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The Qualitative and Quantitative Phytochemical Investigation of *Crinum* Species in Ethiopia

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Abstract: Medicinal plants have a long history of use in most communities all over the world. Plants have the ability to synthesize a wide diversity of chemical compounds that uses to perform important biological functions. Many of Genus Crinum has been broadly used in traditional and ethno-medicines in the world wide. The aims of this study were to investigate the qualitative and quantitative phytochemicals constituents of the four species of the genus Crinum that exists in Ethiopia. All experiments were follow standard procedures. For the purpose of conducting phytochemical analyses on the four species each, three to six bulbs were collected from Field Gene Banks, Botanical Gardens and local fields. The bulb samples were cleaned, dried and crushed into powder. In this study the cold extraction methods were used the extraction solvents such as: n-hexane, ethanol, methanol and water. As a result of the phytochemical analyses, it revealed the presence of alkaloids, flavonoids, saponins, tannins and phenols in Crinum abyssinicum and Crinum bambusetum species. Likewise, it confirmed the presence of alkaloids, flavonoids, phenols and tannins in Crinum macowanii. Moreover, it confirmed the presence of alkaloids, flavonoids, phenols and tannins Crinum ornatum. The chemical constituents revealed the presence of relatively high concentration of alkaloids (9.66%), saponins (19.72%), phenols (10.33%), and tannins (0.61%) in the bulbs of Crinum bambusetum. Similarly, the highest concentration of flavonoids (27.72%) was recorded from the bulbs of Crinum ornatum. As more phytochemicals constituents are being identified and tested, traditional uses of the Crinum are being verified. Accordingly, the evidence on the chemical constituents of the species explains the uses of the plants. Therefore, it is worthwhile to recommend the use of the phytochemical constituents of the species studied for Pharmaceutical use in the treatment of different diseases.

Keywords: Crinum Abyssinicum, Crinum Bambusetum, Crinum Macowanii, Crinum Ornatum, Bulbs, Phytochemical Screening, Qualitative, Quantitative

1. Introduction

Medicinal plants have a long history of use in most communities throughout the world. Nature has been a source of medicinal agents for thousands of years. Plants which have one or more of its organs containing substances that can be used for the healing purpose, are called medicinal plants [1]. Genus *Crinum* is one of the medicinal plants that belong to the family Amaryllidaceae, It comprises of an 'eye-catching group' of tropical and sub-tropical lilies distributed in almost every part of the world, mainly in Africa, Asia, Australia and America. Globally, around 180 *Crinum species* were described [2-4]. Out of these, four species are found in Ethiopia: namely, *Crinum abyssinicum*, *Crinum bambusetum, Crinum macowanii* and *Crinum ornatum* [5]. *Crinum abyssinicum and Crinum bambusetum* are endemic species of Ethiopia [5].

Generally, bulbs of *Crinum* species are used to treat several diseases, like antitumor, anti-coughs and colds, immune-stimulating, analgesic, antiviral, anti-malarial, antibacterial, antifungal activities, etc [6]. In Ethiopia, *Crinum abyssinicum* is used for treatment of Hypertension

[7], animals' internal parasites [8] and Hepatitis B treatment and Skin infection [9]. In addition to this, Ethiopian traditional healers' used *Crinum* species for the treatment of cancer, asthma, colds, malarial, so on. Due to an endless source of bioactive principles, economic importance and medicinal purposes of *Crinum*, it needs especial attention for investigation of phytochemical constituents. Phytochemicals are natural compounds found in plants that are responsible for the colour, taste and aroma of foods [10]. Internationally, about 35 *crinum* species have been investigated for phytochemical constituents, whereas the largest number of the species yet to be studied [11]. The chemistry and economic importance of *Crinum species* have never been studied in-depth in Ethiopia, suggesting that due to lack of awareness about their significance, these species are not accorded the necessary intervention to save them by the rural communities. In order to promote their use at a larger scale and improve their socioeconomic contributions, phytochemical investigations are necessary to isolate diverse classes of compounds contained in their bulbs. The aim of this study was to analyze the major phytochemical constituents of the four species of the bulbs of genus *Crinum* that are known from Ethiopia.

