ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 08 | August - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND TOXICITY SCREENING OF ELECTROHOMEOPATHIC DRUG (SPAGYRIC ESSENCE) SCROFOLOSO – 1

Pharma

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## ABSTRACT

Since a long time medicinal plants have been used in the treatment of various diseases. The therapeutic value of a medicinal plant depends upon the phytoconstituents individually or in the combination. Electrohomeopathic medicine Scrofoloso – 1 (S-1) is an spagyric essence obtained from the selective medicinal plants. It has been extensively using since long time in India as well as across the world to treat several diseases. The current analysis focuses to prove scientifically the phytochemical, antioxidant and toxicity characteristics of Electrohomeopathic medicine S-1.

## KEYWORDS

Electrohomeopathy, Scrofoloso – 1, phytochemicals.

## INTRODUCTION

Over the course of time different scholars carried out specific researches across the world on the use of plant medicines This may be included in Ayurveda, Homeopathy Siddha, Unani, Chinese, , Electrohomeopathy and other traditional medical system. In 1898, an Italian Scholar Count Cesare Mattie originated a new system of medicine and designated as Electrohomeoapathy. The ethics of Count Mattie was that an individual is constituted as complex one and his disease can only be cured by the use of complex remedies. These complex remedies could restore the abnormal physiological function of different organs and biochemical constituents into its original position. So Count Cesare Mattie noted the principle of Electrohomeopathy is "Complexia Complexes Curantor" [1]". In this medical system, spagyric essence is prepared from medicinal plants by using cohobation process. All total 114 medicinal plants are used for the treatment of different diseases. [2. 3] C.C. Mattie distributed all 114 plant medicines in the individual league lean basing on their curative properties and designated as Scrofolso, Canceroso, Angiotico, Fabrifugo, Vermifugo, Venereo, Limphatico, Pettorale and a series of Electricities. [4]

Scrofoloso-1 or S-1 belongs to the lymph remedy league and comprises spagyric essence of different plant combination like *Cochlearia Officinalis*, *Hydrastis Canadensis*, *Nasturtium officinale*, *Scrophularia Nodosa*, *Smilax Medica*, *Tussilago Farfara*, *Veronica Officinalis*, *Strychnos Nuxvomica*, *Matricaria Chamomilla* . It is comprehensively used by local practitioners to treat all most all kinds of lymphatic disorders.

Though this Electrohomeopathy system is claiming to possess curative property for a wide range of disease and being adopted by the practitioners of India as well as the world , no scientific study has done to characterize the phytochemical constituents and toxicity study of this medicines. An effort is made to characterize the phytochemical constituents, antioxidant and toxicity character of this medicine.

## MATERIAL AND METHODS

Electrohomeopathy medicine Scrofoloso-1 is obtained from State Electropathy Medical Society and Research, Cuttack.

### Phytochemical Screening

Scrofoloso-1 was subjected to a preliminary phytochemical screening with following the standard method [5-] for detection of the following constituents and the results were given in Table 1.

### Test for Alkaloids :

**(**Mayer's test, Dragendoff's test, Wagner's test, Hager's test)
Approximately 3ml of Scrofoloso-1 and 3ml of 1% HCl heated for 20 min. The mixtures were cooled and distributed equally in four test tubes names as Test tube – 1 , 2,3,4 respectively.

Mayer's test: To the test tube-1, 1ml of Mayer's reagent was added slowly. The formation of a greenish color precipitate confirmed the presence of alkaloids.

### Dragend off's Test:

To the test tube-2, 1ml of Dragendoff's reagent was added slowly. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

### Wagner's Test:

To the test tube-3, 1ml of Wagner's reagent was added drop by drop. The formation of a reddish-brown precipitate indicated the presence of alkaloids.

### Hager's Test:

To the test tube-4, few drops of Hager's reagent were added. Appearance of yellow color precipitates revealed the presence of alkaloids.

### Test for Glycosides (Keller-Killani Test, Borntrager's test )

### Keller-Killani Test:

2ml of Scrofoloso-1 was treated with 2ml glacial acetic acid containing a drop of $FeCl_3$. No color change is observed which indicated the absence of cardiac glycosides.

