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Physicochemical and phytochemical evaluation of *Aponogeton natans* (Linn.) Engl. & Krause-an important folklore medicine

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ABSTRACT

The objective of the present work is to carry out the physicochemical analysis of powder and to investigate the phytochemicals present in petroleum ether, benzene, chloroform and methanol extracts of Aponogeton natans (Linn.) Engl. & Krause leaf with leaf stalks. Physicochemical analysis was done by determining total ash, acid insoluble ash, water soluble ash sulfated ash, extracting value, dry matter, content, moisture content, crude fiber content, foaming index and swelling index, inorganic elements and fluorescence analysis as per the standard methods. Phytochemical investigations were carried out by using standard preliminary phytochemical tests for the different active constituents present in the plant. From the physicochemical investigation, it was found that the Aponogeton natans (Linn.) leaf and leaf stalks have 5.13 ±0.06, 1.33 ±0.12, 1.2 ±0.06, and 7.02±0. 16 as total ash, acid insoluble ash, water soluble ash and sulphated ash respectively. Pet ether, chloroform, ethanol and water extractive value were found to be 2.19 ±0.29, 9.23 ±0.06, 15.13 ±0.15 and 18.27 ±0.12 respective ly. Dry matter content, moisture content, crude fiber content, foaming index and swelling index were found to be 87.53 ± 0.47, 10.15 ±0.15, 78.20 ±0.22, Less than 100 and 0.79 ±0.02 respectively. The inorganic element, such as iron, sulfate, chloride and nitrates were found in the aerial parts. Florescence analysis of the powder were reported which gives the sensitivity of the chemical in different chemical reagents. From the preliminary phytochemical analysis, it was found that the methanol extract contained maximum important phytoconstituents like carbohydrate, protein, phytosterol, glycoside, saponin, flavonoids and polyphenols. The information gathered from the physicochemical and phytochemical study of Aponogeton natans Linn. delivered the parameters which will serve to determine the quality of the plant material in the future. The physicochemical and phytochemical data obtained from the results of the Aponogeton natans mentioned parts might be useful in determining the authenticity of the drugs.

Key words: *Aponogeton natans* (Linn.), physicochemical analysis, phytochemical screening, methanol extract, flavonoids, polyphenols.

INTRODUCTION

Aponogeton natans (Linn.) Engl. & Krause. belongs to Aponogetonaceae family. The plant occurs in the plains, in the ponds and marshy places in Asia, Australia, India and Srilanka. Leaf pastes are consumed with hot water to treat cuts & wounds [1]**.** Fresh tubers are ground into a paste and boiled with 200 ml of coconut oil and applied on the hair before bath for three days to get rid of fungal infection [2]**.** *Aponogeton natans* (Linn.) Engl. & Krause is an important ingredient in the preparation of an important Ayurvedic preparation Useerasava. This asava is useful for raktapitta (Haemothermia), anemia, impurity of blood and diabetes [3, 4]. A perusal of existing reports reveals that the no detailed physicochemical and phytochemical study had been done earlier. Therefore, the present study has been planned to investigate the physicochemical and phytochemical study of powder and various extracts of *Aponogeton natans* (Linn.) Engl. & Krause. leaf with leaf stalks.

MATERIALS AND METHODS

Physicochemical and phytochemical parameters of plant material

Coarse powder of the plant leaf with leaf stalks was used to perform quality control tests such as total ash, acid insoluble ash, water soluble ash, sulfated ash, extractive matter, loss on drying, crude fiber content, foaming index and swelling index etc. three determinations were carried out for each parameter and the results were expressed as mean value ± S.E.M

Determination of ash values

Aponogeton natans Linn. Leaf with leaf stalks powder was analyzed for total ash, acid insoluble ash and water soluble ash, according to standard procedures [5]**.**

Determination of extractive value

For determination of extractable matter, different solvents viz. petroleum ether, chloroform, ethanol and water were used as the solvents for extraction by maceration method. For preparation of petroleum-ether extract, about 4.0 g of coarsely powdered air dried material was accurately weighed and taken in a glass stoppered conical flask. 100 ml of petroleum ether was added and weighed to obtain the total weight including flask. The conical flask was corked and set aside for 24 h with shaking frequently. The solvent was then filtered through a dry filter paper and 25 ml of this filtrate was transferred to a tarred flat bottom dish and evaporated to dryness on a water bath. The solvent was dried at 105ºC for 6 h and cooled in a desiccator for 30 minutes, was weighed without delay. The content of extractable matter in mg per gram of air dried material was calculated chloroform, ethanol and the water extractable matter was also determined as described above [5].