2. Materials and Methods

2.1. Collection and Identification of Plant Materials

A healthy fresh bulb of *Crinum* species were collected from our institute medicinal plant field gene banks, botanical gardens and local fields that not from protected areas it gazer from highly disturbed grazing fields and no specific permission was required for collection. Identification of the plant was made by a botanist at Ethiopian Biodiversity Institute (Dr Tesfaye Awas) who is expert in the flora of the collection site and a voucher specimen was deposited at the Herbarium of Ethiopian Biodiversity Institute under voucher number Tesfaye Awas and Asnakech Senbeta 2644 (*Crinum abyssinicum*), Tesfaye Awas and Asnakech Senbeta 2645 (*Crinum bambusetum*), Tesfaye Awas and Asnakech Senbeta 2646 (*Crinum ornatum*) and Tesfaye Awas and Asnakech Senbeta 2647 (*Crinum macowanii*).

2.2. Preparation of Sample and Extracts

The bulbs were cleaned, cut into small pieces and air dried in laboratory at room temperature [12]. After drying, the materials were ground into fine powder and stored it until use. The sample extraction were done by macerated in cold solvent in 1: 4 ratios for 72 hours through proceeding from non-polar to polar solvent; i.e., n-Hexane, ethanol, methanol and water [13]. Extracts obtained were filtered, concentrated and evaporated into solid extracts under room temperature. Then the extracted materials were stored in a refrigerator at 4°C until they were used [14]. The laboratory experiments were conducted in Ethiopian Biodiversity Institute, Ethiopian Public Health Institute and National Soil Testing Center.

2.3. Phytochemical Screening

2.3.1. Qualitative Phytochemical Analysis

Phytochemical screening of various constituents of *Crinum* species were carried out by standard methods [1, 14-19] as described below

i. Alkaloids.

For each test 0.5 g extracts were dissolved in 5 ml of dilute (1%) hydrochloric acid on a steam bath and filtered with cotton in test tube.

Mayer's Test: 1 ml of filtrates was treated with a few drops of Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

Hager's Test: 1 ml of filtrates was treated with a few drops of Hager's reagent (saturated picric acid solution) formation of crystalline yellow precipitate indicates the Presence of alkaloids.

Dragendroff's Test: 1 ml of filtrates was treated with a few drops of Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of Orange red precipitate indicates the presence of alkaloids.

ii. Flavonoids.

Alkaline reagent test: 0.1g extract were mixed with 2ml of 2% solution of NaOH, an intense yellow colour was formed which turned colorless on addition of few drops of diluted H_2SO_4 acid which indicated the presence of flavonoids.

Lead acetate Test: 0.1g extracts dissolved with 5ml solvent, 2ml of the solutions were treated with 3-5 drops of 10% lead acetate solution. Formation of yellow or orange color precipitate indicates the presence of flavonoids.

Ammonia test: 0.1g extracts were mixed with 2ml of 10% NH_4OH solution with few drops of concentrated H_2SO_4, yellow color formed which turned colorless indicated the presence of flavonoids.

iii. Phenols.

0.5g of each extracts were dissolved by 10 ml of each solvents than filter it with cotton.

Alkaline reagent: 1ml of extract was mixed with 2ml of 2% solution of NaOH, an intense yellow color fade to colorless.

Lead acetate test: When 2 ml of extract was treated with few drops of 5% lead acetate solution, white precipitates appeared.

Ferric Chloride Test: 2 ml extracts were treated with 3-4 drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

iv. Tannins.

The extract 0.5 g was boiled in 10 ml of each solvent in a test tube for 5 min then the mixture was filtered by cotton and use for the tests.

Test with Iron salts: crude extract show colour reaction with iron salt like $FeCl_3$ and K4Fe (CN) 6 in presence of ammonia. Addition of 2-3 drops of 5% $FeCl_3$ solution to the solutions of tannins forms bluish black precipitate or tannins it forms greenish brown coloured precipitate.

Lead acetate test: When 2 ml of extract was treated with

few drops of 5% lead acetate solution, white precipitates appeared.

Ferric chloride test: To 5 ml of extract few drops of 1% $FeCl_3$ were added. The appearance of blackish-blue or blackish-green colour indicates the presence of tannins.

Gelatin Test: To 2ml extract, 1ml of 1% gelatin solution containing sodium chloride (10% NaCl) was added white precipitate indicates the presence of tannin.

v. Saponins.

