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Determination of Types and Quantities of Secondary Metabolites in Niger Plant (Guizotia Abyssinica L) Found Within Moiben Sub – County, Kenya

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Abstract:
Plants are a major source of active chemical constituents that can be used against other plants. From natural ecosystems, it has been found that exudates from some plants influence the growth and development of other plants. Niger plant (Guizotia abyssinica) exudes secondary metabolites phenols, flavonoids, tannins and saponins that influence plant growth either growing together or in the succeeding season. The aim of this experiment was to extract, identify and quantify the secondary metabolites in Niger plant collected from within Moiben sub-county where it grows wild. Plant materials were collected from all administrative wards in Moiben sub-county and secondary metabolites extracted by standard procedures. The extracts were made from the whole plant, roots and shoots. Data were collected on the levels of alkaloids, flavonoids, phenols, saponins and tannins. The data collected were subjected to ANOVA in Genstat and means separated by Duncan's Multiple Range Test (DMRT). All the five metabolites tested for were present in the plant samples at different quantities. Niger plant contains many metabolites thus further studies should be done for better understanding and application of their allelopathic effect for weed control.

Keywords: *Ecosystems, exudates, metabolites, Niger plant*

1. Introduction

Plants produce chemical compounds as a result of secondary metabolism. The chemical compounds cannot be directly linked with plant growth and development but can influence several processes in the ecosystem. These compounds are known as secondary metabolites or all elochemicals. Important secondary metabolites include alkaloids, terpenes, phenolics and vitamins. Most secondary metabolites are toxic and therefore play defensive roles against biotic factors like protection from attack of pathogens, herbivores pests and weeds [10].

Due to this, plants can regulate the microbial community in their immediate vicinity, endure herbivores, encourage symbiotic improvement, change the physical and chemical properties of the surrounding environment and inhibit the growth of plant competitive species [13]. Depending on the specific allelochemical considered, the target plant and the concentration in the soil, allelochemicals can act either positively or negatively [6]. In agroecosystems, allelopathy can affect weed management, plant reproduction, species consortia, the mulching effect on crops and the succession and rotation of cultivated species [4]. Additionally, allelochemicals have the potential to be used for herbicide synthesis, enabling the discovery of new mechanisms of action.

Wise exploitation of allelopathy in cropping systems may be an effective, economical and natural method of weed management, and a substitute for heavy use of herbicides. Allelochemicals have a great potential as bio-herbicides since they have a shorter half -life and thus can be rapidly biodegraded. Their mode of action is different from synthetic herbicides owing to comparatively fewer halogen substituents and no unnatural ring structures [14]. Allelochemicals, also known as phytochemicals, are environmentally friendly because they have low or no toxicity to animals and beneficial insects, possess an array of activity with varying and diverse sites of action and have a comparatively high degradation rate [5]. Plants in the family Asteracea have been noted to be highly allelopathic [13]. It has been observed that, Niger plant, a plant in this family, is a good precursor for cereals, pulses and oil seeds, because crops following Niger plant have less weed infestation [2]. This experiment was therefore carried out to determine the presence and amount of secondary metabolites in Niger plant within Moiben sub-county.

2. Materials and Methods

A laboratory experiment was conducted in University of Eldoret, Chemistry department laboratory. The metabolites evaluated were phenols, saponins, tannins, flavonoids and alkaloids since they are among the major metabolites in plants.

2.1. Sample Collection and Extract Preparation

Complete Niger plant samples were harvested at flowering stage from the six administrative wards in Moiben sub-county where they grow wild as weeds. The samples were kept in zip lock bags and carried in a cooler box to University of Eldoret Chemistry laboratory. The samples were divided into two whereby in one half, the shoots were separated from the roots. In the other half, the samples were treated as whole plants. The plant samples were washed to remove dirt and oven dried at 72^0 C till constant weight. Using a hand held electric grinder, the samples were separately ground into fine powder. The powder was transferred into labelled 500 ml conical flasks into which distilled water was added. The mixture was stirred then left to stand for 2 hours after which it was filtered using a sieve then by Whatman filter paper (No. 1). The filtrate was collected in a beaker for further analysis.

2.2. Phyto Chemical Tests

2.2.1. Procedure for Phytochemical Test

The following tests were done following a standard procedure adopted by [11].

