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EXTRACTION AND COMPARISON OF POLYPHENOLS IN ALLIUM FISTULOSUM, CYNODON DACTYLON AND HIBISCUS SABDARIFA USING DIFFERENT METHODS OF EXTRACTION

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Abstract- The extracts of many herbs, spices, and other plant species have been used extensively on a global scale to characterize their bioactive property. Edible greens, especially wild greens, play an important role in traditional diets and are rich in polyphenols and other compounds. The polyphenols represent a large group of at least 10,000 different compounds that contain one or more aromatic rings with one or more hydroxyl groups attached to them. They have been known to exhibit antioxidant properties in vitro, and they also play a major role to prevent chronic degenerative diseases caused by using synthetic antioxidants or the presence of free radicals in the body. The present paper aimed to extract and analyze the total polyphenol content, total flavonoid content, and the antioxidant activity in Cynodon dactylon, Hibiscus sabdariffa, Allium fistulosum using soxhlet and ultrasound methods of extraction with solvents ethanol and distilled water and compare their efficiency. The total phenolic content (TPC) and flavonoid content (TFC) was determined by established methods. The In vitro antioxidant activity of the extract was performed by 2,2-diphenyl-1-picrylhydrazyl radical scavenging method. It was observed that plant parts of C.dactylon, have high antioxidant activity with a value of 81.071 when compared to the values, 35.357 and 13.857 of A.fistulosum and H.sabdarifa respectively.

Keywords: Antioxidant, Total phenolic content, total flavonoid content, soxhlet, ultrasound, Allium fistulosum, Cynodon dactylon, Hibiscus sabdarifa

I. INTRODUCTION

There is gaining importance in the intake of antioxidant-rich food for its benefit of reduced risk of degenerative diseases like cardiovascular diseases and cancer.[1] Antioxidants from a variety of sources were studied for the use of food, cosmetics, and other applications. Among the three phenolic compounds found in plants namely, terpenoids, phenolic metabolites, and alkaloids, phenolic compounds have extensive dietary applications. It is also said to modulate many enzymes and cell receptors.

Phenolic compounds include phenolic acids, polyphenols, and flavonoids. 'Flavonoids' is a large group with over 4000 compounds categorized under it. Apart from their physiological roles in plants, flavonoids are important components in the human diet,[2] although they are generally considered non-nutrients. These compounds protect plants and their products from oxidative damage and have been used as antioxidants by humans.[3]

New antioxidants from a natural source are used in functional foods, natural antioxidants, and nutraceuticals. Phytochemical screening[4] is widely used to study antioxidants in plants. The uses of dietary polyphenols have been noticed only in recent years, though there is a consumer and public health awareness, & safety and environmental concern due to the wide use of organic solvents in the extraction processes and the possibility of contamination. This resulted in the search for the best possible extraction and purification methods to obtain better quality.[5] There is a possible inexhaustible resource of raw materials for various food and fashion

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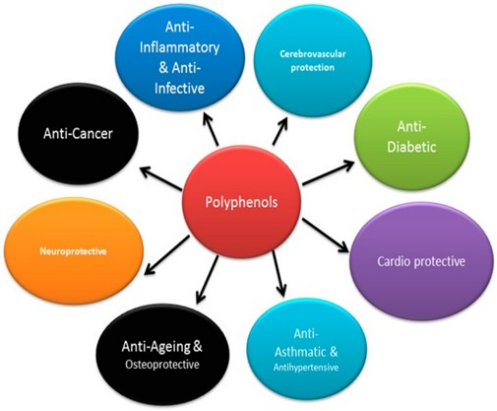
industries and pest control as the latest advancement. There are various medicines prepared from herbs and other plants[6] for thousands of years in traditional Indian and Romania medicines[7] and researches are being carried out to obtain active components from them. To advance these benefits, we require more knowledge of the structure and availability of these components and for this purpose, we study separation and availability of them and there are various existing analysis methods. Polyphenols are of use to human activity in many ways as mentioned in fig.1.

