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Phytochemical analysis of 50 selected plants found in the University Botanic Garden, Maseno, Kenya for their chemotaxonomic values

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ABSTRACT

Qualitative phytochemical analysis of 50 plants, five each from 10 selected families is presented. The objective of the study was to analyze phytochemical constituents of 5 selected plants in each of the 10 families for chemotaxonomic values. The 10 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 50 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 whose leaf extracts were analyzed. They account for 32.43%, followed by alkaloids (27.03%) then flavanoids (14.86%), steroids (12.16%), terpenes (10.81%), and anthraquinones (2.70%). Chi-square analysis revealed that plant families in which the plants are grouped are dependent on the phytochemicals present in plants.

KEY WORDS: Phytochemicals, plant extract, secondary metabolites

INTRODUCTION

The plant kingdom represents an enormous reservoir of biologically active compounds called phytochemicals (Shakeri *et al.,* 2012). 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, etc. (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 the human body (Nonita and Mylene, 2010).

Chemotaxonomy is the classification of organisms on the basis of the nature, content, and/or the distribution of constituent chemical substances (Cammack *et al.,* 2006). In Kenya, less work has been done on chemotaxonomic studies. However, preliminary chemotaxonomic studies have been done on some Kenyan plants. For instance, in 1990, a chemotaxonomic study on selected Lamiaceae species was done, and 37 medicinal plants were analyzed (Githinji, 1990). In the same year, phytochemical tests were carried out to detect presence or absence of compounds in six Apocynaceae species, and it was established that alkaloids and cardiac glycosides were the most abundant followed by triterpenes and saponins while anthraquinones were generally absent (Omino, 1990).

In the recent years, however, a lot of research on phytochemistry has been going on (Njoku and Obi, 2009; Ismaila, *et al.,* 2011; Kavit *et al.,* 2013). Most of these researches are geared toward showing the antimicrobial and antioxidant properties of these phytochemicals. However, minimal research geared toward indicating the use of phytochemicals in plant classification is being done (Ghani, 2012). In Kenya, there have scarcely been any attempts to carry out phytochemical analysis on various *in situ* and *ex situ* plants conserved at the university botanic garden to find out the bioactive components present in

them and find out if these bioactive components can be used for their classification. Therefore, there is need of a more chemotaxonomic research to indicate the use of phytochemicals as taxonomic markers in classification (Ghani, 2012).

MATERIALS AND METHODS

Study Area

The study was carried out in the University Botanic Garden, Maseno (UBGM), which is located in Kisumu County. The 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 center for the Lake Victoria region (Onyango and Onyango, 2005). The garden lies at latitude of 0°00' 16.09" and longitude of 34°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°C with 20°C minimum and 23°C maximum daily temperatures (rice weather station in the botanic garden). The UBGM size is about 7.0 hectares (Onyango and Onyango, 2005).

Collection and Identification of Plant Material

The leaves of the 50 plants, five each from 10 selected plant families were collected from the UBGM. The selection of the 10 plant families used was based on high plant frequencies observed in those families. The 50 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.

Preparation and Extraction of Plant Sample

The leaves from 50 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

About 5 mL of 1% aqueous HCl was added to 5 g of the extract and warmed in 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; 1 ml of the filtrate was treated with a few drops of Wagner's reagent. Reddish brown precipitate indicated the presence of alkaloid (George *et al.,* 2010).

Test for Anthraquinones

About 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 coloration in ammonia phase (lower phase) indicated the presence of anthraquinones (George *et al.,* 2010).

Test for Flavonoids

4 few drops of $Mg(OH)_2$ solution was added to 5 mL of test solution. Intense yellow color was formed which turned to colorless on the addition of few drops of dilute HCl acid. This indicated the presence of flavonoids (Kumar and Gali, 2011).

Test for Steroids and Terpenes

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

Test for Saponins

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 (Astuti *et al.,* 2011).

RESULTS

The phytochemical component of the 50 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

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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	Tp	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

+: 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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	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

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

saponins, it is, therefore, evident that the plants are rich in various phytochemicals and that these phytochemicals may be used in grouping them in their various families.

DISCUSSION

From the Tables 1-10, it is evident that saponins are the most abundant phytochemicals among the plants whose leaf extracts were analyzed. They account for 32.43%, followed by alkaloids (27.03%) then flavonoids (14.86%), steroids (12.16%), terpenes (10.81%), and anthraquinones (2.70%). This observation is in agreement with the qualitative phytochemical experiments done earlier on that found out that alkaloids were abundant in plants (Okello, 2007; Jeruto, 2008). 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 analyzed, followed by alkaloids, steroids and terpenes respectively. The environment could be a factor in determining presence or absence of saponins in plants (Ismaila *et al.*, 2011). This could probably explain why plants in the UBGM are rich in saponins. The environment in the UBGM probably favors 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.

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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	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

+: 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

Plants per family	Phytochemicals					
	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

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

Table 11: Plant families and frequencies of observation of phytochemicals as extracted from the laboratory

Families	Frequency of observation plant 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

Chi-square analysis at $P \leq 0.05$, reveals that there is a relationship between the phytochemical present in the plant and the plant family where the plant is grouped

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 hemolytic activity (Okwu, 2004; Sodipo *et al.*, 2006). Plants produce saponins to fight infection by parasites and probably this could also explain why this phytochemical is common among these plants (Okello, 2007).

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 defense 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 the time of collection. The methodology employed could probably only detect them under high concentrations.

Table 12: Chi-square analysis

Families	Plant $\frac{(o-e)^2}{e}$						
	Terpenes	Steroids	Flavonoids	Anthraquinones	Alkaloids	Saponins	Total
Asteraceae	1.225	2.689	2.2	0.4	0	0.133	6.647
Acanthaceae	0.1	1.8	0.018	0.9	1	0.008	3.826
Araceae	1.6	1.8	2.2	0.4	0	0.008	6.008
Apocynaceae	0.225	0.8	0.018	0.4	0.25	0.008	1.701
Fabaceae	0.225	0.356	0.018	0.4	0	0.008	1.007
Lamiaceae	0.1	0.356	0.018	0.4	0	0.133	1.007
Poaceae	0.225	0.356	3.564	0.9	0.25	0.008	5.303
Rutaceae	1.225	2.689	0.655	0.9	0	0.008	5.477
Solanaceae	0.225	0.022	1.473	0.9	0.25	0.008	2.878
Malvaceae	0.1	0.022	1.473	0.4	0.25	0.008	2.253
Total	5.25	10.89	11.637	6	2	0.33	36.107

36.107< value tabulated (61.656) at d.f - 45; χ^2-0.050 hence not significant

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. 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 the methodology employed.

In this study, the distribution of the constituent secondary chemical compounds found in the members of each plant family was used to show whether these compounds can be used to group these plants in their families or not (Tables 11 and 12).

From the analysis, it is evident that the plants are rich in various phytochemicals and that these phytochemicals can be used in grouping them in their various families. However, it should be noted that phytochemicals are just one of the taxonomic markers that taxonomists use in classification. For conclusive classification to be done, taxonomists also use morphological and anatomical characters from plants such as, chromosomal structure and number, nature of the pollen, inflorescence types and other taxonomic markers (Stace, 1993).

CONCLUSION

The ethanoic plant leaf extracts from the 50 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 these phytochemicals can be used in grouping these plants in their various families. Various researches have also shown that these phytochemicals are responsible for the plants medicinal values (Okello, 2007; Ewaen *et al.,* 2009).

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