2.2.1.1. Tannins

A 100 mg plant powder was taken into a test tube and 3 ml of butanol-HCl reagent (95 ml of n-butanol and 5 ml of concentrated HCl) was added to it. The test tube was plugged cotton and heated at 70 ^{0}C on water bath for one hour. The formation of pink color confirmed the presence of tannins.

2.2.1.2. Phenols

Into a test tube containing 1 ml of extract, 2 drops of 5 % w/v ferric chloride were added. A greenish precipitate indicated the presence of phenols.

2.2.1.3. Flavonoids

To 5 ml of the extract, 2 ml of 10 % w/v sodium hydroxide (0.01 M) was added. There was formation of a yellow color. This was followed by addition of dilute hydrochloric acid. There was a change in color from yellow to colorless indicating the presence of flavonoids.

2.2.1.4. Saponins

- A 500 mg plant powder was extracted in 50% aqueous methanol. The extract was transferred
- Into a test tube and was well shaken by hand. Formation of persistent foam on the liquid surface
- Indicated the presence of saponins.

2.2.1.5. Alkaloids

A 500 mg oven dried plant powder was extracted with 3 ml of methanol containing 10% acetic acid. Ammonium hydroxide was added to it drop wise. Formation of precipitates indicated the presence of alkaloids.

2.2.2. Quantitative Analysis of Compounds

2.2.2.1. Determination of Total Phenols

Total phenol content was estimated using the Folin–Ciocalteau method as proposed by[3]. A measure of 0.5 ml Folin–Ciocalteau reagent was added to 1 ml of sample and incubated at room temperature for 3 minutes. After this, 2 ml of 20% Na2CO3 was added, mixed well and incubated in boiling water bath for 1 minute. The mixture was rapidly cooled and read at 650 nm absorbance against a reagent blank using UV spectrophotometer. The results were expressed as mg/g sample.

2.2.2.2. Determination of Flavonoids

From the sample extract, 1 ml was taken and mixed with 0.075 ml of 5% sodium nitrite solution then incubated at room temperature for 5 minutes. After this, 10% aluminum chloride was added and incubated at room temperature for 6 minutes. To the mixture, 1 N NaOH was added and absorbance read at 510 nm against a reagent blank. This procedure was given by [9].

2.2.2.3. Determination of Tannins

Tannins were determined by a method suggested by[3]. A sample extract measuring 1 ml was mixed with 5 ml of vannilin hydrochloride reagent and incubated at room temperature for 20 minutes. Absorbance was read at 500 nm against a reagent blank. The analysis was performed in triplicates, and the results were expressed as catechin equivalents.

2.2.2.4. Determination of Alkaloids

Determination of alkaloids was done by the procedure given by [8]. Plant material weighing 10 mg was homogenized in a motor and pestle into which 20 ml of methanol: ammonia was added (68:2). The mixture was decanted and after 24 hours fresh methanol: ammonia added. The procedure was repeated thrice and the extracts pooled together evaporated using a flash evaporator. The residue was treated with 1 N Hcl and kept overnight. The acidic solution was extracted with 20 ml of chloroform thrice, pooled together and the organic layers evaporated to dryness. The acidic layer was basified with concentrated sodium hydroxide to pH 12 and extracted with chloroform (20 ml) thrice. The chloroform layers were dried over absorbent cotton and evaporated to dryness. This was weighed and the fraction that contains alkaloids expressed as mg/100 g.

2.2.2.5. Determination of Saponins

Standard saponin solution was prepared by dissolving 10 mg of diosgen in, add 16 ml methanol and 4 ml distilled water. To the aliquots for each tube, 0.25 ml of 8 % vanillin reagent and 2.5 ml of 72 % v/v added slowly on the inner side of the wall. The solutions were mixed well and the tubes were transferred to a 60 ^{0}C water bath. After 10 mins incubation, the tubes were cooled in ice cold water bath for 4 min. A 0.1 g of freeze dried sample was dissolved in aqueous methanol (80%, 0.1 ml) and 0.25 ml of aliquot was taken for spectrophotometric determination for total saponins at 544 nm. This method was suggested by [8].

2.3. Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using Genstat version 14. Means were separated by Duncan's Multiple Range Test (DMRT) at 5 % level of probability.

3. Results

The results of the study presented in Tables 3, 4 and 5 below show the amount of secondary metabolites studied in extracts of the whole Niger plant, shoots and roots from different study sites within Moiben sub-county.

