International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

PHYTOCHEMICAL SCREENING AND IN VITRO EVALUATION OF REDUCING POWER OF ETHANOL EXTRACTS OF SYNEDRELLA NODIFLORA.

Sazzad Hossain*, JonyMallik, Md. Shahed Hossain**

Department of Pharmacy, BGC Trust University, Bangladesh, Chandanaish , Chittagong.

Email: jonymallik@ymail.com

ABSTRACT

Pharmaceutical analysis of the extract of leaf of Synedrella nodiflora (family: Compositae) indicate that compounds having medicinal activity because of presence of alkaloid, gums, steroid, reducing sugar and tannins in the extract of Synedrella nodiflora. The pharmaceutical interest of these compounds coupled with the use of this plant in traditional medicine promoted us to check Synedrella nodiflora for possible antioxidant followed by reducing power determination and antioxidant activities. The dried leaves of the plant were subjected to successive extraction with ethanol, and the extract was used to investigate the activities. The hot ethanol extract of the leaves of Synedrella nodiflora showed low reducing activity. The hot ethanol extract of Synedrella nodiflora (500 µg/ disc) has less activity than the standard. Ascorbic acid is used as standard. The obtained results provide a support for the use of this plant in traditional medicine and its further investigation.

KEY WORDS Synedrella nodiflora , Ethanol extract, Reducing Power, Ascorbic acid.

INTRODUCTION

The subject of phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. If is concern with the enormous variety of organic substances that are elaborated and accumulated by plants and deals with the chemical substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. In all these operations, methods are needed for separation, purification and identification of many different constituents present in plants. Thus advances in our understanding of phytochemistry are directly related to the successful exploration of known techniques, and continuing development of new techniques to solve outstanding problems as they appear. As a result of modern extraction, and isolation techniques and pharmacological testing procedures, new plant drugs usually find their way into medicine as purified substances rather than in the form of galenical preparations. (Trease 1983, A. Ghoni 2003, Dev S. 2002)

The precise mode of extraction naturally depends on the texture and water content of the plant material being extracted and on the type of substance that is being isolated. Generally two type procedure are used for obtaining organic constituents-

- Cold extraction.
- Hot extraction.

The classical chemical procedure for obtaining organic constituents from dried plant tissue (Heartwood, dried seeds, root, leaf) is to continuously extract powdered material in a sox let apparatus with a range of solvents, starting in tern with petroleum ether and chloroform (to separate lipids and terpenoids) and then using alcohol and ethyl acetate (for more polar compounds). The extract obtained is then carried out in a rotary evaporator which will concentrate bulky solution down to small volumes. In most cases, mixtures of substances will be present in the extract and these are subjected to chromatographic fraction (Harbonr, 1984). If a single substance is present, the crystals can be purified by recrystallization. As standard precaution against loss of material, concentrated extracts should be stored in the refrigerator and a trace of toluene added to prevent fungal growth.

Figure 1: Plants of Synedrella nodiflora

METERIALS AND METHODS

Collection

The plant selected for present work was Synedrella nodiflora (Family: Compositae) which was collected from Pahartolly, Chandanish, Satkania (in Satkania Thana), Bangladesh in November, 2010 at day time. The plant is available in winter season and the leaves and stems were collected from fresh plants.

PREPARATION OF CRUDE EXTRACT

Drying and grinding

The collected plant parts were separated from undesirable materials or plant parts. Then the leaves and the stems were cutted into very small pieces and kept in the open dry under shadow for 15 days. Then the plant parts (leaves & stems) were ground into a coarse powder with the help of a suitable grinder (From: BCSIR in Chittagong). The powder was stored in an airtight container and kept in a cool, dark, and dry place until analysis commenced.

Hot extraction

Here we used shocks let apparatus. First we take 50 gm of powder in specially make filter paper and 700 ml of 99.8% ethanol was taken. After 7 days the powder was replace by another 50 gm of powder. Then after 14 days the solution was filter by course filtration.

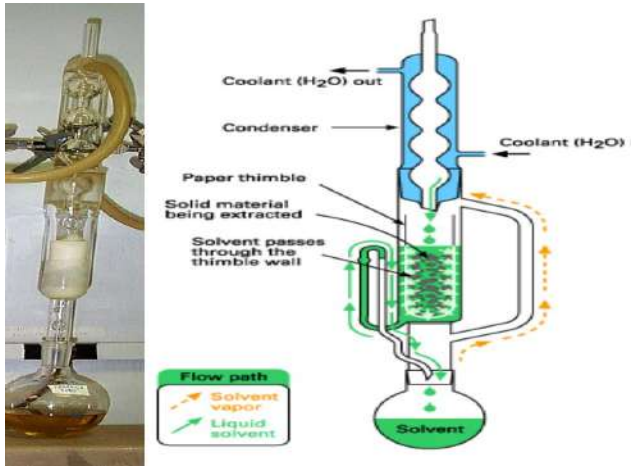


Figure 2: shocks let apparatus

Evaporation of solvent

The filtrate (ethanol extract) obtained was evaporated under ceiling fan and in a tray until dried. It rendered a gummy concentrate of reddish black color. The gummy concentrate was designated as crude extract of ethanol.