Foam Test: 0.5 gm of extract was shaken with 2 ml of distil water. If stable foam produced persists for 15 minutes it indicates the presence of saponins.

Sodium nitrate test: 0.1g of extract was dissolved in to alcoholic or water, then 2ml of the solution was treated with 10%$NaNO_3$ and few drops concentrated H_2SO_4, blood red colors indicate the presence of saponins.

vi. Steroid

Salkowski's test: 0.1g of crude extract was mixed with 2ml of chloroform and few drops of concentrated H_2SO_4 were added sidewise. Shake it and allowed to stand for some minute, a red color produced in the lower chloroform layer indicated the presence of steroids.

3% Vanillin conc.H_2SO_4: 0.1g crude extract was mixed with 2ml of chloroform and 3-5 drop of 3% Vanillin conc.H_2SO_4 were added the development of a rose or reddish brown color indicated the presence of steroids.

vii.Glycosides.

Liebermann's test: 0.1g crude extract was mixed with 2ml of chloroform and 2ml of acetic acid. The mixture was cooled in ice. Carefully, a few drops of concentrated H_2SO4 were added, a color change from violet to blue to green.

Salkowski's test: 0.1g crude extract was mixed with 2ml of chloroform. Then 2ml of concentrated H2SO4 was added carefully and shaken gently a reddish brown colour appear.

viii. Anthraquinones.

Borntrager's test: 0.1g extract was dissolved by 5 ml dil. HCl acid. The hydro alcoholic extract of sample was shaken vigorously with 10ml of benzene. The extract was then filtered and the filtrate was treated with 5 ml of 10% of ammonia solution, the mixture was shaken and the presence of a pink, red or violet color in ammonia phase indicted the presence of free Anthraquinones glycosides.

Modified Borntrager's: 0.1g extracts were hydrolyzed with 5 ml dil. HCl then treated with a few drops of 1%Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonia layer indicates the presence of Anthraquinones glycosides.

ix. Cardiac glycosides.

Liebermann Bruchard's reagent: 0.1g of extract was dissolved with 3 ml of glacial acetic acid followed by addition of a mixture of acetic anhydride and conc. H_2SO_4 (50: 1) was added from the sides of the test tube showed a rose color which turns to greenish blue indicted the presence of Cardiac glycosides.

Keller- Kiliani reaction: 0.1g extract was dissolved with 3 ml of glacial acetic acid and 1 ml of conc. H_2SO_4 was added carefully against the wall of test tube followed by 2 -3 drops of 1 % $FeCl_3$ solution, formation of a bright blue color indicted the presence of Cardiac glycosides sugars.

x. Terpenoids.

0.1g of the extract was dissolve in 2 ml of chloroform and evaporates to dryness. 2 ml of concentrate sulphuric acid was added and heat for about 2 min. A grayish colour indicates the presence of terpenoids.

2.3.2. Quantitative Determination Tests

Quantitative analyses were carried out on the samples to determine the amount of phytochemical composition. The analyses were done based on standard procedures as described below:

i. Determination of total Alkaloid content following the Method [15].

For total alkaloids content determination 5g of the extract was taken into a 250 ml beaker and added 200 ml of 10% acetic acid in ethanol. The mixture was covered and kept it for 4 hrs at room temperature. Then filtered it by Whatman filter paper into a 250 ml conical flask and concentrated it through evaporation on a water bath up to one-fourth of the original volume. On the filtrate adding it concentrated ammonium hydroxide drop wise until the total precipitation was formed, and allowed to settle it. After that the precipitates was washed by dilute ammonium hydroxide then filter, dried, cooled in desiccators and reweighed. The process was repeated three times and the average was taken. The final weight was expressed as percentage of weight of the sample analyzed as shown below [15].

$$\%\ of\ \ Alkaloid\ \ = \frac{W_2 - W_1}{Weight\ \ of\ \ Sample} * 100 \qquad (1)$$

Where, W1 - is weight of filter paper, and W2 - is the weight of filter paper + alkaloid precipitate

ii. Determination of Total Saponins Content Following the Method [20].

