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The Use Of Phytochemical Composition Of Fifty (50) Selected Plants Found In The University Botanic Garden, Maseno, Kenya To Show Classification Relationship Amongst Ten Selected Plant Families

Seline Omondi

School of Biological and Physical Sciences, Maseno University, P.O. Box 333-40105, Maseno, Kenya

J.C. Onyango

School of Biological and Physical Sciences, Maseno University, P.O. Box 333-40105, Maseno, Kenya

Samuel Okello

School of Biological and Physical Sciences, Maseno University, P.O. Box 333-40105, Maseno, Kenya

Pascaline Jeruto

University of Eldoret, School of Sciences, Department of Biological Sciences, P.O Box 1125-30100 , Eldoret, Kenya

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ABSTRACT

Qualitative phytochemical analysis of 50 plants, five each from 10 selected families is presented. The ten plant families were selected based on their high plant frequency of occurrence within the botanic garden. The powdered crude samples of the leaves of the fifty plants were subjected to phytochemical analysis using standard experimental procedures. The ethanoic leaf extracts from the plants were tested for the presence of six phytochemicals. From the research, it is evident that saponins are the most abundant phytochemicals among the plants leaf extracts. They account for 32.43 %, followed by alkaloids (27.03 %) then flavanoids (14.86 %), steroids (12 .16%), terpenes (10.81%) and anthraquinones (2.70 %) respectively. PRM3 statistical programmes was used to analyse the phytochemical data, and ANOVA at $P \leq 0.05$ revealed that plant families Poaceae, Solanaceae and Rutaceae all have phytochemical composition mean of 60.0, indicating that the families are closely related. Apocynaceae, Asteraceae and Malvaceae families had phytochemical composition mean of 53.5, 50.3 and 50.2 respectively.The three means do not significantly differ, indicating the three families are related. Lamiaceae, Fabaceae and Acanthaceae had phytochemical composition mean of 46.8, 43.5 and 40.2 respectively. The means of these three plant families do not significantly differ, indicating that the three plant families are also related. The Araceae family had a mean of 30.7 which differs from all the other means, indicating that this family, based on its phytochemical composition, is not closely related to any of the nine plant families.

Keywords: plant extract, phytochemicals composition, secondary metabolites

1. INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds (Shakeri *et al.,* 2012) called phytochemicals. Phytochemicals are basically divided into two groups, i.e. primary and secondary metabolites, according to their functions in plant metabolism. Primary metabolites comprise of common sugars, amino acid, proteins and chlorophyll while secondary metabolites consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins e.t.c (Nonita and Mylene, 2010). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Many of these have been studied and are still being studied under phytochemistry or natural product Chemistry (Nyunja, 2007). World plant biodiversity is the largest source of herbal medicine and it is now clear that, the medicinal value of these plants lies in the bioactive phytochemical constituents that produce definite physiological effects on human body (Nonita and Mylene, 2010).

2. MATERIALS AND METHODS

2.1 STUDY AREA

The study was carried out in the University Botanic Garden, Maseno, which is located in Kisumu county. The University Botanic Garden, Maseno (UBGM) is found within Maseno University, which is located in the Lake Victoria basin within Western Kenya (Onyango and Onyango, 2005). The UBGM was established in 2001 as a biodiversity centre for the Lake Victoria region (Onyango and Onyango, 2005). The garden lies at a latitude of 0^o 00' 16.09" and longitude of 34^o 36' 08.52"at altitude of 1500 m. Maseno area receives annual rainfall of about 1346 mm per year and the average temperature around the garden is 21.2^{o}C with 20^{o}C minimum and 23^{o}C maximum daily temperatures (rice weather station in the botanic garden). The UBGM size is about 7.0 hectares.

2.2 COLLECTION AND IDENTIFICATION OF PLANT MATERIAL

The leaves of the fifty plants, five each from ten selected plant families were collected from the University Botanic garden, Maseno. The selection of the ten plant families used was based on high plant frequencies observed in those families. The fifty plants selected were a representative of all the three plant habits; trees, shrubs and herbs. This was crucial since partitioning of secondary metabolites is different in plants of various habits (Balick and Cox, 1994). The plants were identified at the University herbarium by the curator, Mr Philip Omondi.