There are various methods through which the extraction and purification process is carried out[8] and the principal method is to either by rupturing the tissue or by diffusion. These methods may result in the degeneration of some chemically sensitive phenols because of mechanical disruption and long extraction periods and severe heating conditions. Most of the methods used in the extraction of polyphenols use organic solvents with low pH and high-temperature conditions but, the extraction efficiency of these methods is low, and it is necessary to purify to improve the antioxidant activity.

Therefore, this work aims to assess the polyphenol content, flavanol content, and antioxidant activities of *Cynodon dactylon, Hibiscus sabdariffa, Allium fistulosum* using both chemical and mechanical extractions and in vitro digestive enzymatic analysis methods.



(Fig.1) Role of polyphenols in human activity

II. MATERIALS AND METHODS:

Preparation of plant material and extracts

All plants: Cynodon dactylon, Hibiscus sabdariffa, Allium fistulosum were purchased and Fresh plant leaves were devoid of dust, impurities and then drained. These leaves were cleaned, sorted, and washed and then dried in an oven at 70°C. Then these dried leaves were stored at room temperature in hermetically sealed black plastic bags under darkness to avoid possible oxidation of the compounds before further treatment. The dried leaves were crushed in a mixer blender and then sieved using a 70/100 mesh sieve-set. 5 grams of each plant material was used to perform the oil extraction by the Soxhlet method. Using ethanol-water mixture as solvent, the oil was extracted. Similarly, 5 grams of the plant material along with ethanol was used for the ultrasound method of extraction at optimum conditions. These quantities were used to ensure that enough oil could be extracted.

Chemicals and Reagents

All the chemicals, solvents, and reagents used were of laboratory grade. Ethanol, Folin-Ciocalteu reagent, sodium carbonate, aluminium chloride, 1,1-diphenyl-2-picrylhydrazyl (DPPH) were used Also detecting reagents like ferric chloride, lead acetate reagent etc. were freshly prepared for qualitative phytochemical screening of plant extracts. Follin's reagent was prepared during the experiment while some of the regents were bought for qualitative detection.

Methods of Extraction:

Soxhlet: In Soxhlet extraction, 5g of powdered samples are sealed in Muslin cloth bags and placed in an extraction chamber located atop a collecting flask beneath a reflux condenser. After the addition of the solvent ethanol-water in 3 different compositions, the system is heated at 50-60°C, and the solvent condenses after reaching a certain level of temperature. In the end, the liquid extract, collected in the flask positioned beneath the system. Soxhlet extraction is a continuous process with the advantage of being less time and less solvent consuming than other methods of extraction.

Sonication: In the Ultrasonic method of extraction, 13g of the powdered samples, mixed with 260mL of pure ethanol. This mixture, then placed inside the Ultrasonic cell crusher with the required working conditions for 40 min and a probe of 20mm. The extract then obtained was filtered using a Whatman filter paper to remove the sediments and other visible particles.

The representation of both the apparatus is given in fig.2.

(Fig 2)-soxhlet and ultrasound apparatus

Determination of Phenolic Content

The determination of total phenolic content was done spectrophotometrically using Folin-Ciocalteu's reagent. The method is based on the reduction of phosphotungstic acid (H_3/P $[W_3O_{10}]_4$ in alkaline solution to phosphotungstic blue (based on $WO_2.nWO_3$). The absorbance value is directly proportional to the aromatic phenolic groups in the sample which were influenced by phosphotungstic blue so formed and is used for the determination, expressed as gallic acid equivalent. During the oxidation of phenolic compounds, phosphomolybdic and phosphotungstic acid, contained in the Folin-Ciocalteu's reagent, were reduced to blue-coloured molybdenum and tungsten oxides. After two hours, the absorbance of blue colouration was measured using a UV-VIS spectrophotometer (Hitachi U2000) at 765 nm against a blank sample. In this work, gallic acid was used as the standard, therefore the results were expressed as mg/g of gallic acid equivalent (GAE).

Total flavonoid content

The total flavonoid content of these plant extracts was determined by the aluminium chloride colorimetric method. The absorbance was measured at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg quercetin equivalent per g dry weight.

