood red colouration. The absorbance was measured at 380 nm and compared to 0-10 ppm standard saponins solutions.

Total Tannins

A 1 mL sample extract was pipetted into 50 mL volumetric flask, and diluted with 20 mL of distilled water. Subsequently, 2.5 mL of Folin-Denis reagent and 10 mL of 17 % Na_2CO_3 were added to the mixture, which was then shaken thoroughly. The final volume was made up with distilled water. The mixture was allowed to stand for 20 min for colour development (bluish-green colour). A standard tannic acid solution of range 0-10 ppm was treated similarly as 1 mL of the sample extract above. The absorbance of samples and standards were then measured at 760 nm.

Total Flavanoids

The plant extract (0.5 mL of 1:10 g mL^{-1}) in ethanol was mixed with 1.5 mL of ethanol, 0.1 mL of 10 % aluminium chloride, 0.1 mL of 1 M potassium acetate and 2.8 mL of distilled water. The mixture was left to stand at room temperature for ~30 min. for colour development. The absorbance was then measured at 415 nm. The calibration curve was prepared using quercetin standard solution.

III. RESULTS & DISCUSSION

Although traditionally, water is used primarily by healers for extracting phytochemicals, absolute ethanol was used in this study. This was due to reports that higher activity is seen with ethanolic extracts relative to aqueous extracts.[20] Additionally, ethanol is more efficient in penetrating the cellular membrane of plant materials to extract the intracellular components.[20] Analyses by UV-Vis have shown that all extracts contain alkaloids, phenols, flavanoids and tannins but no saponins. The negative tests for saponins can be attributed to the method of extraction, as water and methanol have been reported to be more effective solvents for extraction of saponins.[20] The different classes of phytochemicals were detected as present at varying proportions as shown in Table II. *H. nutans* and *A. muricata* contained the highest amounts of alkaloids, 35 mg/g and 30 mg/g, respectively; while *V. amygdalina* and *M. citrifolia* contained the highest amounts of phenols, 31 mg/g and 27 mg/g, respectively. In general, all extracts contained < 10 mg/g of flavanoids and tannins, except for *C. inerme*, which contained 1.5 mg/g of tannins.

Phytochemicals are biologically active compounds produced by plants to flourish, drive away predators or inhibit the growth of competitors (allelopathy). The studied phytochemicals have a wide range of bioactivities that have important applications for humans. Studies have shown that alkaloids possess therapeutic effects against multiple neurodegenerative disorders, such as Alzheimer's disease, Parkinson's disease, Huntington's disease and stroke.[21] Additionally, alkaloids have excellent antioxidant and anti-inflammatory properties.[22–24] Antioxidants regulate and reduce the oxidative damage by either minimizing or preventing oxidation resulting from reactive oxygen species. Like alkaloids, phenol, flavonoids and tannins are phenolic phytochemicals that

Table I: List of 8 Samoan medicinal plants and their usage.

Plant Name	Family	Plant Medicinal Usage	
		Literature	Informant
A. muricata	Annonaceae	-	Leaves are boiled, and the juice is ingested for high blood pressure, cancer, gout and regulating hormones.
V. amygdalina	Asteraceae	-	Leaves are boiled, and the juice is ingested for losing weight, high blood pressure, diabetes, cancer and regulating hormones.
K. pinnata (L.) Pers	Crassulaceae	-	Leaves are chewed to treat diabetes or ground and applied directly to wounds as an antiseptic.
C. hirta (L.) Millsp. also known as *Euphorbia hirta*	Euphorbiaceae	The plant is regarded as an anti-asthmatic and applied to skin eruptions.[10]	The entire weed is ground, and the juice is squeezed onto gums of a baby to soothe symptoms of teething.
H. nutans (G.Forst.) Guill.	Euphorbiaceae	Leaves used to treat circumcision wounds, sores and cuts as well as elephantiasis.[10] Leaves used in water infusions to treat back pains and abdominal swelling.[14]	Leaves are boiled, the juice is ingested for treatment of breast cancer.
C. inerme (L.) Gaertn.	Lamiaceae (formerly Verbenaceae)	Leaves applied with sap of the *Canarian vitiense* A. Gray, to treat sores with appearance similar to impetigo.[11] Leaves used as part of remedies for vomiting blood, fever and as anticoagulant.[10] Plant used for wounds. [10]	Leaves used as poultice for treatment of skin infections and cuts.
S. inophylloides (A. Gray) Müll.Stuttg.	Myrtaceae	Leaves used for sores and as ghost medicine.[10]	The inner bark is grated to treat skin infections and boils.
M. citrifolia L.	Rubiaceae	Fruit for cough, to treat worms, used in eye lotion and to treat tuberculosis.[10]	Juice for treating pimples/acne, high blood pressure and diabetes.