% of yield value of extract of Synedrella nodiflora for leaves in case of hot ethanol extract

Total powder taken for extraction = 380 gm
Each of individual packet contain = 144 gm
Weight of empty vial = 13.64gm
Weight of vial with extract = 35.66 gm
Weight of extract obtained = (35.66-13.64) gm
= 22.08 gm

% yield of plantss = (Weight of extract/ Powder taken for extraction × 100)
= (22.08 /144× 100) gm
= 15.33 gm

Chemical group test

Testing of different chemical groups present in extract is representing the preliminary phytochemical studies. The chemical group test, which were performed are as follows (Trease 1983, Wallis 1985, Plummer 1985). In each test 10% (W/v) solution of extract in ethanol was taken unless otherwise mentioned in individual test.

Reagents used for the different chemical group test

The following reagents were used for the different chemical group test (Trease 1983, Ali 1998, Dev 2002).

Mayer's reagent
1.36 gm mercuric iodide in 60 ml of water was mixed with a solution containing 5 gm of potassium iodide (MW: 166.01 g/mol, Merck) in 20 ml of water.

Dragendroff's reagent
1.7 gm basic bismuth nitrate and 20 gm tartaric acid were dissolved in 80 ml water. This solution was mixed with a solution containing 16 gm potassium iodide and 40 ml water.

Fehling's solution A
34.64 gm copper sulphate was dissolved in a mixture of 0.5 ml of sulfuric acid (37%) and sufficient water to produce 500 ml.

Fehling's solution B
176 gm of sodium potassium tartarate and 77 gm of sodium hydroxide (MW : 40g/mol, Merck) were dissolved in sufficient water to produce 500 ml. Equal volume of above solution was mixed at the time of use.

Benedict's reagent
1.73 gm cupric sulphate, 1.73 gm sodium citrate (MW : 258.1g/mol, Merck) and 10 gm anhydrous sodium carbonate were dissolved in water and the volume was made up to 100 ml with water.

Molish reagent
2.5 gm of pure α- naphthol (MW: 144.17g/mol, The British Drug houses Ltd) was dissolved in 25 ml of ethanol.

Test procedure for identifying different chemical groups
The following tests were performed for identifying different chemical groups (Trease 1983, Md. Ali 1998, Dev 2002).

Test for alkaloids

Mayer's test
2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added.

Dragendroff's test
2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added.

Test for Glycosides
A small amount of an alcoholic extract of the fresh or dried plant material was taken in 1 ml of water. Then a few drops of aqueous sodium hydroxide were added.

A small amount of an alcoholic extract of the plant material was taken in water and alcohol and boiled with Fehling's solution.
Another portion of the extract was dissolved in water and alcohol and boiled with few drops of dilute sulfuric acid, neutralized with sodium hydroxide solution and boiled with Fehling's solution.

Test for Steroids

Sulphuric acid test
1 ml solution of chloroform extract was taken and then added 1 ml Sulphuric acid. Red colour indicates the presence of steroid.

Test for gums
5 ml solution of the extract was taken and then Molish reagent & Sulphuric acid were added. Red violet ring were produced at the junction of two liquids indicates in the presence of gums and carbohydrate.

Test for reducing sugar

Benedict's test
0.5 ml of an aqueous extract of the plant material was taken in a test tube. 5 ml of Benedict's solution was added to the test tube, boiled for 5 minutes and allowed to cool spontaneously.

Fehling's test (Standard test)
2 ml of an aqueous extract of the plant material was added to 1 ml of a mixture of equal volumes of Fehling's solutions A & B and was boiled for a few minutes.

Alpha Naphthol Solution test
5 ml of extract added with 2 drops of 5% Alpha Naphthol Solution (Freshly prepared) and added 1 ml of sulfuric acid on the sides of the test tube.

Test for tannins

Ferric chloride test
5 ml solution of the extract was taken in a test tube. Then 1 ml of 5% Ferric chloride solution was added.

Test for Flavonoids
A few drops of concentrated hydrochloric acid were added to a small amount of an alcoholic extract of the plant material. Immediate development of a red colour indicates the presence of Flavonoids.

Test for Saponins
1 ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. One centimeter layer of foam indicates the presence of Saponins.

