International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 21-09-2021, Accepted: 06-10-2021, Published: 22-10-2021
Volume 6, Issue 5, 2021, Page No. 1357-1362

Proximate analysis, qualitative and quantitative analysis of *Allium fistulosum* L. onion with a focus on its biological activity

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Abstract
Allium fistulosum L. (Welsh onion leaves) is an important traditional medicinal plant belongs to perennial species originated from Eastern Asia and used for the treatment of colds, abdominal pain, influenza, headache and heart disease. The objective of the study was to investigate the phytochemical constituents and proximate composition of *Allium fistulosum* L. extracts was determined using standard method.
Materials and Methods: Three different extractions were made from *A. fistulosum* L. using ethanol, hexane, methanol, acetone, ethyl acetate and chloroform. The qualitative, quantitative and proximate composition were performed and compared among the different solvent extracts.
Results: The total ash, water soluble ash, acid insoluble ash content and loss on drying of dry powder of *Allium fistulosum* L. showed high level of total ash (8.235 w/w) water soluble ash (7.13w/w), acid soluble ash (1.225 w/w) and loss of drying (0.81 w/w). The ethanolic extractive value (8.64%) was found more followed by chloroform (7.545%), ethyl acetate (6.47%), methanol (5.63%) and hexane (1.185%) of *Allium fistulosum* L. The ethanolic extracts contained alkaloids, phenolics, saponins, flavanoids, steroid, tannins and lignin, based on preliminary phytochemical screening. The quantitative assessment contained the total alkaloids contents (TAC) was 22.5 µg, total flavanoids contents (TFC) 25.75 µg and total phenolics contents (TPC) 17.6 µg were obtained. Further study is encouraged on the pharmacological activity of the active components of ethanolic extract that give prospective biological activity.
Conclusion: The quantitative determination of *Allium fistulosum* L. has revealed the presence large quantity of flavanoids amongst all the other phytocompounds. The ethanolic extract of *Allium fistulosum* L. plant contains pharmacological properties that are known and they can be explored for biological potentials.

Keywords: *Allium fistulosum* L, proximate composition, heart disease, pharmacological properties, welsh onion leaves

Introduction

Universally, the utilization of natural plant remedies has produced towards a massive need about the properties for more information and uses of the medicinal plants. The make use of plant in drug formulation and development has been as old as the part of human civilization which dependent on plants for their requirement of healthcare and food. This has referred the medicinal plants as the nature's pharmacy and to the innovation of active compounds and it is too recognized for disease curative (Ajayi *et al*., 2019; Sundar *et al*., 2020) [1, 29]. Medicinal plants play a major role in the health care sector of developing nations for the management of diseases. Scientific and honorable reports indicated that about 25% of approved medicines globally are in use from herb and medicinal plants. Thus herbal medicines have a prominent role to play in the pharmaceutical markets and health care sector of the 21st century (Annan *et al*., 2008; Vidita *et al*., 2013) [2, 32]. Values of the medicinal plant have been assumed a more essential in the past few decades because its play an important role in the progression of alternative drugs devoid of the adverse effects of the man-made drugs (Samira *et al*., 2020) [23]. The phytocompounds synthesized by plants as secondary metabolites that have diverse biological and additional medicinal properties are of enormous attention in the designing of new drug which of growing interest in curative as well as other industrialized applications. These chemical constituents have prominent pharmacological actions on living systems and their organs (Aritra *et al*., 2017; Willy *et al*., 2021) [4, 36].

According to World Health Organization, the stability and excellence to control the herbal drugs, also involves the physicochemical assessment of plant based drug with aspects for selection and management of plant crude material. This estimation based on the quality index including macro and microscopic assessment, moisture content, ash values, extractive values, qualitative and quantitative chemical evaluation, crude fiber and chromatographic examination (WHO; 1996, WHO; 1996 and Fiaz and Saqib, 2015) [34, 34, 11] and its plays a major role for standardization of the native crude drugs (Gupta *et al*., 2018) [12]. The extractive values and phytocompounds analysis of the plant drugs and its formulations are also done to ensure the occurrence of plant actives ingredients and their solubility report. Qualitative phytochemical screening as well as quantitative will help out in understanding of phytochemical compounds occurrence in the exact plant at the same time as it also help for extracting, purification and recognition of the bioactive compounds for more suitable application. This plant possesses several significant medicinal properties however most of the advantages are still restricted to tribal areas since awareness of raw

materials and lack of appropriate scientific standardization. A big quantum of research works in the area of authentication of the correct plant source has been taken.

