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PHYTOCHEMICAL ANALYSIS IN ROOTS AND TUBERS OF BOTSWANA MEDICINAL PLANTS- *Solanum Aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla (Thunb)* AND *Adenia glauca*

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ABSTRACT

Medicinal plants play an important role in our life. Worldwide modern drugs used in healthcare are derived from plants. Several Rhizomes and tubers are important for people due to their edible as well as medicinal properties. The Rhizome and tuberous plants namely *Solanum aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla (Thunb), and Adenia glauca* which are indigenous plants of Botswana have been so far employed in the treatment of cutaneous, subcutaneous parasitic infection, wounds, dermatological diseases, gastrointestinal system disorders, sexual dysfunction, and sexually transmitted infections. This study was conducted to identify the phytoconstituents by subjecting them to Ultrasound-Assisted Extraction (UAE) with distilled water, methanol, ethanol, chloroform, and hexane, each extract of which was further applied to the phytochemical screening through qualitative and quantitative tests. The extracting yields of *Solanum aculeastrum dunal* with different solvents gave the values of 13.0% (hexane), 13.0% (chloroform), 66.6% (ethanol), 66.6% (methanol) and 46.6% (aqueous), *Elephantorrhiza elephentina* 13.0% (hexane), 20.0% (chloroform), 66.6% (ethanol), 80.0% (methanol) and 86.0% (aqueous), *Cadaba aphylla* 60% (ethanol), 66.6% (methanol) and 53.3% (aqueous), *Adenia glauca* 40% (ethanol), 40.0% (methanol) and 46.6% (aqueous). Standards were used to determine the total content of alkaloid, flavonoid, phenol, saponin, and tannin (Atropine, Quercetin, Gallic acid, Tannic acid, and Quillaja). The regression coefficient (R^2) was calculated and for Alkaloids, it is 0.9925, flavonoid (0.9953), phenol (0.9978), tannin (0.9955), and saponin (0.9912) respectively. These medicinal plants have produced many phytochemical substances in quantitative analysis, which will be applied in future drug analysis research.

Keywords: Qualitative analysis, Quantitative analysis, Botswana medicinal plants, UAE method, Healthcare.
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INTRODUCTION

Medicinal plants play a vital role in the lives of both humans and animals. The world survives owing to various unique plant-based drugs; nevertheless, without plants on the earth, human existence would be impossible to live.[1] In ancient times, most diseases like Cholera, Typhoid, Measles, and Gonorrhea were treated with traditional medicinal plants and their extracts. Thus, medicinal plants were a significant source in the past, and now various medications are derived from the phytochemical analysis.[2] *S. aculeastrum dunal* berries and leaves have been used to treat various diseases like different types of cancer, sexually transmitted diseases, skin infections, indigestion, a stomach infection, and gonorrhea.[3,4] *E.elephentina* tuber and root have been reported to possess several medicinal properties, and it's used to treat various illnesses like blood pressure, skin diseases, breast cancer, stomach problems, kidney failure, itching, tuberculosis, hamorrhoids, rheumatic conditions, pain, and peptic ulcer.[5] Traditionally, in Botswana and Southern parts of Africa the leaves, roots, and rhizome decoction are taken orally to clean the womb after abortion and treat fever, menstrual problems, ear pain, and erectile dysfunction.[6,7] *Cadaba aphylla* (Thumb) wild is a non-leafy plant with flowers, fruits, and many branches and is widely distributed in this region. The root is