Determination of foaming index

About 1gm of coarse powder was weighed accurately and transferred to a 500 ml conical flask containing 100ml of boiling water. Maintained at moderate boiling for 30 minutes, cooled and filtered into a 100ml volumetric flask and added sufficient water through the filter to dilute the volume. Poured the decoction into 10 stoppered test tubes (height 16cm, diameter 16 mm) in successive portion of 1 ml, 2 ml, 3 ml its up to 10 ml and adjusted the volume of the liquid in each tube with water to 10 ml. Stoppered the tubes and shaken them in lengthwise motion for 15seconds, two shakes per second. Allowed to stand for 15 min and measured the height of the foam. The results are assessed as follows.

- If the height of the foam in every tube is less than 1cm, the foaming index is less than 100.
- If the height of the foam is 1cm is measured in any tube, the volume of the plant material, decoction in these tubes are used to determine the index. If this tube is the first or second tube in a series, prepare an intermediate dilution in a similar manner to obtain a more precise result.
- If the height of the foam is more than 1cm in every tube, the foaming index is over thousand. In this case the determination was repeated using a new series of dilutions of the decoction in order to obtain a result the foaming index Calculate using the following formula: 1000/a

Where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1cm is observed [5].

Determination of swelling index

1 gram of leaf with leaf stalks powder was weighed accurately and transferred into a 25 ml glass stoppered measuring cylinder (internal diameter 16mm, length of graduated portion 125 mm, marked in 0.2 ml divisions from 0 to 25 ml in an upward direction). 25 ml of water was added and the mixture was shaken thoroughly every 10 minfor 1 hour. Then the mixture was allowed to stand for 3 h at room temperature. The volume occupied by the powder material was measured in ml to determine the swelling index [5].

Determination of dry matter and moisture

The dishes were washed with detergent and dried at 105^{o}C overnight. The dishes were then placed in desiccators for cooling and then weighed. Accurately weighed quantity (2.0 g by difference) of the powdered leaf with leaf stalks was placed into a weighed dish. It was placed in 105^{o}C oven overnight (with lids open until a constant weight loss). The dishes were removed, placed in desiccators and cooled. The cooled dishes were taken out of desiccators and weighed as quickly as possible [6].

Calculation:

$$\textbf{Dry matter}\ (\%) = \frac{(\text{Weight of the dish} + \text{weight of the dried sample}) - \text{Weight of the dish}}{\text{Weight of the sample before drying}} \times 100$$

$$\textbf{Moisture content}\ (\%) = \frac{\text{weight of fresh sample} - \text{weight of dried sample}}{\text{Weight of fresh sample}} \times 100$$

Estimation of crude fiber

2 g of ground sample was extracted with petroleum ether to remove fat (initial boiling temperature 35-38^{0}C and final temperature, 52^{0}C). 2 g of dried sample were boiled with 200 ml of sulphuric acid for 30 minwith bumping chips. It was then filtered through muslin cloth and washed with boiling water until the washings were free from acid. The residue was boiled with 200ml of sodium hydroxide for 30 minutes, filtered through muslin cloth again and washed with 25ml of boiling sulphuric acid. The residue was removed and transferred to pre-weighed ashing dish ('W1', g). Dried the residue for 2 h at 130 ± 2^{o}C, then cooled in a desiccator and weighed ('W2', g). Ignited for 30 minat 600 ± 15^{o}C. Cooled in a desiccator and reweighed ('W3', g) [7].

