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Qualitative Phytochemical Screening of Camellia sinensis and Psidium guajava Leave Extracts from Kericho and Baringo Counties

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ABSTRACT

Medicinal plants are important sources of phytochemicals which are crucial in the treatment of various diseases. *Camellia sinensis* and *Psidium guajava* have been used in Kenya and globally as medicinal plants to treat ailments like diabetes and cardiovascular diseases. However, many of the phytochemicals from these plants responsible for amelioration of these diseases have not been determined in Kenya. In this study, water, ethanol, acetone, ethyl acetate and chloroform were used to obtain the leave extracts of *Camellia sinensis* and *Psidium guajava*. The leaves of these plants were qualitatively screened for phytochemicals using standard methods. The percentage yield of the crude extracts from purple tea was 22.18%, 6.94%, 7.09%, 5.47% and 5.92% while for guava extracts the yield was 16.35%, 12.36%, 5.66%, 10.23%, 8.31% extracted from water, ethanol, acetone, ethyl acetate and chloroform respectively. Results showed the presence of alkaloids, flavonoids, saponins, terpenoids and phenols in both plant extracts. Water, ethanol and acetone which are polar solvents were found to extract major phytochemicals groups than non-polar ethyl acetate and chloroform.

Keywords: Medicinal plants, Purple tea, Guava, Phytochemicals, Solvents, Polarity

[I] INTRODUCTION

Medicinal plants are important for the treatment and management of human diseases due to the presence of plant phytochemical constituents [1]. Phytochemicals are naturally occurring compounds which are of great significance in the defense and protection of plants from various diseases [2]. Research has demonstrated that some of these phytochemicals can also protect human beings and animals against diseases [3]. Since time immemorial, parts of different medicinal plants have been used to treat specific ailments in Kenya [4]. The Tea Research Foundation of Kenya (TRFK) has recently released purple leafed tea for planting by the farmers. The tea has been under development for the last 25 years, and is shown to have high levels of anthocyanins with health enhancing effects [5]. A recent study showed that the purple tea has therapeutic potency against the murine model of Human African Trypanosomiasis (HAT) [6]. However, the presence of various phytochemicals from this new clone of tea has not been widely investigated.

Psidium guajava, also known as common guava is an important food crop and medicinal plant which has been widely used as food and in folk medicine around the world [7]. Many studies have demonstrated the ability of *Psidium guajava* to exhibit hepatoprotective, antimicrobial, cardioprotective, antidiabetic and antioxidant activities supporting its traditional uses [8]. Phytochemicals have been attributed to the success of traditional herbal medicine practiced all over the world [9]. Qualitative screening of phytochemicals is important to the pharmaceutical industry since the presence of a phytochemical of interest may lead to its further isolation, purification and characterization [10].

Successful extraction and determination of biologically active compounds from plants is largely dependent on the type of solvent used during extraction [11]. Extraction techniques used in pharmaceutical industry involves the separation of medicinally active compounds of plant tissues from inactive components using selective solvents [11]. During extraction, solvents will diffuse into the solid plant tissue and solubilize compounds with similar polarity [12].

The aim of the current study is to qualitatively screen and identify major phytochemicals groups from the leaves extract of purple tea and guava extracted from five solvents with different polarities.

[II] MATERIALS AND METHODS

2.1. Sample Collection

The leaves of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Common Guava) were collected from Kericho and Baringo Counties respectively. The plants species were authenticated by a Botanist at Jomo Kenyatta University of Agriculture and Technology. Voucher specimens were deposited at Jomo Kenyatta University of Agriculture and Technology Herbarium for future reference.

2.2. Solvents for extraction

The chemicals used were analytical grade ethanol, acetone, ethyl acetate, chloroform and the standards reagents for phytochemical screening were purchased from Sigma-Aldrich USA.

