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Phytochemicals Extraction in *Craterostigma Plantagineum Hoscht*: Aqueous and Ethanol Solvents

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Abstract

Background: ***Background:*** Phytochemicals are plant bioactive compounds that are produced for their protection with some having medicinal value. *Craterostigma plantagineum hoscht* is a plant that is purported to have medicinal value and is used for treatment of pain, liver and diarrheal ailments. Extensive researches on phytochemicals present in *Craterostigma plantagineum hoscht* are missing. The aim of this study was to determine qualitative phytochemicals yield in ethanol and aqueous extracts of *Craterostigma plantagineum hoscht*. ***Materials and methods:*** *Craterostigma plantagineum hoscht* was harvested and allowed to air dry for one month. Grinding into powder form later followed this. Solvents used included 70% ethanol and distilled water. 500mg of Craterostigma plantagineum ground powder was soaked in 70% ethanol for 48 hours, filtered and vaporized in rotary evaporator. Aqueous extraction involved soaking of 500mg of ground powder in 250 millilitre distilled water, set in a shaker for 2 days, filtered, deep frozen for 48 hours and finally freeze dried for 72 hour. Chemicals used to test phytochemicals included 1% gelatin, chloroform, ferric chloride, sulphuric acid, lead acetate solution, magnesium turnings, 10% sodium chloride, acetic anhydride, Mayer's reagent, ammonia and hydrochloric acid. ***Results:*** The yield included flavonoids, Saponins, tannins, cardiac glycosides, phenols, anthraquinones, alkaloids, terpenoids and sterols. Tannins, anthraquinones, flavonoids, sterols, cardiac glycosides, Saponins, and phenols were highly extracted in ethanol while aqueous extract yielded more alkaloids and terpenoids. ***Conclusion and recommendations:*** Ethanol extraction yielded more phytochemicals than aqueous. Nonetheless, some phytochemicals were better extracted in ethanol while others in aqueous making the choice of extraction method dependent on the phytochemical of interest.

Keywords: Phytochemicals, *Craterostigma plantagineum hoscht*, Ethanol extraction.

1. INTRODUCTION

According to African traditional medicine, the system is disposed into three different strata viz. herbalism, spiritualism and divination, with herbalism being the bedrock of the system [1]. The usage of herbal medicines dates back to over 3000 years with the world's oldest familiar textbook in herbalism.'' The Devine Farmer's Classic of Herbalism'' being traced to 2000 years ago in China [2]. In Africa up to about 90% of the population use traditional medicine while in India up to 70% depend on the same. In United States of America 38% of adult population to some extent depended on traditional medicine in comparison to 12% of their paediatric population in the year 2007. This is an indication that the practice is not only confined to the third world [3].

Medicinal value of plants is based on the phytochemicals present. They are extracted using different assays which include use of various solvents such as acetone, methanol, ethanol and chloroform, with the yield of the phytochemicals being determined by the polarity of the solvent in relation to that of the solute [4]. According to [5] different solvents are used for phytochemical extraction and they include nonpolar solvents (e.g. hexane, ether, acetic acid), intermediate polar agents (e.g. ethanol, dichloromethane) and polar solvents (alcohol, acetone, dimethylformamide,

dimethylsulfoxide, methanol and water). Use of individual solvents (e.g. water, acetone, chloroform) is advised as opposed to use of solvents that are hydro-organic like water: ethanol or water: methanol. This is because some phytochemicals such as phenols form polymerized compounds that have varying dissolubility making it difficult to dissolve in solvents of varying polarity [6].

The purpose of this study was to provide an insight into phytochemicals extracted from *Craterostigma plantagineum hoscht* in the ethanol and aqueous solvents.

2. MATERIALS AND METHODS

Craterostigma plantagineum hoscht were harvested from Sekenani Valley Maasai Mara and allowed to air dry for one month. This was later followed by grinding into powder form. Solvents used included ethanol and distilled water. Chemicals used included 1% gelatin, chloroform, ferric chloride, sulphuric acid, lead acetate solution, magnesium turnings, 10% sodium chloride, acetic anhydride, Mayer's reagent, ammonia and hydrochloric acid.

2.1. Extraction in ethanol

500 grams of *Craterostigma plantagineum hoscht* ground powder was immersed for 2 days in 70% ethanol. It was first filtered using muslin cloth and then afterward using a filter paper. The filtrate was placed in rotary evaporator for 2 days to allow ethanol to evaporate. The derivative was then conserved in a refrigerator at low temperatures of 4°C.

2.2. Extraction in aqueous

500 grams of *Craterostigma plantagineum hoscht* ground powder was placed in a container and 250 millilitres of distilled water was added. The solution was then placed in a shaker for 48 hours. This was then followed by filtering using a filter paper, and was done two times. The product was set into a deep freezer for 48 hours to allow it to freeze. Afterward, the frozen product was placed in a freeze drier where it freeze-dried for 72 hours. A yield of 56 grams was attained and was conserved in a refrigerator at low temperatures of 4 degrees Celsius.

2.3. Phytochemical screening tests

A**nthraquinone**: 5ml of the extract solution was hydrolyzed using sulphuric acid. This was followed by addition of 1 ml of diluted ammonia. Anthraquinones existence was confirmed by appearance of rose pink colour.

Alkaloids: Mayer's reagent was added to 1ml of the extract. Existence of alkaloids was indicated by occurrence of a precipitate, yellow in colour.

Polyphenols: Three drops of five percent lead acetate solution was put in 1ml of the extract. Presence of polyphenols was appreciated by appearance of a precipitate, yellow in colour.