Calculation:

Loss in weight = (W2 - W1) - (W3 - W1)

$$\%\ \textbf{crude fiber content} = \frac{\text{On the ignition}}{\text{weight of sample (g)}} \times 100$$

Fluorescence analysis

A small quantity of dried and finely powdered leaf with leaf stalks sample was placed on a grease free microscopic slide and added 1-2 drops of a freshly prepared solution such as 5 % ferric chloride, concentrated sulphuric acid, concentrated hydrochloric acid, concentrated nitric acid, 1N sodium hydroxide, picric acid solution, iodine solution was mixed by gentle tilting the slide and was left for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in daylight, short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in various radiations were recorded [8].

Test for inorganic elements

The ash was prepared from drug material. To the ash 50% v/v HNO_3 was added and it was kept for 1 hour or longer. It was filtered & with filtrate the following tests were performed to test for calcium, magnesium, sodium, potassium, iron, sulphate, phosphate, chloride [9].

Extraction of *Aponogeton natans* (Linn.) Engl. & Krause leaf with leaf stalks

There is increasing interest in the extraction and isolation of secondary metabolites from plants. Extraction methods were applied to obtain a crude plant extract. The classical chemical procedure for obtaining organic constituents from dried plant tissues was continuous soxhlet extraction with a range of solvents.

Preparation of extracts

The air dried leaf and leaf stalks was loaded into soxhlet apparatus and subjected to extraction for about 72 h with petroleum ether (60-80ºC), benzene, chloroform and methanol successively. After extraction the solvent was distilled off and the extract was concentrated under reduced pressure using a rotary evaporator. The extracts were stored in a closed bottle and kept in refrigerator until tested. The four extracts were then subjected to phytochemical analysis.

Preliminary phytochemical analysis

Therapeutic activity of vegetable drugs depends upon the type of constituents present in them. Plant material was screened for the presence of phytoconstituent(s) using different chemical tests. Powder drug and different extracts were screened for different phytoconstituents [10, 11].

RESULTS AND DISCUSSION

Physicochemical and phytochemical parameters of plant material

The analytical parameters studied include ash values, extractive values, loss on drying, crude fiber content, foaming index and swelling index. The data obtained from the above studies are shown in **(Table 1).** The physicochemical analysis presented in this research will be beneficial in determining plant adulteration with other species.

Fluorescence analysis

Fluorescence analysis of leaf with leaf stalks powder had been carried out in daylight and under U.V light. The powders were treated with different organic solvents and solutions were again observed in normal daylight and under U.V. light and the observations are pooled in **(Table 2).** The analysis shows the presence fluorescence, which may be attributed due to the presence of some phytochemicals under different light conditions.

Inorganic element

Inorganic element found in the ash of leaf with leaf stalks were iron, sulphate, chloride and nitrate shown in **(Table 3).** The inorganic elemental analysis is important as these elements play an important role in a physiological process involved in plant and animals.

Table 1: Physicochemical parameters of *Aponogeton natans* Linn. leaf with leaf stalks.

Sl. No.	Parameters	Values in %
1	**Ash Value**	
	Total Ash	5.13 ± 0.06
	Acid insoluble ash	1.33 ± 0.12
	Water soluble ash	1.2 ± 0.06
	Sulphated ash	7.02± 0.16
2	**Extractive value**	
	Pet ether	2.19 ± 0.29
	Chloroform	9.23 ± 0.06
	Ethanol	15.13 ± 0.15
	Aqueous	18.27 ± 0.12
3	**Loss on drying**	
	Dry matter content	87.53 ± 0.47
	Moisture content	10.15 ± 0.15
4	**Crude fiber content**	78.20 ± 0.22
	Foaming index	Less than 100
	Swelling index	0.79 ± 0.02

Table 2: Fluorescence analysis of *Aponogeton natans* Linn. powder.