Screening for antioxidant activity

It was first done by Oyaizu, 1986 in Dillenia indica extract. This test is done in following way-

At first Synedrella nodiflora extract was mixed with 1 ml of distilled water with 2.5 ml, 0.2 M phosphate buffer (pH 6.6). Then in this mixture 2.5 ml 1% potassium ferrocyanide [$K_3Fe(CN)_6$] was mixed. The mixture was incubated at 50^0C for 20 min. 2.5 ml of trichloroacetic acid was mixed in this mixture. Then the mixture was incubated at 3000 rpm for 10 min. 2.5 ml of upper layer of the solution was mixed with distilled water (2.5ml) and ferric chloride ($FeCl_3$). Then the absorbance was measured at 700 nm. Ascorbic acid was used as standard.

Preparation of 1200µg/ml, 1000µg/ml, 800µg/ml, 600 µg/ml, 400µg/ml, 200 µg/ml, sample & standard.

Preparation of sample

0.1 gm of sample was taken in 100 ml volumetric flasks and adjusted the volume up to 100 ml with water. Then 1200 µg/ml, 1000 µg/ml, 800µg/ml, 600 µg/ml, 400 µg/ml, 200 µg/ml, of sample was made consequently.

Preparation of standard

0.1 gm of ascorbic acid was taken in 100 ml volumetric flasks and the other part was filled with distilled water. Then preparation of 1200 µg/ml, 1000 µg/ml, 800 µg/ml, 600 µg/ml, 400 µg/ml, 200 µg/ml, of standard solution as per procedure of sample.

Preparation of sample for reducing activity test

1 ml of sample and standard (1200 µg/ml, 1000µg/ml, 800µg/ml, 600µg/ml, 400µg/ml, 200µg/ml) was in test tube. 2.5 ml of phosphate buffer was taken in each test tube. 2.5 ml of potassium ferric cyanide was taken in each test tube**.** Then the test tubes was taken in incubator at 50^0C for 20 minutes. 2.5 ml of trichloroacetic acid was taken in each test tube & centrifuged at 3000s for 10 minutes. 2.5 ml of solutions of each test tube was separated & taken in separate test tubes**.** 2.5 ml of distilled water was taken in each test tube. 0.5 ml of ferric chloride was taken in each test tube**.** Then the absorbance of the solution each concentration was measured in 700 nm.

RESULT AND DISCUSSION:

Extract	Alkaloid	Glycoside	Steroid	Gums	Flavonoids	Saponin	Reducing sugars	Tannins
Ethanol extract of Synedrella nodiflora	+	–	+	+	–	–	+	+

+ = Presence **– = Absence**

Table 1:Results for different group test are given below (Table 1)

Phytochemical study showed that alkaloid, gums, steroid, reducing sugar and tannins were present and saponin, glycoside,and flavonoid are absent in the extract of Synedrella nodiflora.

	SAMPLE					
Concentration	**1**	**2**	**3**	**4**	**5**	**Average**
200 µg/ml	0.005	0.005	0.004	0.005	0.004	**0.0046**
400 µg/ml	0.006	0.007	0.007	0.005	0.006	**0.0062**
600 µg/ml	0.006	0.006	0.005	0.007	0.005	**0.0056**
800 µg/ml	0.006	0.007	0.007	0.008	0.006	**0.0068**
1000 µg/ml	0.012	0.011	0.012	0.010	0.011	**0.0112**
1200 µg/ml	0.007	0.008	0.007	0.006	0.008	**0.0072**