3.1. Phenols

Results showed that the whole plant, shoot and root extracts contained phenols though at varying levels. The level of phenols was highest in the whole plant extract, root and shoot extracts respectively (Table 3, Table 4 and Table 5). As pertains to site, there were significant differences in the level of phenols where Chepkoilel, Kimumu and Huruma were not significantly different from one another. Levels in Kimoning and Cheplaskei did not show any significant differences amongst themselves whereas Merewet,Tembelio, Sergoit, Moiben and Sigot did not have any significant differences. In Kaptuktuk and Kapsaos, alkaloid levels were significantly different from one another and distinct from all the rest of the sites. The highest levels of phenols was recorded in Chepkoilel, Sergoit and Moiben at 2.07 mg/g while the lowest amount was in Kaptuktuk (1.83 mg/g). The levels of phenols in the roots showed significant differences among the sites. Chepkoilel, Tembelio and Kapsaos samples had no significant differences amongst them whereas there were no significant differences between Kimoning, Kaptuktuk, Kimumu, Sergoit, Huruma, Moiben and Sigot sites. Kaptuktuk and Cheplaskei sites did not have significant differences amongst them. There were no significant differences in the amount of secondary metabolites in the shoots.

3.2. Tannins

Tannins are naturally occurring polyphenol compounds found in all plant parts. Samples collected from Moiben site had the highest mean of all metabolites (1.63 mg/g) followed by Sergoit site (1.61 mg/g). The least mean for all the metabolites was recorded in samples from Cheplaskei (1.47 mg/g).

Collection Site	Alkaloids(mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Tannins (mg/g)
Chepkoilel	1.60a	1.27a	2.07bc	0.57a	2.17a
Kimoning	1.53a	1.07a	1.90ab	0.53a	2.33a
Kaptuktuk	1.50a	1.03a	1.83a	0.60a	2.47a
Merewet	1.60a	1.17a	2.03abc	0.57a	2.57a
Tembelio	1.57a	1.07a	2.00abc	0.57a	2.47a
Kapsaos	1.50a	1.30a	2.17c	0.57a	2.30a
Kimumu	1.50a	1.23a	2.13bc	0.60a	2.57a
Sergoit	1.53a	1.10a	2.07abc	0.53a	2.63a
Huruma	1.50a	1.10a	2.13bc	0.57a	2.43a
Moiben	1.60a	1.17a	2.07abc	0.63a	2.67a
Sigot	1.57a	1.20a	1.97abc	0.63a	2.43a
Cheplaskei`	1.47a	1.13a	1.90ab	0.57a	2.27a
Mean	1.54	1.15	2.02	0.58	2.44
DMRT	0.0869	0.1428	0.0989	0.084	0.2438
CV%	6.9	15.2	6.0	17.8	12.2

Table 1: Results of Phytochemical Analysis of Niger Plant Extracts from Various Sites (Whole Plant)

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability

Collection Site	Alkaloids (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Tannins (mg/g)
Chepkoilel	1.00a	1.07b	1.77b	0.37a	1.67a
Kimoning	1.13a	0.80ab	1.53ab	0.27a	2.07a
Kaptuktuk	0.97a	0.83ab	1.30a	0.40ab	1.80a
Merewet	1.03a	0.90ab	1.53ab	0.43ab	1.83a
Tembelio	1.03a	0.90ab	1.70b	0.27a	2.07a
Kapsaos	1.07a	1.03ab	1.80b	0.30a	1.93a
Kimumu	1.03a	0.90ab	1.60ab	0.40ab	2.03a
Sergoit	1.20a	0.77a	1.57ab	0.27a	2.23a
Huruma	1.03a	0.90ab	1.60ab	0.40ab	1.97a
Moiben	1.03a	0.93ab	1.57ab	1.23b	2.17a
Sigot	1.07a	0.97ab	1.44ab	0.30a	1.80a
Cheplaskei	0.97a	0.80ab	1.30a	0.30a	1.80a
Mean	1.05	0.9	1.56	0.41	1.95
DMRT	0.13	0.12	0.16	0.37	0.26
CV%	15.80				

Table 2: Results of Phytochemical Analysis of Niger Plant Extracts from Various Parts of Moiben Sub County (Roots)