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used to treat various ailments, including blood cleaning, sores, and fractures. Leaves and stems of *C.aphylla* are consumed orally to treat high blood pressure, gastrointestinal issues, and diabetes. Plant extracts are used as anticancer agents to treat various cancer cell lines.[8-11] *Adenia glauca* is a cactus plant. Africans utilize this herb in traditional medicine. *A.glauca* tuber has a lack of cyanogenic properties, the stem, leaves, and bark do, as well as the toxic fruits.[12,13] This plant was used to treat illnesses such as ear infections, skin infections, and swollen legs. Specific ailments can be cured by inhaling the smoke of this plant.[14] Plant extracts possess bioactive components that are known to be involved in their bioactivity. In earlier studies reported, conventionally phytochemical components are extracted using multiple solvents based on the polarity, and researchers normally employed dried powder of plant material to extract bioactive compounds and eliminate the interference of water at the same time.[15] However, the Ultrasonic assisted extraction method (UAE) is an effective method for plant extraction because it is less expensive, takes less time to complete, requires less equipment, and has less of an impact on the environment than conventional methods. The benefits of this approach include a shorter extraction time without affecting the compound's molar mass or molecular structure. In the UAE, quantitative yield is higher than using conventional methods by enhancing the surface contact between solvents and samples through the mechanical impact of vibrations from ultrasound, UAE helps to increase the penetration of plant cell walls to solvents.[16,17] Results from earlier research suggested that UAE is a more effective method to extract phytochemicals from plants.[15] Phytochemicals from natural sources play a key role in the preparation of novel drugs. Hence, the present study aimed to investigate the bioactive principles present in the rhizome and tuberous plants viz., *Solanum aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla* (Thunb), and *Adenia glauca* were analyzed in this study for phytochemical analysis. The indigenous plants, *Solanum aculeastrum dunal* "Solanaceae*",*[18] *Elephantorrhiza elephentina* "Fabaceae"*,*[19] *Cadaba aphylla* (Thunb) "Capparaceae" [20] and *Adenia glauca* "Passifloraceae" [21] respectively, the native varieties of South Africa to explore its medicinal properties.

EXPERIMENTAL

Collection and Preparation of Plant Samples

Solanum aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla (Thunb), and *Adenia glauca* were obtained from the Kanye, Mogoditshane, and Moshupa villages in Botswana's capital city, Gaborone. Only *Elephantorrhiza elephantina* plant powder was bought from a traditional herb's venter at the capital city center mall of Gaborone, Botswana. The plants were identified and authenticated by the Botswana National Herbarium and the University of Botswana Herbarium. The collected plants were washed with tap water to remove soil and dust particles. After washing with tap water and then rinsed with analytical water to remove unwanted particles, the plant parts were dried in sub-due lightroom for 15 days and ground to powder using Thomas Wiley Model 4 grinder, and the powder sample was stored in an amber bottle before extraction.

UAE Method of Extraction

1.000g of each sample was accurately weighed into a 15ml centrifuge tube and 10ml methanol, ethanol, chloroform, hexane and water to added as an extractant. The plant samples were vortexed for 5 minutes and then put into an ultrasonic bath at 25ºC for 30 min. Again, Vortex shaking was done for 1min then the sample was centrifuged for 10 minutes at 5000rpm. The remaining supernatant solution was filtered through a Whatman No.1 filter paper to remove the undissolved solid particles. This was followed by filtration with a 0.45µm Nylon filter membrane to remove suspended particles. The filtrate was then kept for further analysis.

Preliminary Phytochemical Qualitative Analysis

A conventional method was used to screen and identify the phytochemical components in *Solanum aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla* (Thunb), and tuber of *Adenia glauca* after solvent extraction with methanol, ethanol, chloroform, hexane, and water. Primary and secondary metabolites of phytochemical components such as alkaloids, flavonoids, terpenoids, tannin, phenol, carbohydrates, quinones, glycosides, steroids, lipids, and oils were detected from these solvent extracts.[22-27]

Phytochemical Quantitative Analysis

Quantitative analysis of phytochemical compounds such as alkaloids, flavonoids, tannin, phenol, terpenoids, and steroids was detected using a standard procedure in these roots and tubers. Standards were used to determine the total content of alkaloid, flavonoid, phenol, saponin, and tannin (Atropine, Quercetin, Gallic acid, Tannic acid, and Quillaja). Three times, these estimation processes were carried out at the same time.[28-36]

The Percentage Recovery of Phytochemicals

The dried samples after extracting with the solvents is determined for their solubility of phytochemicals according to their polarity nature and the percentage of compounds extracted is calculated using the formula:

$$\%\ of\ Phytocompounds = \frac{Number\ of\ compounds\ found}{Total\ number\ of\ Compounds} * 100$$

RESULTS AND DISCUSSION

Phytochemical Screening (Qualitative Analysis)

The present investigation evaluated 5 different solvent compositions (hexane, chloroform, ethanol, methanol, and water) for their relative capacity to extract phytochemicals from the indigenous herbs of Botswana, namely *Solanum aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla (Thunb), and Adenia glauca* and the results are shown in Table-1. The results of the screening show that Alkaloids, flavonoids, tannins, saponins, and terpenoids are found to be present in the methanolic, ethanolic, and aqueous extracts of all the species, Anthocyanins and Coumarins are present in methanol, ethanol and aqueous extract of *Elephantorrhiza elephentina, Cadaba aphylla (Thunb),* reducing sugars are found to be present only in *Elephantorrhiza elephentina's* methanolic, ethanolic and water extracts whereas glycosides are found in methanol, ethanol and aqueous extracts of *Solanum aculeastrum dunal* and *Adenia glauca.* Amino acids and proteins are present only in *Solanum aculeastrum dunal, Elephantorrhiza elephentina, and Cadaba aphylla (Thunb),* and absent in *Adenia glauca.* The absence of quinones is seen in almost all the extracts of these roots and tuber varieties.