### Borntrager's Test:

2ml of Scrofoloso-1 was mixed with 2ml of benzene and equal amount of ammonia solution and shook well. A pink red color is formed in ammonial layer revealed the presence of anthraquinone glycoside.

### Test For Flavonoid

Shinoda's test : 2ml of Scrofoloso-1 were treated with 5ml ethanol (95% w/v) and a few drops of concentrated hydrochloric acid and 0.5 g of magnesium metal. Appearance of pink, crimson or magenta color within a minute or two, revealed the presence of flavonoids.

### Test For Phenols And Tannins

### Ferric Chloride Test:

2ml of Scrofoloso-1 were treated with 2ml of 5% solution of ferric chloride. . A blue-green color indicated the presence of tannins and phenols.

### Test For Protein

### Millon's Reagent Test:

To 3ml of Scrofoloso-1, 5ml Millon's reagent was added. No color change is observed, which indicated the absence of amino acid.

### Test For Carbohydrates

Molish test: 2ml of a Scrofoloso-1 in a test tube is treated with two drops of the Molisch reagent. The solution is then tapped slowly into a test tube containing 2ml of concentrated sulfuric acid. No color change is observed, which indicated the absence of Carbohydrate.

### Test for Steroids

Liebermann Burchard test : 3ml of a Scrofoloso-1 was dissolved in chloroform and added to 3ml of acetic anhydride; 3ml of glacial acetic

acid was warmed and cooled. To this a few drops of concentrated sulfuric acid were tapped along the sides of the tube. A bluish green color indicated which confirmed the presence of steroids.

**Test for Saponins**

**Foam test :** Scrofoloso-1 when vigorously shaken with water forms copious lather indicating presence of saponins.

**Antioxidant Activity Test**

The antioxidant activities of the Scrofoloso-1 were determined by DPPH, hydrogen peroxide and reducing power assays. [7]. All the assays were done in triplicate and the data were expressed as mean ± standard deviation (n=3) and the results are given in the Table 2.

**DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical Scavenging Assay:**

The S-1 and DPPH were incubated in the dark for 30 min and then the absorbance was read using a spectrophotometer at 517 nm. BHT methanolic solutions and ascorbic acid were used as standards and methanolic solution of DPPH free radical as negative control. The percentage of radical scavenging activity was calculated using the following formula.

$$\%\ \text{DPPH radical scavenging activity} = 1 - \left(\frac{A_{sample}}{A_{control}}\right) \times 100$$

where $A_{sample}$ and $A_{control}$ are absorbance of sample and control, respectively.

**Hydrogen Peroxide Scavenging Assay:**

20ml of hydrogen peroxide solution was prepared from phosphate buffer saline (PBS) and the pH is adjusted to 7.40. The Scrofoloso-1 (65.25 mg) were previously suspended in 1 ml of DMSO before preparing their solutions at different concentrations 0.5, 1, 1.5, 2 and 2.5 mg/ml) in water. A volume of 2 ml of hydrogen peroxide in PBS were mixed with 1ml of Scrofoloso-1 solution. The mixture was swirled, and followed with the incubation for 10 min before measuring the absorbance at 230nm. The blank solution contained PBS without hydrogen peroxide. The percentage inhibition was calculated by using the equation below.

$$\%\ \text{of}\ H_2O_2\ \text{inhibition} = \left(\frac{A_{control - A\ sample}}{A_{control}}\right) \times 100$$

where $A_{sample}$ and $A_{control}$ are absorbance of sample and control, respectively.