Sl. No.	Reagent	Visible/Day light	UV 254 nm	UV 366nm
1	Powder drug as such	Light brown	Brown	Yellowish brown
2	Powder + Methanol	Light brown	Yellowish brown	Brownish black
3	Powder + 1% glacial acetic acid	Brown	Dark brown	Blackish brown
4	Powder +10% NaOH	Yellowish brown	Dark yellowish	Bluish brown
5	Powder + Dil. NH_3	Yellowish brown	Light brown	Brown
6	Powder + Conc. HNO_3	Brown	Blackish brown	Dark brown
7	Powder + Dil. NH_3 + Conc.HNO_3	Yellowish brown	Light brown	Blackish brown
8	Powder +1M H_2SO_4	Brown	Dark brown	Yellowish black
9	Powder +1M HCl	Brownish yellow	Brown	Dark brown
10	Powder + 10% $FeCl_3$	Reddish brown	Light brown	Brownish yellow
11	Powder + Acetone + Methanol	Light brown	Brown	Black
12	Powder + 10% Iodine	Yellowish brown	Dark brown	Blackish brown

Table 3: Determination of inorganic element of *Aponogeton natans* Linn. leaf with leaf stalks.

Sl. No.	Test for	Inference
1	Calcium	-
2	Magnesium	-
3	Sodium	-
4	Potassium	-
5	Iron	+++
6	Sulphate	+++
7	Phosphate	-
8	Chloride	++
9	Carbonate	-
10	Nitrate	+

Table 4: Description of *Aponogeton natans* Linn. leaf with leaf stalk extracts.

Sl. No.	Extract	Color	Consistency	Color at 365 nm	Color at 254 nm	Percentage yield
1	Petroleum ether	Yellowish	Greasy	Intense yellow	Greenish yellow	2.84
2	Benzene	Yellowish	Greasy	Brownish	Greenish yellow	2.77
3	Chloroform	Brownish	Waxy powder	Reddish brown	Greenish	3.36
4	Methanol	Greenish yellow	Amorphous	Reddish brown	Yellowish green	11.81

Table 5: Preliminary phytochemical analysis of *Aponogeton natans* Linn. extracts					
Experiment	**Powder drug**	**Petroleum ether extract**	**Benzene extract**	**Chloroform extract**	**Methanol extract**
Test for carbohydrates					
1. Molisch's Test	-	-	-	-	+
2. Fehling's Test	-	-	-	-	+
3. Benedict's Test	-	-	-	-	+
4. Barfoed's Test	-	-	-	-	+
5. Bial's Test	-	-	-	-	-
6. Aniline Acetate Test	-	-	-	-	-
7. Cobalt-chloride Test	-	-	-	-	+
8. Tollen's Phloroglucinol Test	-	-	-	-	-
9. Selwinoff's Test	-	-	-	-	-
10. Inversion Test	-	-	-	-	+
11. Iodine Test	-	-	-	-	-
Test for Gums and Mucilages					
Swelling Test	-	-	-	-	-
Test for Proteins and Amino Acid					
1. Ninhydrin Test	-	-	-	-	+
2. Biuret Test	-	-	-	-	-
3. Tannic Acid Test	-	-	-	-	++
4. Heavy Metal Test	-	-	-	-	-
5. Million's Test	-	-	-	-	-
6. Xanthoprotein Test	-	-	-	-	-
Test for Fixed Oils and Fats					
1. Spot Test	-	+++	++	-	-
2. Saponification Test	++	-	-	-	+
Test for Phytosterols					
1. Libermann's Test	++	++	++	-	+
2. Salkowski's Test	-	++	++	-	-
3. Libermann-Burchard's Test	-	++	++	+++	++
Test for Glycosides					
1. Baljet's Test	+	-	+	-	++
2. Legal's Test	+	-	-	-	+
3. Borntrager Test	-	-	-	-	-
4. Modified Borntrager Test	-	-	-	-	-
5. Cyanogenetic Glycoside Test	-	-	-	-	-
6. Raymond's Test	-	-	-	-	-
7. Tollen's Test	-	-	-	-	-
8. Xanthydrol Test	-	-	-	-	-
9. Antimony Trichloride Test	-	-	-	-	++
10. Kedde's Test	-	-	-	-	-
Test for Saponins					
1. Foam Test	+	-	-	-	+
2. Haemolytic Test	+	-	-	-	++
Test for Flavonoids					
1. Ferric Chloride Test	+	-	-	-	++
2. Shinoda Test	+	-	-	-	++
3. Lead Acetate Test	+	-	-	-	++
4. Fluorescence Test	+	-	-	+	++
5. Action of Alkali and Acid	+	-	-	-	+++
Test for Tannins and Phenolic Compounds					
1. Ferric Chloride Test	+	-	-	-	++
2. Test with Heavy Metals	+	-	-	-	++
3. Nitric Acid Test	+	-	-	-	++
4. Goldbeater Skin Test	+	-	-	-	-
5. Gelatin Test	+	-	-	-	++
6. Phenazone Test	+	-	-	-	++
7. Catechin Test	+	-	-	-	++
8. Chlorgenic Acid Test	+	-	-	-	++
9. Vanillin-HCl Test	+	-	-	-	++
Test for Alkaloids					
1. Mayer's Test	-	-	-	++	-
2. Dragendorff's Test	+	-	-	-	-
3. Wagner's Test	-	-	-	++	-
4. Hager's Test	-	-	-	-	-
5. Sonneuschein's Test	-	-	-	-	-
6. Scheibler's Test	-	-	-	-	-
7. Tannic Acid Test	+	-	-	-	-