For total saponins content determination 20 g of samples powder was taken into a conical flask and added it 50 ml of 20% aqueous ethanol. The samples were heated over a hot water bath for 4 hrs with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 100 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 10 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 30 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight and the saponins content was calculated as percentage of the original sample thus

$$\% \text{ of Saponin} = \frac{W_2 \quad W_1}{\text{Wheight of Sample}} * 100 \quad (2)$$

Where, W1- is weight of evaporating dish, and W2 – is weight of dish + sample

iii. Determination of Total Flavonoids Content Following the Method [21-22].

The total flavonoids content was determined by extracting 10 g of the plant sample with 100 ml of 80% aqueous methanol at room temperature. The solutions were filtered through no. 42 filter paper into weighed crucibles. Then, the crucibles' contents were evaporated to dryness over water baths and the final weights were determined as follows.

$$\% \text{of Flavonoid} = \frac{W_2 \quad W_1}{\text{Wheight of Sample}} * 100 \quad (3)$$

Where, W1- is weight of empty crucible and W2- is weight of crucible + Flavonoids extract

iv. Determination of Total Phenols Content.

The total phenol content was determined by Folin-Ciocalteu examine method with spectrophotometry [23-25]. For examine taking 1g of sample powder to extract with 10 ml of 80% methanol. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water was taken in a volumetric flask (25 ml). 1ml of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Na2CO3 solution was treated to the mixture. The volume was made up to 25 ml. A set of standard solutions of gallic acid (20, 40, 60, 80 and 100 mg/ml) were prepared in the same manner as described earlier. Protected for 90 minutes at room temperature and the absorbance for test and standard solutions were determined against the reagent blank at 550 nm with an UV/VIS spectrophotometer. Quantification was done on the basis of a standard curve of gallic acid. Based on the measured absorbance, the concentration of phenols was read (mg/ml) from the calibration line; then the content of phenols in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Total phenol content = GAE x V x D /m, where GAE is the gallic acid equivalence (mg/mL); V is the volume extract (mL), D is dilution factor and m is the weight (g) of the pure plant extract.

v. Determination of Total Tannins Content.

The total tannins content were determined by Folin-Ciocalteu method [25-26]. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35 % Na2CO3 solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 mg/ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract.

3. Statistical Analysis

All laboratory experiments were carried out triplet for each extract (n = 3). The statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Tukey test of multiple mean comparisons at 5% level of significance and SPSS (version 20.0).

4. Results and Discussion

4.1. Qualitative Phytochemical Analysis

Phytochemical screening was carried out on *crinum* species to test the presence of most relevant bioactive compounds uses for medication. The presences of phytochemicals constituents in *crinum* were evaluated from the extracts of *crinum* species bulbs by successive solvent method such as n-hexane, ethanol, methanol and aqueous solvents. The strongest confirmation of the presences or absences of phytochemical constituents in the bulbs of *crinum* were detected with more than one reagent used for each bulbs extracts. In these tests, it reveals the presence of various bioactive secondary metabolites which might be responsible for the medicinal attributes. The investigation of chemical constituents of the plants shows that, the bulbs of genus *Crinums* are rich in phytochemical compositions. As it can be observed in the Table 1 alkaloids, flavonoids, phenols, saponins and tannins were detected accordingly, in the crude extracts of with some reagents in all *Crinum* species except n-hexane solvent. But the detection of Anthraquinones glycosides, cardiac glycosides and terpenoids for all *crinum* species were negative by all reagent tests in all solvent extracts. Qualitative phytochemical investigation results of *crinum* abyssinicum and *crinum* bambusetum revealed that, the presence of alkaloids, flavonoids, phenols, saponins and tannins was confirmed in all reagents with different solvent extracts except n-hexane, in the extract of *crinum* bambusetum the presence of steroid and glycosides detected by only one reagent. In addition to this, they detected in *crinum* abyssinicum in ethanol and methanol extract respectively, but they detected in *crinum* bambusetum in ethanol and aqueous extracts accordingly. Thus, *crinum* bambusetum and *crinum* abyssinicum results almost similar detection of phytochemicals constituents. Moreover, both species are endemic to Ethiopia and had been never investigated for the presence of phytochemicals constituents so far. Most notably, the *crinum* macowanii detection revealed positive test for alkaloids, flavonoids, phenols and tannins with some reagents in ethanol, methanol and aqueous extracts. Similarly, the presence of saponins and steroid were detected in this species by only one reagent in aqueous extract whereas glycosides detected in ethanol extract. The presence of alkaloids in *crinum* macowanii was consistent with previous findings [27-29].