**Reducing Power Assay:**

The reducing power assay consists of evaluating the ability of plant fractions to reduce $Fe^{3+}$ ions to $Fe^{2+}$ by electron donation. This was facilitated by the presence of potassium ferricyanide. Briefly, 65.25 mg Scrofoloso-1 were diluted into 1ml of DMSO, and then diluted with distilled water into 25ml resulting in a stock solution of 2.5 mg/ml. The working plant fraction solutions were prepared by diluting the stock solution to 0.5 mg/ml. 1ml of working solution of Scrofoloso-1 was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.60) and 2.5 ml of a 1% (w/v) solution of potassium ferricyanide . The mixture was vortexed, and then incubated in a water bath at 50°C for 20 min. Thereafter, 2.5 ml of a 10% (w/v) trichloroacetic acid was added, and then centrifuged at 3000 rpm for 10 min. A volume of 2.5 ml of upper layer was carefully shifted into a test tube before being mixed with 2.5 ml of distilled water and 0.5 ml of a 0.1% (w/v) solution of ferric chloride . The mixture was well shrilled before the measurement of absorbance at 700 nm using the spectrophotometer. The increase absorbance of the reaction mixture reflected the elevated reducing power of Scrofoloso-1 at different concentrations. The percentage inhibition was calculated using the following equation:

**Table 1: Results Of Preliminary Phytochemical Screening Of Electrohomeopathic Medicine Scrofoloso – 1**

| Sl. no | Phytochemical constituents of Electrohomepathy medicines Scrofoloso-1 | | Result |
|---|---|---|---|
| 1. | Alkaloid | 1. Mayer's reagent<br>2. Hager's reagent<br>3. Wagner's reagent<br>4. Dragendorff's reagent | ++<br>++<br>++<br>++ |
| 2. | Glycosides | 1. Cardiac glycoside<br>2.Anthraquinone glycoside | -<br>++ |
| 3. | Flavonoid | Shinoda's test: | ++ |
| 4. | Phenols and tannins | Ferric chloride test: | ++ |
| 5. | Amino acid | Millon's reagent test: | - |
| 6. | Carbohydrates | Molish test | - |
| 7. | Steroids | Liebermann–Burchard test | + |
| 8. | Saponins | Foam test | + |

++ = strongly present, + = slightly present, - = absent

$$\%\ \text{of reducing power} = \left(\frac{A_{control - A\ sample}}{A_{control}}\right) \times 100$$

where $A_{sample}$ and $A_{control}$ are absorbance of sample and control, respectively.

**Table 2: IC50 Values (mg/ml) Of Scrofoloso – 1in DPPH Scavenging, Hydrogen Peroxide And Reducing Power Assays.**

| Name of sample | DPPH ($IC_{50}$[a]) | Hydrogen peroxide ($IC_{50}$[a]) | Reducing power ($IC_{50}$[b]) |
|---|---|---|---|
| Scrofoloso – 1 | 0.3 ±0.01 | 2.16±0.01 | 2.4±0.05 |
| Ascorbic acid (Std) | 0.3±0.01 | 0..5±0.01 | 0..6±0.02 |
| BHT (Std) | 0.3±0.05 | 0..42±0.01 | 0.47±0.01 |

The data were represented as mean via statistics as a standard deviation (SD) in triplicate under standard graph pad prism software (mean ± SD).

a $IC_{50}$ (mg/mL): inhibition concentration at which 50% of DPPH radicals and hydrogen peroxide are scavenged.

b $IC_{50}$ (mg/mL): inhibition concentration at which 50% of ferric ion are reduced.

**Acute Toxicity Study**

The oral acute toxicity study of Scrofoloso-1 evaluated according to Organization for Economic Co-operation and Development (OECD) guideline 423 [8]. Acute toxicity test was carried out on albino mice weighing about 18 to 26 g. A total of 24 adult mice were kept in poly-carbonated cages. They were randomly divided into 4 experimental groups of 6 mice each. (One control group and three treatments.) The Scrofoloso-1 was given in the doses of 300, 1000 and 10000 mg/kg of body weight orally to different groups of mice, each group consisting of six mice. The behaviors and mortality of animals were observed for 2, 4, 6, 8, 12 and 24 h. and also carefully monitored for 14 days. A normal control was also run parallel which was receiving normal saline (10 ml/kg). The mice received tap water and a normal diet.

## RESULTS AND DISCUSSION

**Qualitative Phytochemical Analysis**

The results of the phytochemical screening tests (positive and negative) obtained from Scrofoloso – 1 is presented in Table 1.