Antioxidant-activity

The antioxidant activity of the extract was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay, as described earlier. The absorbance of the mixture was then measured at 517 nm. Ascorbic acid was used as a positive control. The ability of the sample to scavenge DPPH radical was determined from:

DPPH scavenging effect = (control OD – sample OD) / control OD x 100

1. RESULTS AND DISCUSSION:

Determination of Total Phenolic Content:

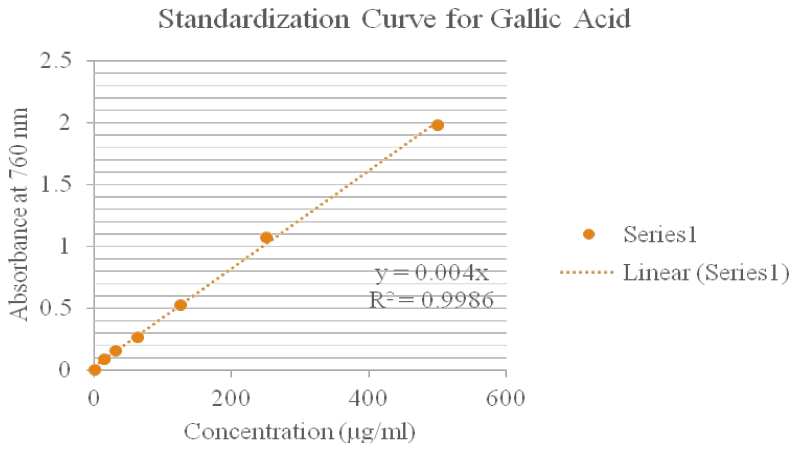
The total phenols content in the obtained extracts of Cynodon dactylon, Hibiscus sabdariffa, Allium fistulosum was determined using Folin-Ciocalteu reagent. The absorbance was measured at 760 nm in a UV spectrophotometer. The amount of total phenolic compounds was expressed as mg gallic acid equivalents

(GAE)/g of plant material using a regression equation that was obtained using a gallic acid calibration curve (y = 0.004x, R^2 = 0.9986) represented in fig.3. Extracts from each of the species were taken for further studies based on the high phenolic content obtained

The total phenolic contents of the aqueous and ethanolic extracts of the leaves were estimated using the Folin Ciocalteau reagent (FCR). This was then compared with a blank solution of gallic acid. The value of absorbance for each concentration is given in table.1.

CONCENTRATION(mg/ml)	ABSORBANCE
15	0.084
31.25	0.156
62.5	0.266
125	0.52
250	1.07
500	1.98

(Table.1) Standardization of gallic acid



(Fig.3)-Gallic acid standardization curve

TPC = "C x V" /"m"

C- Concentration of Gallic acid(µg/ml)

V- Volume of extract(ml)

m – mass of the extract (g)

The value of absorbance obtained when the extracts were analysed using the UV spectrophotometer was recorded below:

Sample	**Number of cycles**	**Sample solution (mg/mL)**	**Weight of dry extract (mg)**	**Absorbance**	**GAE concentration C (µg/ml)**	**TPC-GAE equivalent A=(C*V)/M**
A(1:1)	5	1000	0.05	0.430	101.25	101.25
G(1:1)	5	1000	0.05	0.11	21.12	21.12
S(1:0)	5	1000	0.05	0.082	14.125	14.125
A(1:0)	5	1000	0.05	0.21	51.125	51.125
G(0:1)	5	1000	0.05	0.442	2.125	2.125

A(40-U)	-	1000	0.05	0.8	75.625	75.625
G(1:0)	5	1000	0.05	0.058	10.625	10.625
S(1:1)	5	1000	0.05	0.024	19.125	19.125
A(0:1)	5	1000	0.05	0.026	0.125	0.125
A(20-U)	-	1000	0.05	0.44	33.625	33.625
S(40-U)	-	1000	0.05	0.4	21.625	21.625
S(20-U)	-	1000	0.05	0.32	15.625	15.625
G(20-U)	-	1000	0.05	0.07	4.625	4.625
G(40-U)	-	1000	0.05	0.18	9.125	9.125

(Table.2) Value of TPC obtained for all samples

A- Arugampul, S- Spring onion, G- Gongura leaves *

U- Ultrasound method of extraction *, ((1:0)(1:1)(0:1)) – Cycles of soxhlet extraction *

These were the amounts of polyphenol calculated in terms of GAE equivalent in all the extracts obtained. From the above data, it is determined that soxhlet extraction proved to be the more efficient method when compared to the ultrasonic method of extraction. Also, among the 3 species, Cynodon dactylon exhibited the maximum content of polyphenol present by 35% than the other two. And it was found that the samples with (1:1) ethanol-water ratio gave the highest result. So, from hereon only the (1:1) are used for analysis.