The qualitative phytochemical investigation result of *Crinum* ornatum showed that, the presence of alkaloids, flavonoids, phenols and tannins in all solvent extracts expect n-hexane. But, in this species saponins, steroid and

glycosides were absent by all reagent in all solvent extracts. The absence of saponins in *Crinum* ornatum was contradicted with the opinion of the person [30] who noted that saponins are one of the active constituents. On contrary, the absence of saponins in *Crinum* ornatum was consistent with the observation of previous study [31]. In addition, the absence of Anthraquinones glycosides in the species was in line with the reports of previous study [30] and the absence of cardiac glycoside was observed against the previous findings [31]. Some *Crinum* species that aren't found in Ethiopia are investigated for alkaloids and flavonoids constitutes and results a positive test [32] Such as: *Crinum* jagus, *Crinum* latifolium etc, which is go in line with the results of Ethiopia *Crinum*. According to the phytochemical screening of *Crinum* jagus indicated the presence of tannins, alkaloids, sterols, triterpenes, flavonoids, phenols and saponins [33]. In the same manner, phytochemical studies on *Crinum* defixum bulbs exposed the presence of alkaloids, Saponins, tannins, phenols and Flavonoids [34]. Many plant extracts are reported to have health beneficial properties due to secondary metabolites such as alkaloids, tannins, phenols, steroids, saponins, flavanoids, terpenoids, glycosides, anthroquinones glycosides, cardiac glycosides and so on. These bio-constituents are known for their multipurpose biological effects and are implicated in treatment of various diseases. Some of the literature reveals that, lot of pharmacological investigations have been carried on *crinum* species. Most notably, almost all reported that pharmacological uses the bulbs of *crinum* species to treat several diseases, like antitumor, anti-coughs and colds, immune-stimulating, analgesic, antiviral, anti-malarial, antibacterial, antifungal activities, etc [6].