A. fistulosum L. is a non-bulbing, cluster forming and tender green belongs to onion species under Liliaceae family. It is also called as spring onion, welsh onion, scallion, salad onion or Japanese bunch onion (Pleasant, 2013) [18] and it is highly civilized in the Northern area of Nigeria and northern Hemisphere temperate to tropical regions. Almost the entire plant parts of the *A. fistulosum* that is, shoots, leaves and non-developed bulbs are eaten raw in salad, boiled as soup, cooked as vegetable or used as healing herbs (Singh and Ramakrishna, 2017). It has medicinal properties such as antioxidant and antifungal due to sulphur-containing compounds, flavonoids and fatty acids (Vlase *et al*., 2013) [33]. It has also been reported that this plant can be used for the treatment of eyesight problems, common colds, headaches, heart problems, wounds and festering sores; reduces fat accumulation and serum lipid concentrations; and the root exudates in soil root-zone have anti-termite, anti-fungal and anti-microbial activities. Onionins A1, A2 and A3 are some of the bioactive compounds that have been successfully isolated and characterized from the leaves of *A. fistulosum* using various spectroscopic techniques (Nohara *et al*., 2014) [16].

D-Limonene is a naturally occurring monoterpene compound which has chemotherapeutic and chemopreventive activity against many rodent tumor types (Crowell and Gould, 1994) [9]. This compound is found in the rind of citrus fruits, such as oranges, lemons and limes. It is concentrated in orange peels, comprising approximately 97% of this rind's essential oils (Sobel, 2019) [27]. It belongs to a group of compounds called terpenes which offers several health benefits and its strong aromas protect plants from predators. D-Limonne has been reported to possess anti-oxidant (Yu, 2017) [38], anti-inflammatory (Souza *et al*., 2003; Yilmaz and Ozbek, 2018) [28, 37] and anti-carcinogenic properties. The present investigation is aimed to investigate and characterize the leaf powder of *A. fistulosum* L. for phytochemical analysis, by qualitative and quantitative analysis.

Materials and Methods

Sample collection and identification of *Allium fistulosum* L.

Fresh leaves of *Allium fistulosum* L. (Family Amaryllidaceae) were collected from Perambalur District. The plant was authenticated with common name spring onion by Senior Scientist and Head, ICAR- Krishi Vigyan Kendra, Hans Rover campus, Valikandapuram.

Preparation of extracts

The collected bulbs were washed with distilled water and then kept for shade dry after that the air dried leaves were ground into fine powder. Fifty (50) grams of the powder was mixed with 500ml of Methanol, Ethanol, Hexane, Chloroform and Ethyl acetate in a sterilized conical flask separately and placed for 3 days with alternating shaking. The residual solvents extracts of sample were removed at 48- 49°C under reduced pressure using a rotary evaporator. Each extract was kept in a sterile bottle and cooled at 4 °C for further experiment and used to assay the biological activity.

Physcio-chemical analysis

Powder Studies

Fluorescence behavior of powder

To study the fluorescence behavior of root powder, by treating with different chemical reagents *viz*.1N sodium hydroxide, 1N sodium hydroxide in methanol, 1N hydrochloric acid, picric acid, acetic acid, 1N nitric acid, acetone, nitric acid, 50% sulphuric acid in ammonia solution and observed under daytime light, long UV (365 nm) and short UV light (254 nm). The fluorescence analysis was carried out by the technique of Chase and Pratt, 1949 [8] and Singh *et al*., 2013 [26] when cut surface or powder is exposed to UV light.