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability

Collection site	Alkaloids (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)	Saponins (mg/g)	Tannins (mg/g)
Chepkoilel	0.67a	0.37a	0.50a	0.47ab	0.60a
Kimoning	0.43a	0.40a	0.47a	0.43ab	0.53a
Kaptuktuk	0.50a	0.37a	0.53a	0.40ab	0.80a
Merewet	0.53a	0.40a	0.57a	0.37ab	0.77a
Tembelio	0.50a	0.37a	0.53a	0.47ab	0.50a
Kapsaos	0.43a	0.57a	0.67a	0.33a	0.53a
Kimumu	0.60a	0.43a	0.47a	0.47ab	0.70a
Sergoit	0.50a	0.47a	0.53a	0.40ab	0.57a
Huruma	0.50a	0.40a	0.60a	0.33a	0.63a
Moiben	0.63a	0.33a	0.50a	0.43ab	0.57a
Sigot	0.53a	0.43a	0.63a	0.50b	0.73a
Cheplaskei	0.60a	0.47a	0.50a	0.33a	0.70a
Mean	0.54	0.42	0.54	0.41	0.64
DMRT	0.12	0.11	0.15	0.07	0.13
CV%	27.7	32.1	34.8	20.1	24.6

Table 3: Results of Phytochemical Analysis of Niger Plant Extracts from Various Parts of Moiben Sub County (Shoots)

Means followed by different letter(s) in a column are statistically significant at 5 % level of probability.

3.3. Saponins

Saponin levels were significantly different amongst the sites both in the shoots and roots. In the root samples, Chepkoilel, Kimoning, Tembelio, Kapsaos, Sergoit, Sigot and Cheplaskei did not have significant differences among them. Kaptuktuk samples had significant differences from all the rest whereas Merewet, Kimumu and Huruma did not have significant differences amongst them but were significantly different from the rest. In shoot samples, there were three clusters of similarity: Chepkoilel, Kimoning, Kaptuktuk, Merewet, Tembelio, Kimumu, Sergoit and Moiben were not significantly different from one another. Kapsaos, Huruma and Cheplaskei samples were significantly different from the rest of the sites. Samples from Sigot showed unique results that were significantly different from all the rest.

3.4. Alkaloids

There were no significant differences in the level of alkaloids from all the sites both in the roots and shoots.

3.5. Flavonoids

There were significant differences in the level of flavonoids in the roots. Here, Chepkoilel and Sergoit sites were distinctly different from one another and from all the rest of the sites. However, there were no significant differences in the levels of flavonoids in the shoots.

4. Discussion

Results of the study revealed that Niger plant contains secondary metabolites at varying levels in the whole plant, roots and shoots.

Extracts from the whole plant had significant differences only in phenols. Here, Kaptuktuk had significant differences from all the other sites. The highest levels of phenols were recorded in Kapsaos while the lowest were in Kaptuktuk. All the other sites had intermediate levels between the two ranges.

In the root extract, there were no significant differences in the level of alkaloids and tannins. Flavonoid levels were not significantly different in Kimoning, Kaptuktuk, Merewet, Tembelio, Kapsaos, Kimumu, Huruma, Moiben, Sigot and Cheplaskei. However, there were significant differences between all the above sites, Chepkoilel and Sergoit. On levels of phenols, Kaptuktuk and Cheplaskei were significantly different from Chepkoilel, Tembelio and Kapsaos which were in turn significantly different from Kimoning, Merewet, Kimumu, Sergoit, Huruma, Moiben and Sigot. Saponins revealed Chepkoilel, Kimoning, Tembelio, Kapsaos, Sergoit, Sigot and Cheplaskei sites were significantly different from Kaptuktuk, Merewet, Kimumu and Huruma sites. Sergoit site was significantly different from all the other sites too.

In shoots, significant differences were recorded only in saponins. The highest levels of saponins were in Sigot (0.50 mg/g) and he lowest was in Kapsaos, Huruma and Cheplaskei all with 0.33 mg/g. The remaining sites had between 0.37 mg/g to 0.47 mg/ g and were not significantly different from one another.

There were differences in the levels of secondary metabolites in different parts of the plant. However in some, the levels in the roots were the same as those in the shoots. This can be attributed to the natural distribution of the metabolites in the plant parts where they are produced.

Different sites also revealed differences in the level of some metabolites in Niger plant. According to [12], ecological factors play a big role in affecting plant secondary metabolites. It is an inherent trait in plants that they resist biological, physical and chemical environmental stresses by regulating the accumulation of secondary metabolites in long periods ofadaptation to the environment [7]. Thus the present results can be attributed to the environmental conditions in the area that the Niger plants were collected from. Some factors have been singled out as having an effect on metabolite concentration in plants. Studies by [16] have shown that heat stress is an important environmental factor in promoting accumulation of secondary metabolites for Hypericum (Hypericum perforatum). This means that same plants exposed to different temperature levels can have different levels of metabolites.