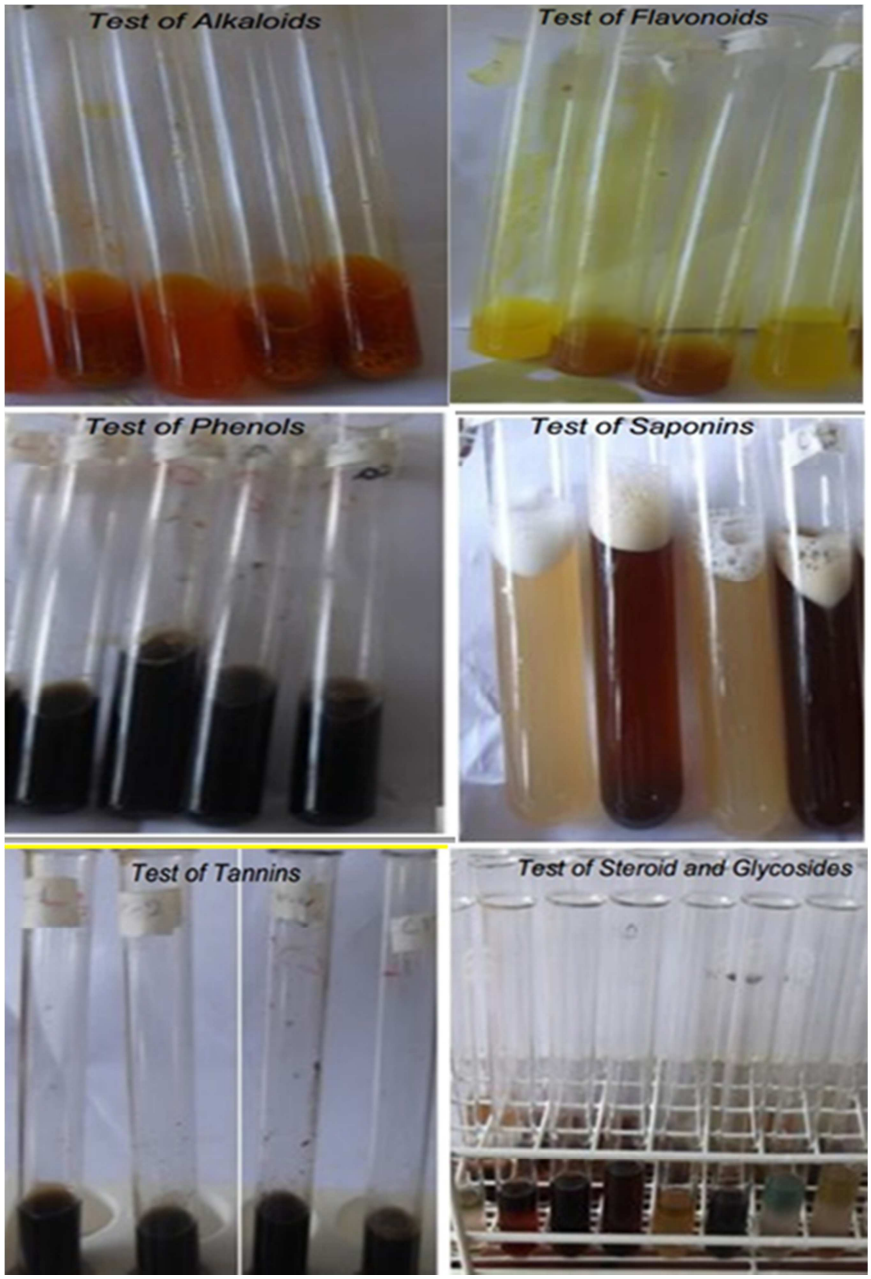

Figure 1. *The positive tests results of phytochemical constituents.*

Table 1. *Result of qualitative phytochemical screening of Crinum species.*

Phytochemical test	Reagents	Results of Different *Crinum* species extract by different solvent							
		Crinum abyssinicum				*Crinum* bambusetum			
		n-Hexane	Ethanol	Methanol	Water	n-Hexane	Ethanol	Methanol	Water
Alkaloids	a). Mayer's reagent	-	+	-	-	-	+	+	-
	b). Hager's reagent	-	+	+	+	-	+	+	-
	c). Dragendorff's reagent	-	+	+	+	-	+	+	+
Flavonoids	a). Alkaline reagent	-	+	-	-	-	+	-	-
	b). Lead acetate test	-	+	-	-	-	+	+	-
	c). Ammonia test	-	+	-	-	-	+	-	-
Phenols	a). Alkaline reagent	-	+	+	+	-	-	+	+
	b). Lead acetate test	-	+	-	+	-	+	+	+
	c). Ferric chloride	-	+	+	+	-	-	+	+
Tannins	a). Iron salts test	-	-	+	+	-	-	+	+
	b). Lead acetate test	-	+	-	+	-	+	+	+
	c). Ferric chloride	-	+	+	+	-	-	+	+
	d). 1% gelatin test	-	+	-	-	-	+	-	-
Saponins	a). Foam	-	-	-	+	-	-	-	+
	b). 10%NaNO3 test	-	+	-	+	-	+	-	+
Steroid	a). Salkowski's test	-	-	-	-	-	-	-	-
	b). 3% Vanillin conc.H_2SO_4	-	-	+	-	-	+	-	+
Glycosides	a). Liebermann's test	-	-	-	-	-	-	-	-
	b). Salkowski's test	-	+	-	-	-	+	-	-
Anthraquinones	a). Borntrager's test	-	-	-	-	-	-	-	-
glycosides	b).Modified Borntrager's	-	-	-	-	-	-	-	-
Cardiac	a).Liebermann Bruchard's reagent	-	-	-	-	-	-	-	-
glycosides	b). Keller- Kiliani reaction	-	-	-	-	-	-	-	-
Terpenoids	Chloroform plus sulphuric acid	-	-	-	-	-	-	-	-