Table-1: Phytochemical Qualitative Analysis of Five Extracts on *S.aculeastrum dunal* (root), *E.elephentina* (root), *C.aphylla* (Thunb) (root), and *A.glauca* (tuber).

S. No.	Plants	*S.aculeastrum dunal*					*E. elephentina*					*C.aphylla (Thunb)*					*A.glauca*				
	Compounds	H	C	E	M	W	H	C	E	M	W	H	C	E	M	W	H	C	E	M	W
1.	Alkaloids	-	C	A	A	A	-	-	A	A	A	-	-	A	A	A	-	-	A	A	A
2.	Flavonoids	-	C	A	A	A	-	-	A	A	A	-	-	A	A	A	-	-	A	A	A
3.	Amino acid	-	-	C	C	-	-	-	C	C	B	-	-	-	C	-	-	-	-	-	-
4.	Protein	-	-	C	C	-	-	-	C	C	B	-	-	C	-	-	-	-	-	-	-
5.	Anthraquinones	C	-	C	C	-	-	C	B	A	C	-	-	-	C	-	-	-	-	-	-
6	Steroids	C	-	B	B	-	C	-	C	A	C	-	-	B	-	-	-	-	-	-	-
7.	Terpenoids	-	-	B	B	B	B	-	B	A	A	-	-	B	B	B	-	-	C	C	C
8.	Tannins	-	-	A	A	A			A	A	A	-	-	A	A	A	-	-	A	A	A
9.	Saponin	-	-	B	B	B	-	-		A	A	A	-	A	A	A	-	-	B	B	B
10.	Anthocyanins	-	-	-	-	-	-	C	C	B	B	-	-	C	B	C	-	-	-	-	-
11.	Coumarins	-	-	-	-	-	-	-	C	C	C	-	-	C	C	C	-	-	-	-	-
12.	Reducing sugar	-	-	-	-	-	-	A	-	A	A	-	-	-	C	-	-	-	-	-	-

13.	Oils & fats	-	-	-	-	A	-	-	-	-	A	-	-	-	-	C	-	-	-	-	A
14.	Glycosides	-	-	C	B	C	-	-	-	-	-	-	-	-	-	-	-	-	C	B	C
15.	Quinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

A = Excellent, B = Good, C = Present, - = Absent
H -Hexane, C- Chloroform, E – Ethanol, M – Methanol, W – Water

Thus, from the qualitative screening, it is observed that these four herbs contain essential secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids essential for the growth, development, innate immunity, defense response signaling of plants acting as signals for symbiosis between plants and microbes, and modifying microbial communities associated with hosts, pest and pathogen repellents[37,38,39,40] and many positive beneficial effects in human health care.[41]

Percentage Yield

The dried plant samples after vortexed in the respective solvents were calculated for the percentage of phytocompounds separated in each extract. The results of extraction are shown in Fig.-1. Based on the polarity the solvents were chosen and the study attempted to identify the best solvent suitable for the efficient separation of compounds. The results showed a difference in the percentage of compounds extracted wherein the solvent polarity has directly influenced the extraction of phytocompounds as well as the chemical nature of the plant part (roots and tuber) varies in their solubility towards the given solvent. From the data, it is concluded that water had the highest yield of 86% for *Elephantorrhiza elephentina* along with methanol at 80% and ethanol at 66.6%. The methanol and ethanol recovery was 66.6% for *Solanum aculeastrum dunal*, and *Cadaba aphylla* 66.6% recovery in methanol, 60% recovery in ethanol, and 53.3% in the water while only 40% of the components were recovered in methanolic, ethanolic and 46.6% in water extracts of *Adenia glauca*. The non-polar solvents namely hexane and chloroform had the least % recovery of 13% and 20.0% for *Solanum aculeastrum dunal* and *Elephantorrhiza elephentina*. This leads to the conclusion that among the four medicinal herbs, *Solanum aculeastrum dunal, Elephantorrhiza elephentina, and Cadaba aphylla* contain potent phyto components compared to *Adenia glauca* of pharmacological importance responsible for curing chronic and acute ailments.