2.3 PREPARATION AND EXTRACTION OF PLANT SAMPLE

The leaves from fifty plants were collected in labeled polythene bags from the botanic garden and transported to the University laboratory. They were then air dried for 2-3 weeks, and then ground into powder form using a blender mill. Each ground leaf sample was extracted using 95% ethanol in a soxhlet apparatus (Njoku and Obi, 2009). The solvent was removed by distillation under reduced pressure and the resulting semisolid mass, thick syrup of about 12-15 g from 50 g of extracted dry powder was vacuum dried using flash evaporator (Okello, 2007). This extract was used for carrying out tests to determine presence or absence of secondary metabolites. The standard phytochemical bioactive component identifications were carried out according to the following methods:

Test for alkaloids

According to a method of George *et al.*, (2010), 5 mL of 1% aqueous HCl was added to 5 g of the extract and warmed in a water bath while stirring. It was then filtered and the filtrate was used to test for alkaloid as follows; 1 mL of the filtrate was treated with a few drops of Mayer's reagent. Creamy turbid dispersion indicated the presence of alkaloid. This observation was further confirmed by carrying out another alkaloid test thus; 1ml of the filtrate was treated with a few drops of Wagner's reagent. Reddish brown precipitate indicated the presence of alkaloid.

Test for anthraquinones

According to a method of George *et al.,* (2010), 2.5 g of extract was boiled with 5 mL of 10% H_2SO_4 and filtered. The filtrate was shaken with 2.5 mL benzene. The benzene layer was separated, and 10% NH_4OH added. A rose pink colouration in ammonia phase (lower phase) indicated the presence of anthraquinones.

Test for flavonoids

According to a method of Kumar and Gali (2011), few drops of $Mg(OH)_2$ solution was added to 5ml of test solution. Intense yellow colour was formed which turned to colourless on addition of few drops of dilute HCl acid. This indicated the presence of flavonoids.

Test for steroids and terpenes

According to a method of George *et al.,* (2010), few drops of acetic anhydride was added to the 0.5g of extract then boiled and cooled. Concentrated H_2SO_4 was then added from the side of test tube. A pink coloration formed at the junction of two layers indicated presence of terpenes and the green colouration formed in upper layer showed presence of steroids.

Test for saponins

According to a method of Astuti *et al.,* (2011), 0.5 g of extract was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion, which indicated the presence of saponins.

3. RESULTS AND DISCUSSION

The phytochemical component of the fifty plants is presented in Tables 1-10. Results obtained from the powdered samples revealed that the samples contained a wide array of phytochemicals, ranging from alkaloids, anthraquinones, flavonoids, steroids, terpenes and saponins. , it is therefore evident that the plants are rich in various phytochemicals.

Table 1: Phytochemicals present in leaves of 5 plants from Acanthaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
ACANTHACEAE *Acanthus pubescens* Thomson ex Oliv.	+	_	+	_	+	+
Justicia flava (Forssk.) Vahl.	_	_	_	_	_	+
Thunbergia alata Bojer ex Sims.	+	_	+	_	+	+
Sanchezia speciosa J.Leornard	_	_	_	+	_	+
Aphelandra squarosa Nees.	_	_	_	_	_	+
Frequency	2	0	2	1	2	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 2: Phytochemicals present in leaves of 5 plants from Apocynaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
APOCYNACEAE *Tabernaemontana stapfiana* Britten.	_	_	+	_	+	+
Mondia whitei (Hook f.) Skeels.	_	_	+	_	+	+
Plumeria rubra (Poir) L.H.Bailey	_	+	_	_	+	+
Thevetia peruviana (Pers.) K.Schum.	_	+	_	_	+	+
Acokanthera schimperi (A. DC.) Schweinf	+	+	_	_	+	+
Frequency	1	3	2	0	5	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 3: Phytochemicals present in leaves of 5 plants from Araceae family

Phytochemicals / Plants per family		St	Fl	An	Al	Sp
ARACEAE *Caladium bicolour* (Aiton.) Vent.	_	_	_	_	+	+
Colocasia esculenta (L.) Schott.	_	_	_	_	_	+
Monstera deliciosa Liebm.	_	_	_	_	+	+
Dieffenbachia seguine Schott.	_	_	_	_	+	+
Syngonium podophyllum Schott.	_	_	_	_	+	+
Frequency	0	0	0	0	4	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 4: Phytochemicals present in leaves of 5 plants from Asteraceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
ASTERACEAE *Tithonia diversifolia* Hemsl.	–	+	–	–	+	+
Vernonia amygdalina Del.	+	+	_	_	+	+
Conyza bonariensis L.	+	+	_	_	+	+
Bidens pilosa L.	_	+	_	_	_	+
Spilanthes mauritiana (Rich ex Pers.) DC	+	–	–	–	+	–
Frequency	3	4	0	0	4	4