Proximate analysis

(Vidita *et al*., 2013 & Willy *et al*., 2021**)** [32, 36] The parameters determined for proximate analysis include ash value, extractive value and loss of drying and it was determined as mentioned in guidelines of WHO (2002) [35]

Loss on drying

The plant powder material weighed 2 gm was cited on a tared evaporating dish and distributed the material as consistently as feasible by gentle sidewise quaking to a depth not greater than 10mm. Placed the loaded bottle in the oven or desiccators, then detached the stopper and kept as it in the chamber. The samples were dried for the specified time then it was allowed to cool at room temperature in desiccators prior to weighing. Weigh the crucible and the contents until two successive readings match each other is used to determine the amount of moisture.

Loss of drying (%) = Loss in weight x 100/w

W=Weight of drug in gm.

Determination of ash values

Total ash

About 2-4g of air-dried powdered plant material was accurately weighed and kept in a silica crucible or dish and burn up at a temperature not in excess of 450°C waiting free from carbon. Then it was cool in desiccators, weighed and calculates the percentage of total ash with reference to the dried plant material.

Total ash (% w/w) = (Weight of ash/Weight of sample) x 100

Water soluble ash

The total ash obtained by mixing with 25 ml of water and boiled, about 5 minutes and then an ashless content with insoluble material was collected in the filter paper, ignited at a temperature not beyond 450°C. Finally, calculate the content of insoluble ash in mg/g of dried-material by subtracting the weight of this ash in mg from the weight of water soluble ash.

Water soluble ash (% w/w) = (Weight of ash - weight of insoluble ash/ Weight of sample) × 100

Acid-insoluble ash

The total ash obtained, by boiling with 25ml of hydrochloric acid about 5 min covered with a watch glass, collect the insoluble matter on an ash less filter paper and washed with

boiled water then it was ignited finally cooled in dessicator for 30 min and weighed. Calculated the content of acid-insoluble ash content in mg per g air plant dried material.

Acid insoluble ash (% w/w) = (Weight of ash/ Weight of sample) x 100

Extractive value

Extractive value is used as an evaluating crude drug which is not readily estimated by other means. It is employed for that material for which no suitable chemical or biological assay method exist.

Alcohol extractive

The powdered plant material accurately weighed 5 gm was macerated in the course of 100 ml of ethanol, methanol, ethyl acetate, chloroform and Hexane in a closed flask incubated for 24 hrs and mixed at regular intervals with continuous shaking. After 24 hrs incubation, the solution was rapidly filtered without any loss of solvent. Then from the filtrate about 25 ml of solution was taken in a flat bottomed shallow disc and evaporated at 100°C till it was completely dried and weighed. The rate of solvent extractive was computed with reference to air dried plant material. The %w/w alcohol soluble extractive value with reference to the air-dried plant material was calculated as follows

$$\text{Alcohol soluble extractive} = \frac{\text{Weight of residue x 100 Alcohol soluble extractive}}{\text{Volume of extract evaporated x Weight of sample}} \text{ Value (\% w/w)}$$

Phytochemical screening

The phytochemical screening of the Garlic for various phytochemical constituents such as terpenoids, flavonoids, alkaloids, reducing sugars, steroid, glycoside, phenol, anthraquinones, saponin, sugar, quinones, coumarins, lignin and tannin were carried out using standard methods as described by Edeoga *et al*., 2005 [10].

Quantitative analysis

Total phenolic content assay

The total phenolic content of plant extracts were calculated with Folin- Ciocalteau reagent, with slight modification. Briefly, 2 mL of Folin–Ciocalteu reagent (1:9; Folin-Ciocalteu reagent: distilled water) and 1 mL of sample (5 mg/mL) was put into a 10ml volumentric flask. At 5 in, the mixture was permitted to stand at room temperature and mixed gently with 3 mL of 7.5% (w/v) Na2CO3 was added to the mixture. The mixture was homogenized and a set of Gallic acid standard solutions (20, 40, 60, 80 and 100 µg/ml) allowed situating at room temperature for 90 min. Total polyphenol content absorbance was observed using a spectrophotometer at 760 nm. The total phenolic content was expressed as Gallic Acid Equivalents (µg GA/mg of dried extract) in mg/mL plant extract (Mariah *et al*., 2021) [14].