- No literature available

Table II: Quantitative estimation of phytochemicals (mg/g).

Species	Alkaloids	Phenols	Flavonoids	Tannins
S. inophylloides	11	6	2	2
C. inerme	1	13	9	15
K. pinnata	14	6	5	10
V. amygdalina	11	31	5	9
H. nutans	35	20	5	9
A. muricata	30	4	3	5
C. hirta	20	8	1	3
M. citrifolia	20	27	3	3

make up the largest category of phytochemicals and are the most prevalent across the plant kingdom.[25] These phenolic compounds have versatile health benefits for humans due to their antioxidant, anticancer, antibacterial and skin protective properties.[25–28]

The literature was searched for comparison of data; this was difficult to do as experimental conditions, such as extraction solvent, solvent concentration, and extraction method, varied from one group to another. For example, Shibula and Velavan[29] have carried out a phytochemical investigation on the leaf of *A. muricata*. The results of this study are compared to the data of the present study as shown in Table III. The phytochemicals content is significantly higher in the leaf extract analyzed by Shibula and Velavan. This is most likely a result of different extraction solvent concentration as Shibula and Velavan used 70 % ethanol, as opposed to 90 % used in this study. On the other hand, concentrations reported by another group for *A. muricata* were two orders of magnitude lower for flavanoids (0.049 vs 2 mg/g) and three orders of magnitude lower for alkaloids (0.031 vs 30 mg/g), no total phenols, tannins or saponins concentration were reported.[34] This group used 10 % ethanol for extraction. The majority of other similar investigations contain only qualitative, rather than quantitative, analyses of phytochemicals. [19,30,31]

Table III: Comparison of data obtained in the present study (A) with those presented by Shibula *et al.* (B) and Onuah *et al.* (C)

	Alkaloids	Phenols	Flavanoids	Tannins	Saponins
A	11	6	2	2	0
B	85	179	121	20	36
C	0.035	-	0.049	-	-

According to Table I, only *C. hirta* is being used as an anti-asthmatic medicine. Alkaloids are also known for their muscle-relaxant property.[32] This property makes alkaloids useful for treating coughs and asthma. In fact, salbutamol one of the short-acting beta agonists compounds well known as the first-line therapy for some types of asthmas, is an alkaloid. The high alkaloid content in *H. nutans*, *A. muricata, C. hirta and M. citrifolia* suggest that these in addition to their local usage, can also be applied to treat asthma and coughs. Other properties of alkaloids not previously mentioned are anti-diabetic, anti-cancer and anti-microbial.[32] The presence of alkaloids in appreciable amounts in *H. nutans, A. muricata, C. hirta and M. citrifolia* provides scientific basis for their usage as anti-diabetic, anti-microbial (sores, elephantiasis, coughs, skin eruptions, tuberculosis), anti-asthmatic, anti-inflammation (acne, cough, sores) and anti-cancer medicines. *C. inerme* exhibits characteristics that can be attributed to alkaloids, however, the alkaloid concentration is relatively low (1 mg/g). This suggests that phenols (13 mg/g), flavonoids (9 mg/g) and tannins (15 mg/g) might be responsible for its therapeutic effects, as these phytochemicals also have anti-inflammatory and anti-microbial properties. Results obtained by Mujeeb *et al.*[33] have shown that phytochemical concentrations comparable to the ones reported here have antimicrobial activity.

There is no clear cut correlation between the local medicinal usage of the tested plants and data presented here. However, the presence of a variety of phytochemicals at appreciable amounts provides scientific basis for their medicinal usage. Because, the different classes of phytochemicals have similar properties, as well as encompass a sizable amount of diverse bio-active compounds, future studies will aim to identify specific compounds that are responsible for therapeutic effects, as it was done by Gustafson and group for prostratin.

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