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Total Phenol Quantification and Anthelmintic Activity of *Sarcostemma acidum* (ROXB.) VOIGT

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Abstract

Helminthic infections are chronic illnesses both in human beings and cattles. *Pheretima posthuma* earthworms are a known helminthic and they have anatomical and physiological similarities with intestinal roundworm parasite present inside human beings. Medicinal plants have been using as health care products from a long time ago that have been used by humans in the form of traditional medicines all over the world. The use of herbal drugs extract and their remedies have increased throughout the world against the increasing use of synthetic drug medicines. In this study stem extracts of *Sarcostemma acidum* is evaluated for its anthelmintic property along with preliminary phytochemical analysis and total phenol quantification. Preliminary photochemical investigation reflected the presence of many phytoconstituents. Presence of phenols were detected in all the extracts studied. Methanol extracts showed maximum (40mg/gm) phenolic content. Anthelminthic property of crude methanolic extract of *Sarcostemma acidum* showed maximum response followed by ethanol, ethyl acetate, petroleum ether least by distilled water.

Key Words: Phenol Quantification, Phytochemical studies, Pharmacognosy, Anthelmintic studies ,*Sarcostemma acidum*

INTRODUCTION

Plants play an important role in traditional medicine and are widely consumed as home remedies. The WHO supports the use of traditional medicine and they are proven to be efficacious and safe. Over three quarters of the world population relies mainly on plants and plant extracts for health care. It is well known that plant produce these chemicals to protect them, but recent research demonstrate that they can also protect the humans against diseases. These drugs are derived either from whole plant or from different plant organs. A knowledge of these chemical constituents of plants is desirable not only for the discovery of therapeutic agents ,but also because such information may be great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies[1].

Helminthes infections are among the most common infections in human beings in which human intestinal parasitic worms are vectored through air, food, and water, which causes disease state, secretes toxins, and steals the vital nutrients from host bodies[2] .They cause severe threat to people especially in the third world countries by contributing to the prevalence of anaemia, malnutrition, eosinophilia, and pneumonia. Anthelmintics are a group of antiparasitic drugs which acts locally to expel worms from the gastrointestinal tract or systematically to eradicate adult helminthes without causing significant damage to the host[3].Anthelmintics from natural sources may play a key role in the treatment of these parasitic infections[4].

Sarcostemma acidum is an important endangered medicinal plant which is a member of Asclepiadaceae family and is typically found in the valleys and sub tropical mountains in the Himalayas[5].The plant is religiously related to Hinduism and is believed to be a major ingredient of the soma in ancient India. Members of *Sarcostemma* genus are also known as “climbing milkweeds” because the plant yields an abundance of a mildly acidulous milky juice and travelers suck its tender shoots to allay thirst[6]. The plant possess many medicinal uses like acrid, cooling, narcotic, emetic, antiviral and rejuvenaing, useful in vitiated conditions of pitta, dipsia, hydrophobia, psychopathy and general dibility, the plant is used by the various tribal communities of India in the treatment of various diseases and disorders[5,7].

Phytochemicals alkaloids, saponins, flavonoids, phenols ,steroids were reported in Sarcostemma acidum extracts and phenols is detected in all the extracts studied[8]. The present study evaluates the total phenolic content and anthelmintic activity of water, ethanol, methanol, petroleum ether and Ethyl acetate extracts of *Sarcostemma acidum* .

MATERIALS AND METHODS

Preparation of crude plant extract

Sarcostemma acidum (Roxb.) Voigt plant was collected from the Urapakkam Scrub Jungle, Kancheepuram District Chennai. The collected material were cleaned, washed ,shade dried, and powdered to coarse powder using an electric grinder . 10 g of dried, ground plant materials were soaked in 100ml of respective solvents distilled water, ethanol, methanol, petroleum ether or ethyl acetate in closed flasks and was allowed to stand for one week and shaken frequently. The soaked material was stirred and the final extracts were passed through Whatman filter paper No.1. The filtrates obtained were concentrated by keeping in the hot water bath. The extraction yield is expressed as the percentage of total mass of extracts (*Mext*) with respect to the mass material used (*Mo*)[9]Yield percentage (%) = (*Mext* / *Mo*) x 100

Preliminary phytochemical analysis

Detection of active phytochemical constituents was carried out for all the extracts using the standard procedures[10].