Tannins: One percent gelatin and ten percent sodium chloride was added to 1millilitre of the extract. Presence of tannins was confirmed by presence of blackish blue or greenish black colour.

Saponins: A millilitre of *Craterostigma plantagineum hoscht* extract was poured into a test tube. 50-millilitre tap water was then added, which was then followed by powerful shaking. Existence of saponins was confirmed by appearance of colours red, blue or green with an accompanying pink ring.

Test for flavonoids: 4 drops of HCL acid and magnesium turnings were added to a millilitre of *Craterostigma plantagineum hoscht* extract in a test tube. Appearance of a magenta red or pink colour confirmed presence of flavonoids.

Test for cardiac glycosides: Two millilitres of ferric chloride was added to 5ml of *Craterostigma plantagineum hoscht*. Cardiac glycosides were appreciated by appearance of a pink precipitate.

Test for sterols and steroids: A millilitre of *Craterostigma plantagineum hoscht* was poured into a test tube. Then there was addition of 0.5milliliter of sulphuric acid, 0.5 millilitre of acetic anhydride and 0.5 millilitre of chloroform. Sterols were confirmed by appearance of a red colour. Moreover, appearance of green colour confirmed presence of steroids.
Qualitative yield of phytochemicals in aqueous and ethanol extracts

Screening of phytochemicals was done as per the protocol. There was high yield of flavonoids, Saponins, phenols, cardiac glycosides, anthraquinones and tannins in ethanolic extract while alkaloids, sterols, and terpenoids were higher in aqueous extract.

Table 1: Phytochemicals in aqueous and ethanolic extracts

Phytochemical	Ethanolic extract	Aqueous extract
Alkaloids	Absent	Trace
Flavonoids	+++	++
Sterols	++	Absent
Saponins	+++	+
Tannins	+++	+

Phytochemical	Ethanolic extract	Aqueous extract
Terpenoids	Absent	++
Cardiac glycosides	+++	++
Phenols	+++	Absent
Anthraquinones	+	Absent

Key: *+++ = Highly present; ++ = moderately present; += mildly present; Absent =not detected*

3. DISCUSSION

Craterostigma plantagineum hoscht is a perennial herb purported to have medicinal value by the Maasai of Sekenani valley of Maasai Mara as well as Oromo community of Ethiopia. Qualitative screening of different phytochemicals in air-dried *Craterostigma plantagineum hoscht* plant leaves, stems and roots in ethanol and aqueous extract had different yields of various phytochemicals. Ethanolic extract yielded more flavonoids, sterols, phenols, saponins, tannins, cardiac glycosides and anthraquinones. On the other hand, aqueous extract yielded more alkaloids and terpenoids in comparison to ethanolic extract. There is paucity of information regarding phytochemicals present in *Craterostigma plantagineum hoscht.*

There was high phenolics extraction in the ethanolic extraction compared to aqueous extraction. High extraction of phenolics in ethanol compared to aqueous has been reported in other studies. In a study done by [7] it was observed that ethanolic extract of *Beijing propolis* yielded higher phenols content compared to aqueous extract. Similar finding have been documented in a study by [8] that found that ethanol extraction of *Juniperous procera* yielded more phenolics. Phenols have been shown to be more soluble in ethanol and sparingly soluble in water [9].

High content of flavonoids was detected in ethanolic extraction. Ethanol has been shown to yield more flavonoids in comparison to aqueous extract [10]. Flavonoids have varying polarity with some being highly polar while others being nonpolar e.g. flavones, flavones and flavonols. Non-polar flavonoids are best extracted in organic solvents [11]. It is therefore, highly likely that the flavonoids in *Craterostigma plantagineum hoscht* were non-polar since they were best extracted in ethanol. Terpenoids are best extracted in water compared to ethanol. According to a study done by [12] terpenoids were highly extracted using aqueous in comparison to ethanol. Equally, terpenoids have been associated with evaporation when extracted in ethanol [13].

Saponins levels were higher in ethanolic extract. Extraction of saponins is higher in ethanol or methanol and other weak alcohols as compared to aqueous [14]. Similar findings have been documented by [15] who found increasing concentrations of ethanol was associated with linear increase in saponins yield compared to aqueous extraction. This study found trace amount of alkaloids in aqueous extract while they were absent in ethanol extract. Water has been documented as a better solvent for alkaloids in comparison to ethanol extract, however this extraction depends on the specific alkaloid present.

Anthraquinones were mildly present in the ethanolic extract. According to a study by [16] ethanol extraction was the best for anthraquinones when compared to aqueous extraction. Similarly aqueous ethanol extraction of *Polygoni multiflori* had higher yield of anthraquinones compared to aqueous extract [17].

Sterols were absent in aqueous as they are not soluble in water, however, they are better soluble in ethanol and solvents that are not polar [18]. In a study done by [19] of the less polar solvents ethanol yielded the highest tannins in comparison to methanol and acetone. However, tannins can also be extracted in water or a mixture of water with ethanol, methanol, or acetone [20]. Cardiac glycosides from the extract were higher in ethanolic extract, which agrees with other studies that have documented high ethanolic extraction of cardiac glycosides in comparison to aqueous [21].

4. Limitations of the study

The study was on qualitative phytochemicals yield thus unable to quantitate the various phytochemicals present in the plant.

5. CONCLUSION

This study concludes that ethanol extraction of phytochemicals in *Craterostigma plantagineum hoscht* yielded most of the phytochemicals with exception of alkaloids and terpenes that were found in aqueous extract. The choice of extraction method would then be determined by the phytochemical of interest.

Conflict of interest: None declared

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