Estimation of total content of alkaloids

Alkaloids content were determined using Harborne method About 1 ml of 1 mg/ml of sample was transferred to a separating funnel whereby there was an addition of 5 ml of bromocresol green solution and 5 ml of 4.7 pH phosphate buffer. The mixture was shaken with 1, 2, 3 and 4 ml of chloroform by vigorous shaking, collected in a 10 ml volumetric flask and diluted to the volume with chloroform. The absorbance was determined on the reagent blank at 470 nm with an UV/Visible spectrophotometer for standard solutions and test solutions. A set of Atropine (20, 40, 60, 80 and 100 µg/ml) was prepared as test solution. The total content of alkaloids was calculated as Atropine equivalents (µg AE/mg of dried extract). Reagent blank was prepared in the same manner but without extracts (Mariah *et al*., 2021) [14].

Total flavonoid content assay

Total flavonoid content was calculated using the Dowd method as modified by Arvouet-Grand *et al*., (1994). For each extract, 1 mL of methanolic solution (100 µg mL-1) was mixed with 1 mL of aluminium trichloride (AlCl3) in methanol (2%). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a 1 mL of methanol and 1 mL of plant extract without AlCl3. The total content of flavonoid was expressed as Rutin equivalents (µg RE /mg dried extract) as standard graph. Reagent blank was prepared as test solutions but without extracts.

Results and Discussion

Physicochemical parameters are also very important for the equivalence and quality management of herbal drugs which can be identified by loss on drying, ash content and extractive values Herbal resources should be devoid of any type of contamination, so this analysis of powdered drugs can be measured as an important factor in order to ensure the purity of plant drug material (Anonymous, 1998) [3].

Ash content and loss on drying

Ash value is helpful in determining validity and purity of sample and also these values are significant qualitative standards like carbonate, oxalate, and silicate. The total ash content, acid insoluble ash, water soluble ash was established to be 8.235, 1.225 and 7.13 (Table 1 & Figure 1). This value clearly indicates that the root is best for drug exploit and effects. The water soluble ash helps us to get the quantity of inorganic material present in the crude medicine, though acid insoluble ash helps us to find the amount of sand and other waste in the crude substance (Chanda, 2014) [7].

Table 1: Physicochemical Constants of Ash and Loss on drying of *Allium fistulosum* L.

Parameters	Value W/W
Loss on Drying	0.81
Total Ash Content	8.235
Acid Insoluble Ash	1.225
Water Soluble Ash	7.13

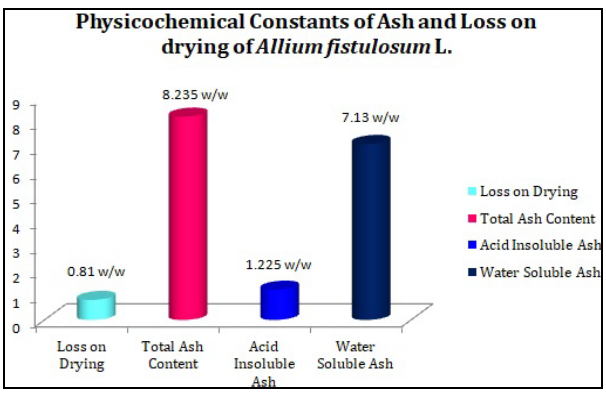

Fig 1: Physicochemical constants of ash and loss on drying of *Allium fistulosum* L.

Extractives values

Different solvents of various extractive values have been determined the quantity of the active constituents in a given amount of plant material when extracted with an exacting solvent (Table 2 & Figure 2). The ingredients of these chemical constituents based on the nature of the drug and the solvent used. A maximum extractive value was found with ethanol (8.64) followed by Chloroform (7.545). The extractive value helps us to choose what solvent will be valuable for extraction of maximum active principle and also helps to decide whether the crude material has previously exhausted or not. Less extractive value indicates addition of exhausted material, ruination or incorrect dealing throughout drying or storage. The alcohol-soluble extractive value was also investigative for evaluation of crude drugs. This shows that the constituents of the drug are more extracted and soluble in water as compared to alcohol (Chanda, 2014; Tatiya *et al*., 2012) [7, 30].