Differences in soil conditions may contribute to the differences in plant metabolite levels in different sites. Inorganic elements in the soil have a major impact on the accumulation of secondary metabolites. It has been shown that different kinds of ecological factors have different extents of effects on different secondary metabolites of the root of S. baicalensis [1]. Inorganic elements in the soil including Mg, Mn, Cr and Fe affect different secondary metabolites [15].

5. Conclusions

- Niger plant contains phenols in both the roots and shoots at varying concentrations.
- Tannins were the least secondary metabolites in Niger plant among those that were studied.
- Saponis were present in Niger plant but the levels were significantly different amongst the sites both in the shoots and roots.
- Niger plant contains alkaloids though there were no significant differences from all the sites both in the roots and shoots.
- There were significant differences in flavonoid levels in the roots but the levels were not significantly different in the shoots.

6. Recommendations

Follow-up studies should aim to extrapolate these results and extend to the phyto chemistry and biological activity of secondary metabolites.

7. References

i. Abdul, K. K. A., Palwash, A., Ayeesha, M., Safdar, A. K. and Rasool, B. T. (2009): Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. Pakistan Journal of Botany. 41 (5): 2129-2135.

ii. Adarsh, V. M, Ajay, K. P., Kavitha, D., Anurag, K. B. (2014): Anti-denaturation and antioxidant activities of Annona cherimola in vitro. International Journal of Pharmacology and Biological Science. 2:1–6.

iii. Bray, H. G. and Thorpe, W. V. (1954): Analysis of phenolic compounds of interest in metabolism.Methods of Biochemistry. 1: 27-52.

iv. Chon, S. U., Jennings, J. A. and Nelson, C.J. (2006): Alfalfa (Medicago sativa L.) autotoxicity: Current status. Allelopathy Journal. 18: 57–80.

v. Cloyd, R. (2004): Natural instincts: Are natural insecticides safer and better than conventional insecticides? American Nurseryman 200: 41.

vi. Eichenberg, D., Ristok, C., Kroeber, W. and Bruelheide, H. (2014): Plant polyphenols - implications of different sampling, storage and sample processing in biodiversity- ecosystem functioning experiments. Chemical Ecology. 30:676 – 692.

vii. Ferreyra, M. L. F., Rius, S. P. and Casati, P. (2012): Flavonoids: Biosynthesis, biological functions, and biotechnological applications. Frontiers in Plant Science.

viii. Harborne J. B. (1973): Phytochemical Methods. London: Chapman and Hall, Ltd. p. 49-78.

ix. Jia, Z., Tang, M. and Wu, J. (1999): The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 64 (4): 555-563.

x. Khan, T., Zahid, M., Asim, M., Iqbal, Z., Choudhary, M. I., Ahmad, V. U. (2008): Pharmacological activities of crude acetone extract and purified constituents of Salvia moorcraftiana. Phytomedicine. 9 (8): 749-752.

xi. Makkar, P.S.H. and Goodchild, J. (1996): Quantification of secondary metabolites: A Laboratory Manual. International Centre for Agricultural Resources, Aleppo, Syria.

xii. Ncube, B., Finnie, J. F. and Van S. J. (2012): Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. African Journal of Botany. 82: 11–20.

xiii. Pedrol, J. and Salgueiro, M. (2012): Allelopathic effect of orange (Citrus sinensis L.) peel essential oil. Acta Botanica Brasilica. 26 (1): 256-259.

xiv. Roth, C. M., Shroyer, J. P. and Paulsen, G. M. (2000): Allelopathy of sorghum on wheat under several tillage systems. Agronomy Journal. 92:885–860.

xv. Zhao, M. Q, Lv, J. R, Guo, L. P. (2010): The effects of inorganic elements in soil on content of inorganic elements and baicalin in Sbaicalensis. Journal of Clinical Laboratory Science. 16: 103–106.

xvi. Zobayed, S. M. A., Afreen, F. and Kozai, T. (2005): Temperature stress can alter the photosynthetic efficiency and secondary metabolite concentrations in St. John's wort. Plant Physiology and Biochemistry. 43: 977–984.