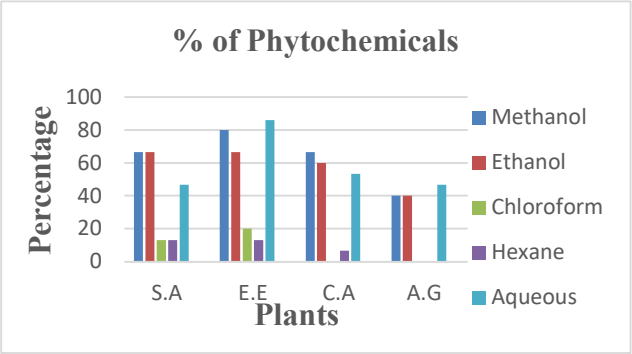

Fig.-1: Percentage Yield of Plant Extracts

Determination of Quantitative Analysis

The plant-based bio-active compounds as therapeutic agents have an effective dosage response with minimal side effects[42] hence quantification is needed as the results obtained could be utilized for drug development, standardization of these medicinal herbs, and interpret their medicinal value of them. The phytochemical alkaloids, flavonoids, terpenoids, phenol, tannin, and saponin were quantified in all four plants using a conventional method. Quantification of phytocompounds is reported in Table-2. The total amount of phenol (Gallic acid), flavonoid (Quercetin), tannin (Tannic acid), an alkaloid (Atropine), and Saponin (Quillaja) content were measured with standards and calibration curve calculated (Y = mx +b) and the graph plotted absorbance vs concentration (g /ml) are shown in Fig.-2, 3, 4, 5 and 6.

For precision purposes, all of the experiments were repeated in triplicate, and the data was calculated using the mean and standard deviation. When comparing the quantitative analyses of these plants, alkaloids and

flavanoids are present in the order *Elephantorrhiza* > *solanum* > *Cadaba* > *Adenia* and *Elephantorrhiza* > *Cadaba* > *Solanum* > *Adenia* respectively. While phenol components were abundant in *Solanum* and *Elephantorrhiza*, additional phytocompounds like tannin, saponin, and terpenoids were also detected. The study concludes that all the pharmacologically important secondary metabolites are present in ample amounts in the selected plant material except terpenoids. This confirms that these roots and tubers could be subjected to isolation of valuable Phyto materials to associate them in the drug discovery processes to cure diseases.

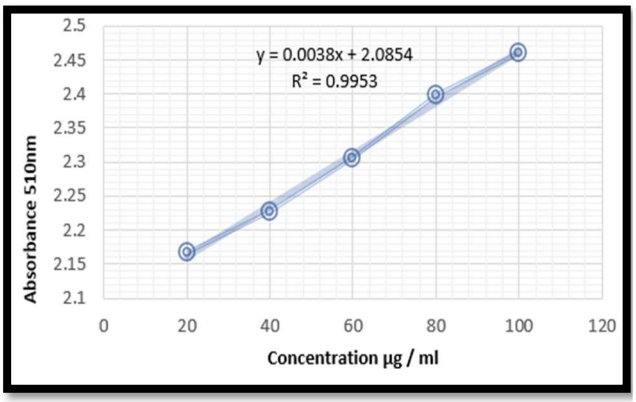

Fig.-2: Calibration Curve for Flavonoid Using Standard Quercetin (µg / ml)

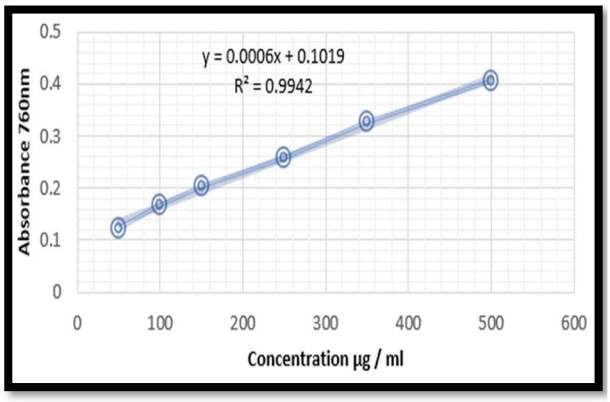

Fig.-3: Calibration Curve for Phenol Using Standard Gallic Acid (µg / ml)