Table 2: Extractive values of *allium fistulosum* l.

Parameters	Value W/W
Ethanol extractive value	8.64
Hexane extractive value	1.185
Chloroform extractive value	7.545
Ethyl acetate extractive value	6.47
Methanol extractive value	5.63

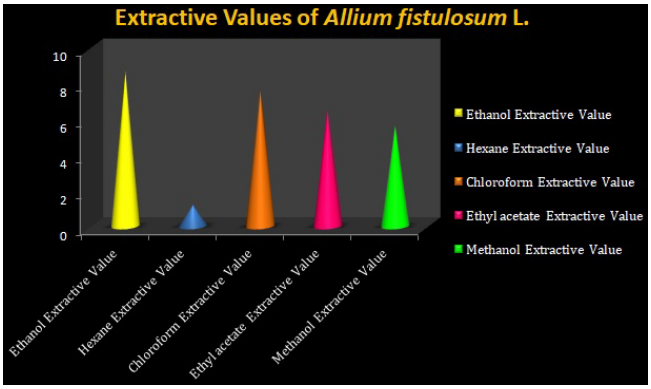

Fig 2: Extractive Values of *Allium fistulosum* l.

Fluorescence analysis of *Allium fistulosum* L.

The behavior of different extracts in day light and under UV light at 254nm and 365nm and represent in Table.3. It was experimental that the ethanolic, acetone, chloroform, benzene, ethyl acetate, water, HCL, Sulphuric acid and hexane extracts were Dark green, orange, yellowish orange, blue, dark orange, dark brown, brown, dark green and dark orange under UV light at 365nm. These parameters are helpful for checking the quality and transparency of the plant in crushed form.

This analysis is also a significant pharmacognostic parameter to show fluorescence of the some constituents in the visible range in daytime. The UV light emits fluorescence in numerous natural products which do not clearly shine in daylight. If plant material themselves are not luminous, they may frequently be converted into glowing derivatives or breakdown products by applying diverse reagents. Hence, plant drugs are repeatedly assessed qualitatively in this fashion, and it is a vital constraint for pharmacognostic estimation (Zhao *et al*., 2011; Khandelwal, 2008) [39, 13].

Table 3: Fluorescence analysis for dry powder of *Allium fistulosum* L.

Treatment	Day light (24 Hrs)	UV light (24 Hrs)
Dry powder	Green	Green
Dry powder + Hexane	Light Green	Dark Orange
Dry powder + chloroform	Pale Green	Yellowish Orange
Dry powder + Benzene	Straw Yellow	Blue
Dry powder + Ethyl acetate	Green	Dark Orange
Dry powder + Ethanol	Dark Green	Dark Green
Dry powder + Acetone	Yellow	Orange
Dry powder + 50% H_2SO_4	Pale Brown	Dark Green
Dry powder + 1N HCL	Pale Brown	Brown
Dry powder + alc.1N NaoH	Brown	Dark Brown
Dry powder + water	Pale Brown	Dark Brown

Phytochemical screening of *Allium fistulosum* L.

The results obtained for the preliminary phytochemical screening of the ethanolic extract of *Allium fistulosum* L. Leaves were presented in Table 4. It showed the presence of alkaloids, steroid, sugar, flavanoids, terpenoids, phenolics compounds, tannins, saponins, coumarins, lignin and terpenoid, glycosides, quinines, Anthroquinone were absent. Phytocompounds give plants their color, essence, aroma and are component of a plant`s natural protection system and defend them against herbivorous insects and fungi, vertebrates, pathogens, and parasites (Muhammad and Ibrahim, 2019) [15]. Based on the analysis, flavanoids are present which act as a potent water-soluble antioxidant and free radical scavenger, which avoid oxidative cell injure, managing diabetes induced oxidative stress and in addition it have strong anticancer action (Okwu, 2001) [17]. Steroids are significance in pharmacy as they possess drugs like sex hormones and can be used for medicine preparation (Salah *et al*., 1995) [22].