Preparation of extracts

The powder material was refluxed successively with pet-ether (60-80^{o}C), chloroform, and ethanol (90%) in a Soxhlet extractor for 18 h in batches of 50 g each cycle. The aqueous extract was prepared by macerating 400 g of the powdered material with 1000ml of distilled water for three days with intermittent stirring and then filtered to obtain the aqueous extract. The color and consistency of the four extracts were studied and the percentage yield of each extract was determined. The results are depicted in **(Table 4).** The percentage yield obtained from pet-ether, benzene, chloroform and methanol extracts were 2.84, 2.77, 3.36, and 11.81% respectively. From the extraction it was found that methanol extract may contain more quantity of phytoconstituents than other extracts. Further, this indicates that the plant may contain higher quantities of polar Phyto constituents than non polar Phyto constituents.

Preliminary phytochemical analysis

All the four extracts were screened for phytochemical investigation by different phytochemical tests to check the presence or absence of a group of phytochemical constituents. These phytochemical tests shown the presence of proteins, carbohydrates, alkaloids, saponins, tannins, flavonoids, steroids, triterpenoids etc. as mentioned in **(Table 5).** Petroleum ether extract gave positive tests for fats and phytosterol; benzene extract gave positive results for fats, phytosterol and glycoside; chloroform extract shown positive tests for phytosterol, glycoside, flavonoids and alkaloids; methanol extracts were found to contain carbohydrate, protein, phytosterol, glycoside, saponin, flavonoids and polyphenols. Preliminary phytochemicals analysis relieves the presence of important phytoconstituents such as phytosterol, glycoside, saponin, flavonoids and polyphenols in methanol extract. The presence of phytoconstituents like flavonoids and polyphenols gives the inference that the extract can be pharmacologically more active than other extracts.

CONCLUSION

The present work focuses on the physicochemical analysis and phytochemical screening of *Aponogeton natans* (Linn.) Engl. & Krause. leaf and leaf stalks. As there was no physicochemical and phytochemical work in this folklore valued drugs, present work is taken up in the view to lay down the physicochemical standards and presence of phytochemicals, which could be used in deciding the genuineness of the selected folklore drug. The presence of phytochemicals was investigated by subjecting all the four extracts to phytochemical screening. Preliminary photochemical investigation revealed the presence of fixed oil and phytosterol in pet ether, benzene and chloroform extracts. Glycoside was found in benzene and methanol extracts, flavonoid in chloroform and methanol extract, alkaloid in chloroform, carbohydrate, protein, saponin, phenolic and polyphenolic compounds were present in methanol extract. The physicochemical and phytochemical data obtained from the results of the *Aponogeton natans* mentioned parts might be useful in determining the authenticity of the drugs.

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