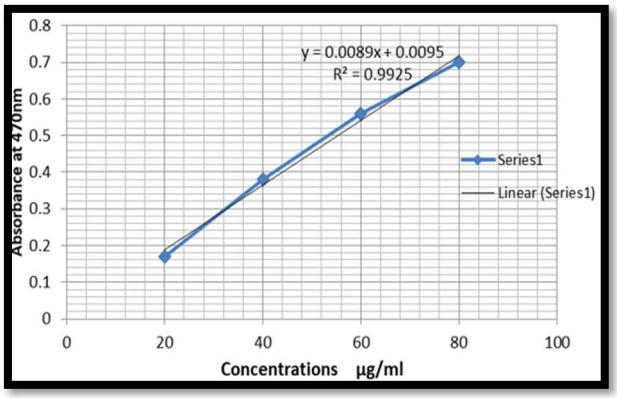

Fig.-4: Calibration Curve for Alkaloid Using Standard Atropine (µg / ml)

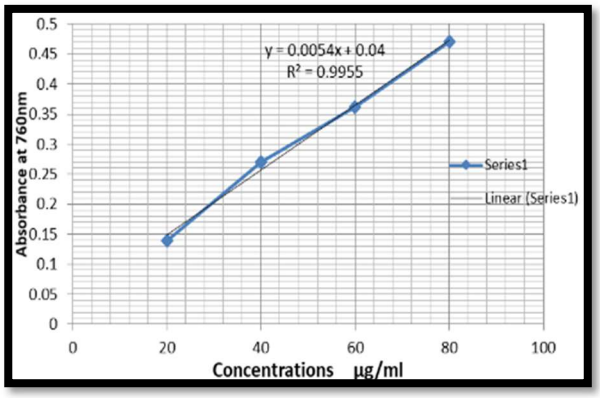

Fig.-5: Calibration Curve for Tannin Using Standard Tannic Acid (µg / ml)

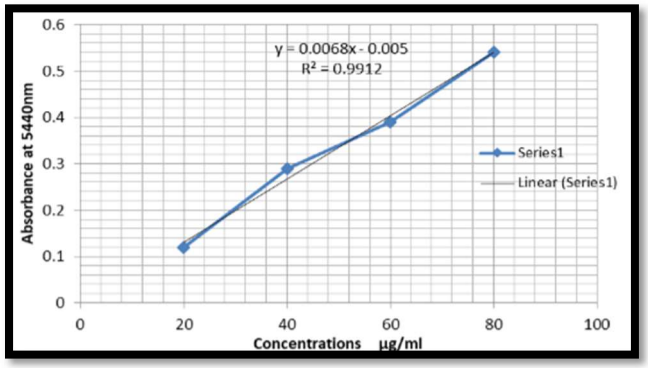

Fig.-6: Calibration Curve for Saponin Using Standard Quillaja (µg/ml)

Table-2: Phytochemical Quantitative Analysis of Four Plants

S.No.	Phytochemicals	Weight mg/gram			
		Solanum	Elephantorrhiza	Cadaba	Adenia
1.	Alkaloids	233.5 ± 14.21	265.5 ± 19.15	249.5 ± 17.43	201.5 ± 14.21
2.	Flavonoids	350.0 ± 28.9	108.03 ± 0.086	262.73 ± 11.98	267 ± 83.36
3.	Saponin	147.8 ± 10.34	168.2 ± 11.76	154.6 ± 10.82	141 ± 9.87
4.	Tannin	175 ± 12.25	207.4 ± 14.49	191.2 ± 13.38	169.6 ± 11.83
5.	Terpenoids	25.34 ± 0.4	81.44 ± 0 .23	10.66 ± 0.1	12.62 ± 0.8
6.	phenol	369.6 ± 10.37	411.4 ± 91.52	91.16 ± 0.10	107.16 ± 0.10

CONCLUSION

According to a survey conducted by the World Health Organization (WHO), plants are the primary source of basic health care. Because they contain more secondary metabolites, they have been utilized to cure various ailments. In a previous analysis, most researchers used conventional methods like Soxhlet, Percolation, Maceration, etc. The advantage of modern extraction methods like UAE and MAE is time-consuming and environmentally friendly. This is the first time the modern extraction method (UAE) was used to extract these four Botswana medicinal plants with different solvents. The results conclude that *Solanum aculeastrum dunal, Elephantorrhiza elephentina, Cadaba aphylla (Thunb),* and *Adenia glauca* are a source of phytochemicals. The presence of secondary metabolites is responsible for their therapeutic effects in folk medicine. It thus helps to develop many novel therapeutic agents from these plants or their templates. Thus, these plant parts (Roots and Tuber) have a wide range of medical applications.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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