Alkaloids used to authenticate their traditional use due to their presence of pharmacological properties for antibacterial activities however for antifungal, anticancer, antiviral and antimalarial (Thawabteh *et al*., 2019; Casciaro *et al*., 2020) [31, 6]. Saponins shield against antibiotics and hypercholesterolemia properties also it have antioxidant, antitumor and anti-mutagenic activities and can reduce the risk of cancer (Roa *et al*., 1995) [21]. Tannin inhibits the growth of numerous bacteria, fungi, yeast and viruses (Prohp and Onoagbe, 2012) [29]. The result of this study found that onion extract possessed a broad spectrum of antimicrobial activity exhibited for both bacteria and fungi due to presence saponin, tannin, flavonoid and alkaloids.

Table 4: Phytochemical screening of *Allium fistulosum* l.

Test	Dry powder of *Allium fistulosum* l.
Terpenoid	-
Flavanoid	+
Steroid	+
Glycosides	-
Sugar	+
Alkaloids	+
Quinones	-
Phenols	+
Tannin	+
Saponins	+
Coumarins	+
Anthroquinone	-
Lignin	+

(+) Present; (-) Negative

Quantitative analysis of *Allium fistulosum* L.

The total phenolic contents of the study plant extracts with FC reagent is articulated in terms of mg of GA/g of the extract and in between the range of 10mg to 50mg.The concentration of phenols was measured in ethanol extract is 17.60 GA/g. The flavanoid content of *A. fistulosum* was determined using with the reagent using aluminium chloride by spectrophotometric method and it was expressed in terms of quercetin equivalent. The flavonoid content was observed in ethanolic extract was 25.75 mgQE/g which shows that concentration depends on the polarity of the solvent used for extraction. The alkaloid content was examined and expressed in terms of capsaicin equivalent as mg of CP/g of extract was 22.50 CP/g. (Table 5 & Figure 3)

Table 5: Quantitative analysis of *Allium fistulosum* L.

Parameters	Value mg/g
Phenol	17.60
Alkaloid	22.50
Flavonoid	25.75

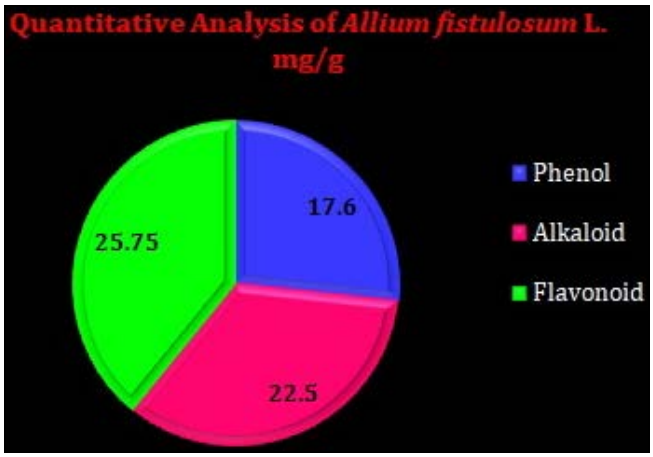

Fig 3: Quantitative Analysis of *Allium fistulosum* L.

Conclusion

From the present study it can be concluded that the ash content, extractive value and fluorescence analysis is useful to find the efficient solvent for extraction process. It gives suggestion about the nature of the chemical constituents present in a plant material. The phytochemical components of *A. fistulosum* L. bulb contain alkaloid, lignin, saponins, sugar, flavonoids, steroid, tannin, coumarins and phenols. We found ethanol is the best solvent for extraction process to high yield of extract. The quantitative and qualitative assessment is helpful for authentication of bioactive phytochemical constituents in *Allium fistulosum* (L) plant further it could be used in drug innovation and development.

Conflicts of Interest

The authors declared no conflicts of interest.

Acknowledgments

Authors of this study wish to thank the Dr's Bio Research Centre, MC road, Thanjavur.

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