2.3. Preparation of plant extracts

Two kilograms of collected leaves of *Camellia sinensis* and *Psidium guajava* were air dried under a shade for two weeks and then crushed to coarse powder. The powdered leaves were extracted with solvents of different polarities. The solvents which were used included chloroform, ethyl acetate, acetone, ethanol and water solvents by cold maceration method [13]. The powdered dry leaves of the two plants were separately soaked in the above solvents (1:6 w/v) at room temperatures for 7 days. After the completion of extraction, the supernatants were filtered through Whatman No.1 filter paper. The filtrate obtained was concentrated under reduced pressure at 40°C using R-200 Rotavapor (Buchi, USA). The obtained semi solid extract was stored at 4°C in the refrigerator for qualitative phytochemical assays [14]. The extraction and phytochemical screening were done in duplicates.

2.4. Qualitative Phytochemical Analysis

Qualitative phytochemical analysis of the Kenyan purple leaf colored tea and common guava extracts from different solvents were done using standards methods.

2.4.1. Test for Alkaloids

One ml of crude extract was dissolved in 5 ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayers reagent separately. Formation of white or creamy precipitates with the reagents indicated the presence of alkaloids [15-16].

2.4.2. Test for Flavonoids

One ml of each of the plant extract in a test tube was added 1 ml of 5% lead acetate and the mixture was allowed to stand at room temperature (25°C) for two minutes. The formation of white precipitates in any of the samples showed that the extract contained flavonoids [15-16].

2.4.3. Test for phenols

Five ml of the extract was dissolved in distilled water. To this solution, 3 ml of 10% lead acetate solution was added. A dark-green colour indicated the presence of phenolic compounds [15-16].

2.4.4. Test for Tannins

Two ml of extract was treated with 10% alcoholic ferric chloride solution and observed for the formation of blue or green colour which indicated presence of tannins [9].

2.4.5. Test for Saponins

Two ml of the extract was added to 6 ml of water in a graduated cylinder. The mixture was shaken vigorously and observed for formation of persistent 2cm foam that indicated the presence of saponins [16].

2.4.6. Test for Steroids and Terpenoids

Five ml of the extract was treated with 2 drops of chloroform, acetic anhydride and conc. H_2SO_4. The mixture was observed for the formation of dark pink or red colour which indicated presence of steroids and terpenoids [9].

2.4.7. Test for Cardiac glycosides

Five ml of each plant extract was treated with 2 ml of glacial acetic acid in a test tube and one drop of ferric chloride solution was added to it. One ml of conc. H_2SO_4 was carefully added to form separate layer. A brown ring at the interface due to the presence of deoxy sugar characteristic of cardenolides and a pale green colour in the upper layer indicated the presence of cardiac glycosides [17].

2.4.8. Data analysis

Data from yields are expressed as mean ± SEM. The statistical significance of the yields from each solvent was carried out using one sample student's 't' test on SPSS statistical software package version 16. The level of significance was set at $P<0.05$.

[III] RESULTS

The percentage yield of the crude plant extracts obtained from these results showed that different solvents yielded different quantities of the crude extract. The water or aqueous solvent yielded the highest percentage of 22.18% of crude tea extract and 16.35% of guava crude extracts (Table 1 and 2). The mean values of the yields were not statistically significant since all the p-values were higher than 0.05 ($P>0.05$).

Table 1: Yields of purple tea leaf extracts from different solvent extracts

Solvent	Amount of purple tea used (g)	Volume of the solvent used (ml)	Extract yield(g)	Percentage (%) yield	P-values (2-tailed)
Water	90	540	19.96 ± 2.54	22.18	0.081
Ethanol	90	540	6.25 ± 1.13	6.94	0.114
Acetone	90	540	6.38 ± 0.60	7.09	0.060
Ethyl acetate	90	540	4.92 ± 0.62	5.47	0.080
Chloroform	90	540	5.33 ± 0.83	5.92	0.098

Mean values of yields are presented as mean ± SEM. Values are statistically significant when $P<0.05$

Table 2: Yields of Guava leaf extracts from different solvent extracts

Solvent	Amount of Guava used (g)	Volume of the solvent used (ml)	Extract yield (g)	Percentage (%) yield	P-values (2-tailed)
Water	150	900	24.52±4.41	16.35	0.113
Ethanol	150	900	18.54±1.57	12.36	0.054
Acetone	150	900	8.49±2.05	5.66	0.151
Ethyl acetate	150	900	15.35±4.59	10.23	0.185
Chloroform	150	900	12.46±1.25	8.31	0.064

Mean values of yields are presented as mean ± SEM. Values are statistically significant when $P<0.05$

From the results of phytochemical screening, water or aqueous extract of both purple tea and common guava had the highest presence of phytochemicals. This was followed by Acetone, Ethanol, Ethyl acetate and Chloroform extracts having few phytochemicals (Table 3).