Table 1. *Continued.*

Phytochemical test	Reagents	Results of Different *Crinum* species extract by different solvent							
		Crinum macowanii				*Crinum* ornatum			
		n-Hexane	Ethanol	Methanol	Water	n-Hexane	Ethanol	Methanol	Water
Alkaloids	a). Mayer's reagent	-	+	+	-	-	+	+	-
	b). Hager's reagent	-	+	+	+	-	+	+	-
	c). Dragendorff's reagent	-	+	+	+	-	+	-	+
Flavonoids	a). Alkaline reagent	-	+	+	-	-	+	+	+
	b). Lead acetate test	-	+	+	-	-	+	-	-
	c). Ammonia test	-	+	+	-	-	+	+	+
Phenols	a). Alkaline reagent	-	-	+	-	-	+	+	-
	b). Lead acetate test	-	+	+	-	-	-	+	+
	c). Ferric chloride	-	+	+	+	-	+	+	-
Tannins	a). Iron salts test	-	-	-	+	-	-	+	-
	b). Lead acetate test	-	+	+	-	-	-	+	+
	c). Ferric chloride	-	+	+	+	-	+	+	-
	d). 1% gelatin test	-	+	+	-	-	-	+	-
Saponins	a). Foam	-	-	-	+	-	-	-	-
	b). 10%NaNO3 test	-	-	-	-	-	-	-	-
Steroid	a). Salkowski's test	-	-	-	-	-	-	-	-
	b). 3% Vanillin conc.H2SO4	-	-	-	-	-	-	-	-
Glycosides	a). Liebermann's test	-	-	-	-	-	-	-	-
	b). Salkowski's test	-	+	-	-	-	-	-	-
Anthraquinones	a). Borntrager's test	-	-	-	-	-	-	-	-
glycosides	b).Modified Borntrager's	-	-	-	-	-	-	-	-
Cardiac	a).Liebermann Bruchard's reagent	-	-	-	-	-	-	-	-
glycosides	b). Keller- Kiliani reaction	-	-	-	-	-	-	-	-
Terpenoids	Chloroform plus sulphuric acid	-	-	-	-	-	-	-	-

Note: The (+) represents presence, while (-) represents absence of a given phytochemical constituent.

Besides the findings presented in the Table 1, the positive tests results of phytochemical constituents were presented in the Figure 1.

4.2. Quantitative Phytochemical Analysis

The concentration of total alkaloids, flavonoids, saponins,

phenols and tannins in the bulbs of *Crinum* species were presented in the Figure 2. It revealed that, the percentage concentration of flavonoids were higher in all *Crinum* species as compared with others phytochemical constituents. Similarly, the percentage concentrations of saponins were higher next to flavonoids in all species except *Crinum ornatum*. Moreover, the percentage concentration of alkaloids and phenols ranges from nearly 4% to 10% in all *Crinum* species. But, the percentage concentrations of tannins were smaller for all species.

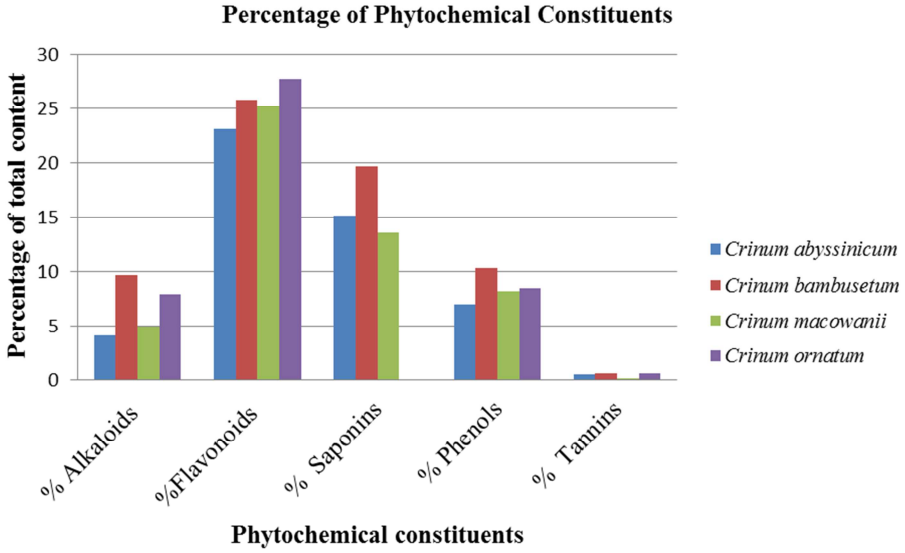

Figure 2. *Percentage of the different phytochemical constituents in the bulbs of the four Crinum species.*