Alkaloids are used as a sympathetic stimulation acting directly on alpha- and beta-receptor, It gives anti-inflammatory, demulcent, ganglionic blocking, anti-plasmodic activity, insecticidal and a hepatoprotective activity [9]. Scrofoloso – 1 contains various types of alkaloids which have been proved by Mayer's test, Hager's test, Wagner's test, and Dragendorff's test. This corresponds to the divergent use of the Scrofoloso – 1 for the treatment of nausea, vomiting, gastritis, asthma and different skin diseases.

Flavonoids have been reported to possess a wide variety of biological activities among which are antimicrobial, anti-inflammatory, antiangiogenic, analgesic, antiallergic, antioxidant, antiviral, anticarcinogenic, anticancer as well as anti-diarrheal properties [10]. As Shinoda's test confirms the presence of flavonoid in Scrofoloso – 1, this justifies the use of the same for the treatment of oral fungal infections, gastrointestinal ailments, fever and colds.

Saponins also have a range of important pharmaceutical properties, for example, anti-inflammatory, antifungal, antibacterial, anti-parasitic, anti-cancer and antiviral activities [11]. As Scrofoloso – 1 contains saponin, it is a prime part of formulation used for the treatment of above diseases.

Coumarins have a significant effect on physiological, bacteriostatic and anti-tumor activity [12]. As Scrofoloso – 1 contains cumarin it is extensively used to inhibit any abnormal growth in the body.

Steroids are responsible for reducing cholesterol levels, for regulating the immune response [13], This corresponds to the miscellaneous use of the Scrofoloso – 1 for the for reducing cholesterol level and as immumobuster also.

**Antioxidant Activity**

The DPPH radical, the hydrogen peroxide scavenging abilities and the reducing power of Scrofoloso – 1 was done in comparison with that of Ascorbic acid and BHT as standards and their $IC_{50}$ values are represented in Table 2. The radical scavenging activity of the Scrofoloso – 1 against DPPH,( $IC_{50}$= 0.3) Hydrogen peroxide( $IC_{50}$= 2.16) and reducing power( $IC_{50}$= 2.4) is quite significant as compared with Ascorbic acid and BTH. Scrofoloso – 1 cotains phenolic compounds which possess significant antioxidant property used to treat various oxidative stress disorders [14].

**Acute Toxicity Study**

Mortality was the criteria for determining acute toxicity [15]. No mortality was recorded even at high doses of Scrofoloso – 1 up to 10 g/kg body weight. In the case of acute toxicity study, it was observed that all the animals survived after 24 h and even after 48 h. This showed that the oral $LD_{50}$ of Scrofoloso – 1 was greater than 10gm/Kg body weight. Hence, it was proved from the acute toxicity test that the Scrofoloso – 1 is quite safe and has no acute toxicity up to the dose of 10 g/kg of body weight orally which is considered a very high dose.

The other monitored parameters included properties of skin and fur, eyes, respiratory pattern, autonomic nervous system features such as salivation, diarrhea, and urination, central nervous system features such as tremors, ptosis, relaxation, changes in the level of activity, gait, and posture, and any other abnormal behavior was observed all round the study period but could not found any significant change. This justified the safety of extensive use of Scrofoloso – 1 by the local Electrohmeopathy practitioners.

## CONCLUSION

In this study, Electrohomeopathic medicine Scrofoloso-1 found to be safe and contain the most phytocompounds validating their traditional use in the treatment of various ailments such as fever, colds, asthma, , high blood pressure, gastroenteritis, dermatitis, and immunoboosters well. Due to the multifarious therapeutic effect of Scrofoloso – 1, it is named as a universal remedy by the local Electrohomeopathic practitioners. Further research is required to isolate, identify, characterize, and elucidate the structure of these bioactive compounds.

## ACKNOWLEDGEMENT

The authors are thankful to all Electrohomeopathy practitioners Dr. K. K. Nayak, Dr. Parsuram Khatua, Dr. Saroi Sahoo, Dr. Manoj Mahala, and Dr. Ajaya Pati and Dr. Satya Patro for their valuable suggestions during the study.

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