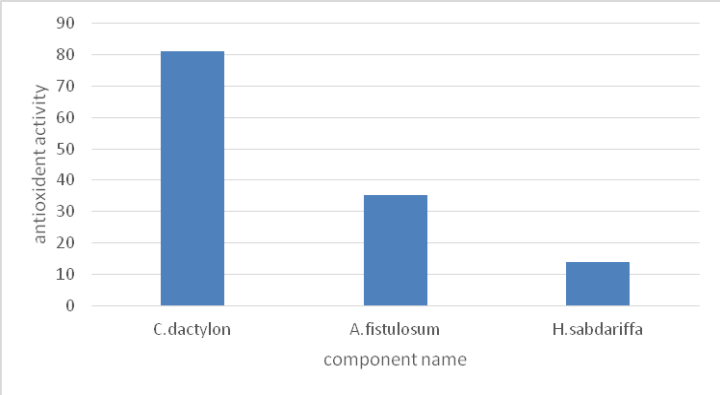
Determination of Antioxidant-activity:

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was performed by the above-reported method. The reaction mixture was incubated for 30 min at room temperature, protected from light to prevent any reaction. The absorbance was measured at 517 nm. The percentages and amount of the DPPH free radical scavenging activity were calculated.

It was determined that Cynodon dactylon (Arugampul) had the highest antioxidant capacity when compared to the other two components as given in the table below.

Cynodon dactylon (Arugampul)	**81.071**
Allium fistulosum (spring onion)	35.357
Hibiscus sabdariffa (Gongura)	13.857

(Table.3) Value of Antioxidant – the activity of Cynodon dactylon, Hibiscus sabdariffa, Allium fistulosum



(Fig.4) – Analysis of antioxidant activity

These were the antioxidant activity in %w/w calculated in all the extracts obtained. From the above data in (Fig.4), it is determined that among the 3 species Cynodon dactylon exhibited the maximum antioxidant activity content

From the above results, we can conclude that the antioxidant activity will be the highest in the component whose total phenolic content is the most. So, the antioxidant activity of Cynodon dactylon was found to be the highest and 50% than the other two components.

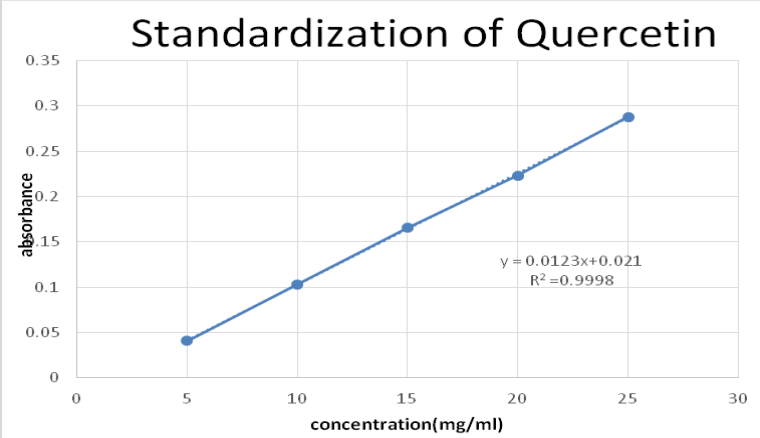
Determination of total flavonoid content

The amount of flavonoid in the plant extracts was determined by the aluminium chloride method. The mixture was incubated for 30 minutes at room temperature. The absorbance of the sample was measured at 420 nm spectrophotometrically. Quercetin was used as a standard compound (1mg/ml). Concentrations of 5,10,15,20 and 25 mg/100ml of quercetin were prepared in methanol.

All the readings were recorded in triplicates. The results were determined from the standard calibration curve of Quercetin which is represented in fig.5 using the values from table.4 and total flavonoid contents were expressed as quercetin equivalents (mg/g) of QE of the extracted compound).