As it can be depicted in the Table 2, the highest percentage of alkaloids content was found in *Crinum* bambusetum, followed by *Crinum* ornatum. The smallest percentage of alkaloids was found in *Crinum* macowanii and *Crinum* abyssinicum. In addition to this it revealed a statistically significant mean difference of the content of alkaloids between all *Crinum* species. Similarly, it found statistically significant mean difference of the content of flavonoids in all *Crinum* species except *Crinum* bambusetum and *Crinum* macowanii. Moreover, there was statistically significant mean difference of contents of saponins in all *Crinum* species except *Crinum* ornatum. And also, the mean difference of the content of phenols was found statistically significant across all *Crinum* species whereas no difference was found between *Crinum* macowanii and *Crinum* ornatum. Furthermore, less content of tannins with statistically significant average difference was found among *Crinum* species but, indifference was found between *Crinum* bambusetum and *Crinum* ornatum. From over all finding of chemical constituents, all *Crinum* species have low percentage of total contents of tannins than the other chemical constituents.The total phenol content of *Crinum* ornatum was determined to be much smaller (84.7 mg/g) than the amounts reported (271.5 mg/g) by literature [30]. The quantities of total flavonoids are also less (27.72 ±0.22%) in amount than reported, 52.4±0.02% by the literature [35]. In other hand, leaves of Crinum latifolium exposed the presence of glycosides, alkaloids, tannins, phenols and the total phenol contents of methanol extract was 17.50±2.64 mg of GAE/ gm of extract [36].

Table 2. *Proportion of phytochemical constituents extracted from bulbs Crinum species.*

No	Phytochemical	Species			
		C. abyssinicum [mean±SE]	C. bambusetum [mean±SE]	C. macowanii [mean±SE]	C. ornatum [mean±SE]
1	Alkaloids	4.09 ± 0.07^{a}	9.66 ± 0.02^{b}	4.99 ± 0.04^{c}	7.92 ± 0.07^{d}
2	Flavonoids	23.13 ± 0.15^{a}	25.78 ± 0.12^{b}	25.22 ± 0.2^{b}	27.72 ± 0.22^{c}
3	Saponins	15.18 ± 0.43^{a}	19.72 ± 0.18^{b}	13.57 ± 0.41^{c}	-
4	Phenols	6.98 ± 0.1^{a}	10.33 ± 0.32^{b}	8.23 ± 0.2^{c}	8.47 ± 0.01^{c}
5	Tannins	0.47 ± 0.02^{a}	0.61 ± 0.03^{b}	0.13 ± 0.00^{c}	0.55 ± 0.00^{b}

All values are expressed as mean ± standard error (SE). Means indicated by the same lettered superscripts represent are not significant difference, Values were found out by using one-way analysis of variance (ANOVA) followed by Tukey test. Significance level P = 0.05.

5. Conclusions

This research work is initiated to detect the presence of bioactive compounds in the *crinum* species to study their importance for medicinal aspect. Due to the reason that, the concentration of phytochemicals varies accordingly, different solvents extract of the plant were selected for the analysis of phytochemicals compositions. The phytochemical screening of *Crinum* species showed that the bulbs were rich in phytochemical constituents. As results, the current study revealed the positive test for alkaloids, flavonoids, phenols, saponins and tannins in the crude extracts of with some

reagents in all *Crinum* species except n-hexane solvent. On contrary, the Anthraquinones glycosides, cardiac glycosides and terpenoids for all *crinum species* were detected negative with all reagent tests in all solvent extracts. The presence of phytochemical constituents in bulbs of *Crinum* species extracts supports their traditional uses, pharmacological agents and modern drug discovery. Therefore, all *Crinums* species are useful for Antitumor, anti-coughs and colds, anti-allergic, anti-inflammatory, antimicrobial, anticancer, antifungal activities and so on. In particular, the endemic *Crinums* species were rich of alkaloids, flavonoids, phenols, saponins and tannins.

Authors' Contributions

Asnakech	Participated on sample collection, investigated all laboratory experiments and written the paper
Tesfaye (Phd)	Participated on sample collection, sample identification, supervised the experiments and edited the paper
Abdella (Phd)	Edited the paper

Competing Interests

The authors declare that they have no competing interests.

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