Table 3: Phytochemical screening of *Camellia sinensis* (Purple tea) and *Psidium guajava* (Common guava) leaves from different solvents extracts

	Water		Ethanol		Acetone		Ethyl acetate		Chloroform	
	Tea	Guava	Tea	Guava	Tea	Guava	Tea	Guava	Tea	Guava
Alkaloids	+	+	+	+	+	+	-	-	-	-
Flavonoids	+	+	+	+	+	+	-	-	-	-
Phenols	+	+	+	+	+	+	-	-	-	-
Tannins	+	+	+	+	+	+	-	-	-	-
Saponins	+	+	-	-	+	-	-	-	-	-
Steroids & terpenoids	+	+	-	-	-	-	+	+	+	+
Cardiac glycosides	-	-	-	-	-	-	-	-	-	-

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

[1V] DISCUSSION

From the above results, it can be noted that successful extraction of biologically active compounds from plants like *Camellia sinensis* and *Psidium guajava* is largely dependent on the type of solvent used during extraction. Different solvents with differing polarities extract specific phytochemicals in plants [11]. In this study, polar solvents like water, ethanol and acetone yielded highest amount of crude extracts and also had the highest presence of phytochemicals. This study therefore validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract [18]. It also implies that the choice of a solvent is affected by different factors like class of phytochemicals, diversity and polarity of the compounds to be extracted [19]. From these results, extracts of chloroform and ethyl acetate indicated the presence of steroids and terpenoids. Terpenoid lactones have been obtained by successive extractions of dried barks with hexane, chloroform and methanol. Occasionally tannins and terpenoids will be found in aqueous phase, but they are more often obtained by treatment with less polar solvents [11].

Qualitative screening of *Camellia sinensis* and *Psidium guajava* showed that the leave extracts of the two medicinal plants have alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids. This variation in phytochemical constituents makes plants potential medicinal plants [20]. Investigations of alkaloids have revealed many pharmacological properties including antidiabetic, antiprotozoal and cytotoxic [21] and anti-inflammatory properties [22]. Flavonoids and phenolic compounds in various plants have been reported to have multiple biological effects like antioxidant, free radical scavenging abilities, anti-inflammatory and anti carcinogenic properties [23]. Steroids in plants are known for their insecticidal, analgesic properties, cardiotonic and central nervous system activities, antimicrobial and anti-inflammatory properties [24]. Tannins isolated from medicinal plants have also been reported to exhibit remarkable toxicity against bacteria and fungi [25].

[V] CONCLUSION

Results showed that water, ethanol and acetone solvents are important in extraction of polar phytochemicals and thus crucial in isolation of alkaloids, flavonoids, phenols and tannins.

Phytochemicals found present in the leaves of *Cammelia sinensis* (Purple tea) and *Psidium guajava* (Common guava) indicates their potential as source of bioactive compounds that may supply novel medicines. Further studies are suggested to quantitatively determine these phytochemicals and ascertain their medicinal activities.

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REFERENCES

1. Nostro A, Germano MP, D'angelo V, Marino A, Cannateli MA, (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett Appl Microbiol, 30:379-384.
2. Doss A and Anand SP, (2012). Preliminary phytochemical screening of *Asteracantha longifolia* and *Pergularia daemia*. World Applied Sciences Journal, 18 (2): 233-235.
3. Ngbede J, Yakubu RA and Nyam DA, (2008). Phytochemical screening for active compounds in *Canarium schweinfurthii* (Atile) leaves from Jos North, Plateau States, Nigeria. Research Journal of Biological Sciences, 3 (9): 1076-1078.
4. Karinge JW, (2006). A survey of traditional health remedies used by the Maasai of Southern Kajiado District, Kenya. Ethnobotany Research and Application, 4: 061-073.
5. Kerio LC, Wachira FN, Wanyoro JK and Rotich MK, (2012). Characterization of anthocyanins in Kenyan teas: Extraction and identification. Elsevier, 131:31-38.
6. Khalid R, Wachira FN, Nyariki NJ and Isaac OA, (2014). Kenyan purple tea anthocyanins and coenzyme-Q_{10} ameliorate post treatment reactive encephalopathy associated with celebral human African trypanosomiasis in murine model. Parasitology international, 63 (2):417-426.