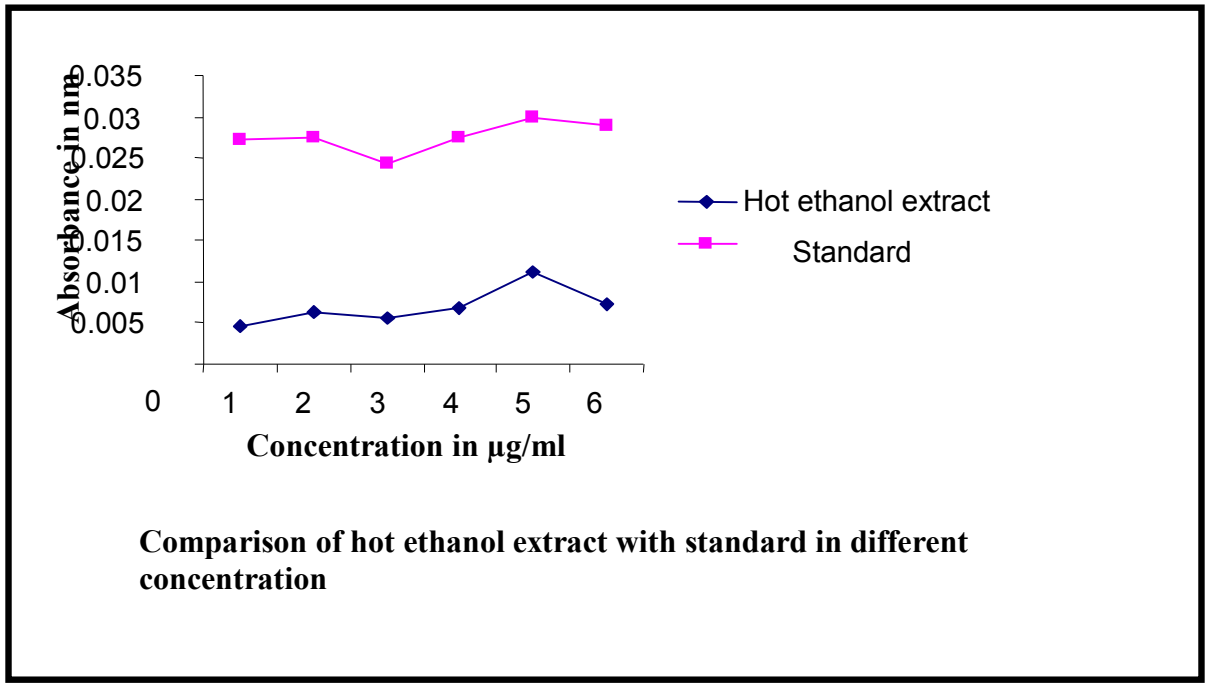
Table 2:The absorbance of the hot ethanol extract at 700nm in given in the table 2

STANDARD						
Concentration	**1**	**2**	**3**	**4**	**5**	**Average**
200 µg/ml	0.028	0.025	0.027	0.028	0.028	**0.0272**
400 µg/ml	0.027	0.030	0.027	0.026	0.027	**0.0274**
600 µg/ml	0.025	0.022	0.024	0.025	0.025	**0.0242**
800 µg/ml	0.027	0.027	0.025	0.029	0.029	**0.0274**
1000 µg/ml	0.030	0.031	0.030	0.029	0.030	**0.0300**
1200 µg/ml	0.029	0.029	0.028	0.030	0.029	**0.0290**

Table 3:The absorbance of standard at 700nm in given in the table 3

SAMPLE				STANDARD			
Concentration	**Average**	**SD**	**SE**	**Concentration**	**Average**	**SD**	**SE**
200 µg/ml	0.0046	0.00049		**200 µg/ml**	0.0272	0.0012	
400 µg/ml	0.0062	0.00075		**400 µg/ml**	0.0274	0.0012	
600 µg/ml	0.0056	0.00080		**600 µg/ml**	0.0242	0.0012	
800 µg/ml	0.0068	0.00075		**800 µg/ml**	0.0274	0.0015	
1000 µg/ml	0.0112	0.00075		**1000 µg/ml**	0.0300	0.0006	
1200 µg/ml	0.0072	0.00100		**1200 µg/ml**	0.0290	0.0006	

Table 4Comparism of the absorbance of hot ethanol extract Vs standard at 700nm in given in the table 4



Graphical representation of comparison of hot ethanol extract with standard in different concentration (Figure 3)

DISCUSSION

Synedrella nodifloraa (Family: Compositae) is a medicinal plant which has many therapeutic effect. The reducing activity of a compound depends on the presence of redactors, which has been exhibited antioxidative potential by breaking the free radical chain, donating a nitrogen atom. The presence of reductants in the fraction Synedrella nodiflora extracts causes the reduction of the Fe^{2+}/ ferric cyanide complex to the ferrous form. Therefore the Fe^{2+}/ can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. In the graph will be show the reductive capability of the plant extract in compare with ascorbic acid. Hot ethanol extract has low antioxidant activity than standard. The entire test has been done in laboratory base chemical & Synedrella nodiflora has great aspects of research.

CONCLUSION

Alkaloids have different pharmacological activity such as they are used as analgesic, stimulant, some are used to raise blood pressure and some are used to fall blood pressure. Due to presence of alkaloids it is possible to have any one of the pharmacological activity. So further experiment should be need to examine pharmacological activity. Tannins are used as anti-insecticidal and tannin acid is used as astringent in burn case. Steroids are used as stimulant so due to absence of steroids it has less possibility of stimulant effect. All these uses of different type of chemicals in the extract of **Synedrella nodiflora** indicate that the necessity of further examination.

ACKNOWLEDGEMENT

We are grateful to the Bangladesh Council of Scientific & Industrial Research (BCSIR) for providing the laboratory support.

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