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 5: Phytochemicals present in leaves of 5 plants from Fabaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
FABACEAE *Crotalaria ochroleuca* G.Don.	–	+	+	_	+	+
Vigna unguilata (L.)Walp.	_	_	_	_	+	+
Erythrina abyssinica Lam. ex DC.	+	_	_	_	+	+
Sesbania sesban (L.) Merr.	_	_	+	_	_	+
Phaseolus vulgaris L.	_	_	_	_	+	+
Frequency	1	1	2	0	4	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 6: Phytochemicals present in leaves of 5 plants from Lamiaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
LAMIACEAE *Leonotis nepetifolia* (L) R.Br.	–	–	+	–	+	+
Leucas martinicensis (Jacq) W.T. Aiton	_	_	+	_	+	+
Hyptis pectinata Poit. Lam.	_	_	_	_	+	_
Plecranthus verticillatus (L.f.) Druce	+	+	_	_	+	+
Rosmarinus officinalis L.	+	_	_	_	+	+
Frequency	2	1	2	0	5	4

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 7: Phytochemicals present in leaves of 5 plants from Malvaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
MALVACEAE *Hibiscus rosa- sinensis* L.	_	+	+	_	+	+
Sida cordifolia L.	_	_	_	_	+	+
Urena lobata ssp. Lobata L.	+	_	+	_	_	+
Sida rhombifolia L.	_	+	+	_	_	+
Urena lobata ssp.sinuata L.	+	_	+	_	_	+
Frequency	2	2	4	0	2	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 8: Phytochemicals present in leaves of 5 plants from Poaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
POACEAE *Cymbopogon citrates* (DC ex Nees.)Stapf.	+	_	+	+	+	+
Oryza sativa L.	_	_	+	_	+	+
Pennisetum purpureum Schumach.	_	+	+	_	+	+
Phragmites australis (Cav.)Trin.ex.Stend	_	_	+	_	+	+
Bambusa vulgaris Nees.	_	_	+	_	+	+
Frequency	1	1	5	1	5	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 9: Phytochemicals present in leaves of 5 plants from Rutaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
RUTACEAE *Clausena anisata* (Willd.) J.Hk. ex Benth.	_	_	_	_	_	+
Fagaropsis angolensis (Engl.)H.M.Gardner	_	+	+	_	+	+
Teclea nobilis Del.	+	+	_	+	+	+
Citrus limon (L.) Osbeck	+	+	_	_	+	+
Citrus sinensis Osbeck	+	+	_	_	+	+
Frequency	3	4	1	1	4	5

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

Table 10: Phytochemicals present in leaves of 5 plants from Solanaceae family

Phytochemicals / Plants per family	Tp	St	Fl	An	Al	Sp
SOLANACEAE *Datura stramonium* L.	_	_	+	_	+	+
Physalis minima L.	+	+	+	_	+	+
Brugmansia candida Pers.	_	_	_	_	+	+
Solanum incanum L.	_	_	+	_	+	+
Solanum scabrum Mill.	_	+	+	+	+	+
Frequency	1	2	4	1	5	5
Total Frequency	16	18	22	4	40	48

Key: + Present, -Absent, Tp –Terpenes, St – Steroids, Fl- Flavonoids, An-Anthraquinones, Al – Alkaloids, Sp - Saponins

From the Tables 1 - 10, it is evident that saponins are the most abundant phytochemicals among the plants whose leaf extracts were analysed. They account for 32.43 %, followed by alkaloids (27.03 %) then flavanoids (14.86 %), steroids (12 .16%), terpenes (10.81%) and anthraquinones (2.70 %) respectively. This observation is in agreement with the qualitative phytochemical experiments done by Okello (2007) and Jeruto (2008) who found out that alkaloids were abundant in plants. Alkaloids, saponins, terpenes and steroids are some of the phytochemicals known to be abundant in the leaves of plants (Kavit *et al*., 2013). This observation is in agreement with this research which found out that saponins were the most abundant in the leaf extracts of plants analysed, followed by alkaloids, steroids and terpenes respectively. According to Ismaila *et al*., (2009), environment could be a factor in determining presence or absence of saponins in plants. This could probably explain why plants in the UBGM are rich in saponins. The environment in the UBGM probably favours saponin synthesis in plants found there. However, more research needs to be carried out to establish exactly why saponins are abundant in the leaf extracts of the plants found in the UBGM.

Saponins were detected in all the plant leaves apart from the leaves of *Hyptis pectinata* and *Spilanthes mauritiana.* Saponins exhibit unique properties of formation of foams in aqueous solutions, cholesterol building properties, bitterness and haemolytic activity (Sodipo *et al*., 2000; Okwu, 2004). Plants produce saponins to fight infection by parasites (Okello, 2007) and probably this could also explain why this phytochemical is common among these plants.

Alkaloids were detected in leaves of all the plants apart from 10 plants. These phytochemicals are normally abundant in plants and their absence in the 10 plants leaves can be explained in the light of the fact that alkaloids are often restricted to specific organs within the plant among the angiosperms (Okello, 2007). Many alkaloids have antibiotic properties suggesting defence against microbial infection (Okwu, 2004).