Concentration(µg/ml)	Absorbance
5	0.048
10	0.1032
15	0.1655
20	0.2231
25	0.2789

(Table.4) Standardization of Quercetin



(Fig.5) standardization curve for Quercetin

Upon calculating it was determined that Arugampul (Cynodon dactylon) had shown the highest content of Flavonoids amongst the three components and the values are in table.5.

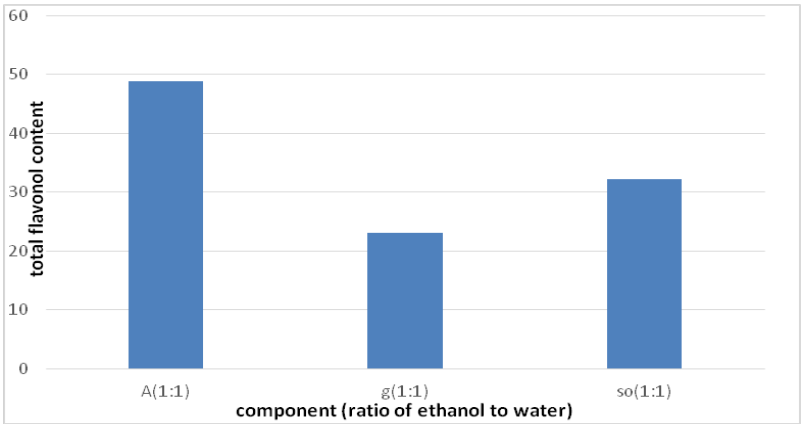
Sample	Total Flavonoid Content (mg/g of Quercetin)
Cynodon dactylon (Arugampul)	**48.81**
Allium fistulosum (Spring onion)	32.16
Hibiscus sabdariffa (Gongura)	23.17

(Table.5) Analysis of Total Flavonoid content

These were the Total Flavonoid content in %w/w calculated in all the extracts obtained as in Fig.6. From the data, it is determined that among the 3 species Cynodon dactylon exhibited the maximum Total Flavonoid content which was 51% more than spring onion and 58% higher than gongura.

From the above results, we can conclude that the Total Flavonoid content will be the highest in the component whose total phenolic content is the most. So, the antioxidant activity of Cynodon dactylon was found to be the highest.

The problem with soxhlet extraction is that it cannot be used on an industrial scale as the equipment is highly expensive to be set up and be processed. So, an experimental working design of equipment which carries out a process like a soxhlet has to be configured. After the extracts are obtained using the soxhlet and ultrasound method, these extracts contain a considerably high amount of the solvent as well. The presence of such solvents in the extract might provide hindrance while analyzing the presence of the necessary compounds. So, the extract must be separated from the mixture to obtain pure and efficient results. To carry out this separation process a rotary vacuum evaporator is to be used. But again, this can be used only on a lab-scale and cannot be industrially used. So, a working design of a separator to perform this specific function would provide a yield of better quantity as well as quality and efficiency.



(Fig.6) – Analysis of Total Flavonoid content

Similarly, the Ultrasound equipment is a highly expensive one and thus another working model of this equipment that can produce high yield at an Industrial level and eco-friendly will make the process efficient. The remaining solid feed after extraction process must be disposed of properly, draining of the remaining solvents or other chemicals present before being used as a cattle feed.

III. CONCLUSION

The results of the present study have revealed that extraction by soxhlet using ethanol-water mixture achieved high yields of polyphenols and from A.fistulosom, C.dactylon, and H. sabdariffa when compared to Ultrasound method of extraction. It was observed that the antioxidant capacity of C.dactylon extract was higher than that of A.fistulosum and H.sabdariffa extracts. Ultraviolet spectrometry analysis revealed the presence of phenolic compounds and flavanoids in all 3 compounds. It was determined the C.dactylon produced at least 18% and 51% more TPC and TFC values than the other two species respectively. Finally, the phenolic compounds and flavanols could be isolated from C.dactylon extracts due to their application as natural antioxidant compounds, which is important because of the increasing use of synthetic antioxidants in the food industry.

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