7. Yariko D and Kouji M, (2010) Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf extract. Nutrition & Metabolism, 7:9.
8. Shirur DS, Adhikari R, Sanjay ST and Sajjekhan S, (2013) A review on the medicinal plant *Psidium guajava* linn. (Myrtacea). Journal of Drug Delivery & Therapeutics, 3 (2), 162-168.
9. Kumar SR, Balasubramanian P, Govindaraj P and Krishnaveni T, (2014) Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Coriandrum sativum* L. roots (Coriander). Journal of Pharmacogmosy and Phytochemistry, 2 (6):74-78.
10. Ugochukwu SC, Arukwe UI and Ifeanyi O, (2013). Preliminary phytochemical screening of different solvent extracts of stem bark and roots of *Dennetia tripetala* G. Baker. Asian J of plant sci and Res, 3(3):10-13.
11. Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H, (2011). Phytochemical screening and Extraction: A review. Internationale Pharmaceutica Sciencia, 1(1): 98-106.
12. Chaitanya JP, Lalagoud M, Chandrashekar R, Bhavani NL and Kudle, (2014). Phytochemical and Antimicrobial screening of the polar and non-polar solvent root extracts of *Vanda tessellate* (Roxb.)Hook.ex G.Don. Int. J. Phytochemical and Antimicrobiology, 5 (4): 315-320.
13. Handa SS, Khanuja SPS, Longo G, Rakesh DD, (2008). Extraction technologies for medicinal and aromatic plants. International centre for science and high technology, Trieste, 21-25.
14. Kumar SA, Paul AD and Tanveer N, (2013). Bioactivity guided extraction of Seabuckthorn (*Hippophae rhamnoides* L. ssp. *turkestanica*) leaves. Journal of Scientific & Industrial Research, 72: 307-311.
15. Harborne JB, (1998). Phytochemical Methods: A guide to modern techniques of plant analysis,3rd Edn, Chapman and Hall, London, UK, pp.302
16. Trease GE and Evans WC, (1989). Pharmacognosy, 11th Edn, Brailliar Tiridel Can, Macmillian Publishers, pp.35-38.
17. Sofowara, A, (1993). Medicinal plants and Traditional Medicine in Africa, Spectrum Books Ltd, Ibadan. pp. 289
18. Ncube NS, Afolayan AJ, Okoh AI, (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African J. Biotechnology, 7 (12): 1797-1806.
19. Eloff JN, (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants. Journal of Ethnopharmacology, 60:1-8.
20. Maobe AGM, Gatebe E, Gitu L and Rotich H, (2013). Preliminary Phytochemical Screening of Eight Selected Medicinal Herbs Used for the treatment of Diabetes, Malaria and Pneumonia in Kisii Region, Southwest Kenya. Euro. J. Appl. Sci, 5 (1):1-6.

21. Akindele AJ and Adeyemi OO, (2007). Anti-inflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus*. Fitoterapia, 78: 25-28.
22. Malairajan P, Geetha G, Narasimhan S and Veni KJ, (2006). Analgesic activity of some Indian medicinal plants, Journal of Ethnopharmacology, 19:425-428.
23. Thamaraiselvi, Lalitha P and Jayanthi, (2012). Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia (Mart.) solms. Asian J. Plant Sci. Res, 2 (2):115-122.
24. Argal A and Pathak AK, (2006). CNS activity of *Calostropis gigantean* roots. Journal of Ethnopharmacology, 106:142-145.
25. Banso A and Adeyemo SO, (2007). Evaluation of antibacterial properties of tannins isolated from *Dichrostachys cinerea*. Afr. J. Biotechnol. 6 (15): 1785-1787.