Anthraquinones were only detected in four plants (Table 10). Their rare occurrence could be due to environmental, ecological and even diurnal fluctuations depending on time of collection. The methodology employed could probably only detect them under high concentrations.

Flavonoids were detected in 22 plants (Table 10). These phytochemicals are potent water soluble antioxidants and free radical scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu, 2004). Flavonoids are important taxonomic markers as specific types of flavonoids exist in specific plants and plant parts (Stace, 1993).

Terpenes and steroids were present in 16 and 18 plants respectively (Table 10). Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers. Steroids are derivatives of triterpene squalene (Wikipedia encyclopedia, 2012). This probably explains why the leaf extraction of some plants tested positive for the presence of terpenes and steroids. In some plant extracts however, steroids were detected but not terpenes. This could be as a result of methodology employed.

The analysis of the phytochemical data, that is, the means of the composition of phytochemical compounds in the selected plant families as shown in Table 11 shows that certain plant families are closely related based on their phytochemical composition. The plant families Poaceae, Solanaceae and Rutaceae all have phytochemical composition mean of 60.0, indicating that the families are closely related. The close relationship between the Solanaceae and Rutaceae plant families is supported by Selvakumare *et al.* (2011) in their "Molecular evolution of miraculin-like proteins study" which indicates that members of these two plant families contain these proteins. Many of them possess trypsin inhibitory activity and are involved in plant defense.

The plant families Apocynaceae, Asteraceae and Malvaceae have phytochemical composition means of 53.5, 50.3 and 50.2 respectively. The three means do not significantly differ therefore indicating that the three plant families are closely related. The plant families Lamiaceae, Fabaceae and Acanthaceae has phytochemical composition means of 46.8, 43.5 and 40.2. The means of these three plant families also do not significantly differ, indicating that the three plant families are closely related. This close relationship between the Lamiaceae and Acanthaceae plant families is supported by Reddy *et al.* (2004). However the two families differ from each other by the nature of their inflorescences, ovaries as well as fruits.

Lastly, the Araceae family has a mean of 30.7 which significantly differs from all the other means, indicating that this family, based on its phytochemical composition, is not related to any of the nine plant families (Table 11 and Appendix 1).

Table 11: Composition Of Phytochemical Compounds In Leaves Of Plants From Selected Plant Families

Phytochemical Compounds / Plant Families	Alkaloids (%)	Anthraquinones	Flavonoids (%)	Saponins (%)	Steroids (%)	Terpenes (%)	PLANT FAMILIES MEANS
Acanthaceae	40d	20e	40d	100a	1.0f	40d	40.2
Apocynaceae	100a	1.0f	40d	100a	60c	20e	53.5
Araceae	80b	1.0f	1.0f	100a	1.0f	1.0f	30.7
Asteraceae	80b	1.0f	1.0f	80b	80b	60c	50.3
Fabaceae	80b	1.0f	40d	100a	20e	20e	43.5
Lamiaceae	100a	1.0f	40d	80b	20e	40d	46.8
Malvaceae	40d	1.0f	80b	100a	40d	40d	50.2
Poaceae	100a	20e	100a	100a	20e	20e	60.0
Rutaceae	80b	20e	20e	100a	80b	60c	60.0
Solanaceae	100a	20e	80b	100a	40d	20e	60.0
PHYTOCHEMICAL MEANS	80.0	8.6	44.2	96.0	36.2	32.1	49.5
	$LSD_{(0.05)}$ = 4.2 Standard Deviation = 2.57 Covariance = 5.20						

Means, (3-replication) followed by same letter along the column do not significantly differ (Duncan's MRT, P = 0.05).

4. CONCLUSION

The ethanoic plant leaf extracts from the fifty plants collected from the UBGM are rich in phytochemicals such as alkaloids, anthraquinones, flavonoids, steroids, terpenes and saponins. From the research, it is evident that the means of the composition of these phytochemicals in the plants can be used to show which plant families are related.

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Appendix 1: Plant families and frequencies of observation of phytochemicals as extracted from the laboratory

Plant Families ↓ / Freq. of observation of phytochemicals →	Terpenes	Steroids	Flavonoids	Anthraquinones	Alkaloids	Saponins	Total
Asteraceae	3	4	0	0	4	4	15
Acanthaceae	2	0	2	1	2	5	12
Araceae	0	0	0	0	4	5	9
Apocynaceae	1	3	2	0	5	5	16
Fabaceae	1	1	1	0	4	5	13
Lamiaceae	2	1	2	0	5	4	13
Poaceae	1	1	5	1	5	5	18
Rutaceae	3	4	1	1	4	5	18
Solanaceae	1	2	4	1	5	5	18
Malvaceae	2	2	4	0	2	5	16
Total	16	18	22	4	40	48	148