Quantitative estimation of total phenols

Total phenols were estimated by employing Folin - Ciocalteau reagent[11] For estimation, Folin - Ciocalteau reagent was diluted to IN with equal volume of distilled

water. 1ml of the same was added to 1ml of the extract in a 25 ml test tube followed by 2 ml of 20% Sodium Carbonate. The mixture was heated in a boiling water bath for exactly one minute. The blue colour was diluted to 25ml with distilled water. The percentage of light transmittance was determined at 725nm using Labtronics NT 290 Spectrophotometer. Total phenol was calculated from Standard curve prepared using Catechol.

Anthelmintic activity

Adult earthworms (*Pheretima posthuma),* was used to evaluate anthelmintic activity *in vitro*. Identified earthworms were collected from Kerala Agricultural University, Mannuthy. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. All the test solution and standard drug solution were prepared freshly at 5mg/ml concentration before starting the experiments. Observations were made for the time taken for paralysis and death of individual worm's upto 4 hours of test period. Time for paralysis was noted when no movement of any sort could be observed except the worms were shaken vigorously. Time for death of worms was recorded after ascertaining that the worms neither moved when shaken vigorously[12]. Experiments were repeated in triplicates.

RESULTS AND DISCUSSION

The various products from plants have been used for preparation of drugs from the time immemorial. Particularly in the present modern scenario of increased demands for new drugs, it become very essential to discover plant based drugs with negligible side effects. The dried powder of stem was extracted with various solvents i.e., water, ethanol, methanol, petroleum ether and ethyl acetate. The solvents were removed by evaporation in water bath to obtain the extract. The percentage yields of various extract was presented in Table 1.the extractive yield differed among the solvents. High polar solvent water showed maximum yield and minimum yield obtained in petroleum ether extract, which is highly non polar. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time and composition of sample[12].

Table 1: Percentage of extracts obtained from *Sarcostemma acidum stem powder*

Parameters	Observations
Water Soluble extractives	11.9%
Methanol Soluble Extractive	5.9%
Ethanol Soluble Extractive	6.0%
Ethyl acetate soluble extractive	5.2%
Petroleum ether Soluble extractive	5.0%

Preliminary phytochemical screening of the crude extracts revealed the presence of primary and secondary metabolites (Table 2). This indicates the therapeutic potential of *Sarcostemma acidum* plant as the phytochemicals are non-nutritive, chemical compounds occurs naturally on plants during metabolic processes and they have diverse proactive properties or disease preventive properties[13].

The preliminary phytochemical screeing of all the extracts showed the presence of phenols. The results of total phenolic content of various extracts are shown in Table 3. Methanol extract showed maximum(40 mg/gm) phenolic content,least in peteoleum ether (6mg/gm). Plant materials rich in phenolics are used in the food industry because they decrease the oxidative degradation of lipids and maintain the quality and nutritional value of food. Phenolic compounds in the plants are very impotant because their group scavenging abilities.

Table 2 : Preliminary screening for primary and secondary metabolites of the crude exracts of *Sarcostemma acidum*

PHYTOCHEMICAL CONSTITUENTS	Test	METHANOL	ETHANOL	DISTILLED WATER	ETHYL ACETATE	PETROLEUM ETHER
Carbohydrates	Molish	+	+	+	++	+
Starch	Iodine	-	-	-	-	-
Sugar	Benedicts	-	+	-	-	-
Protein	Biuret	+	++	++	+	+
Amino acid	Ninhydrin	-	-	-	-	-
Saponin	Foam Test	+++	+	-	-	-
Phenol	Folin	+	+	+	+	+
Tannin	Ferric Chloride	-	-	+	-	+
Steroids	Salkowski	-	+	++	+	++
Alkaloids	Meyers	+	-	+	-	-

+ indicates the intensity of occurrence of the compound tested

- absence of metabolite

Table 3: Quantification of phenols in *Sarcostemma acidum* stem extracts

Plant extract	Phenol quantification (mg/gm)
Methanol	40
Ethanol	12
Petroleum ether	6
Ethyl acetate	18
Distilled water	14

The Phenol quantification is an important step in drug discovery. Natural phenolic compounds play an important role in cancer prevention and treatment. Phenolic compounds from medicinal herbs and dietary plants include phenolic acid, flavonoids, tannins, coumarins and others. Various bioactivities of phenolic compounds are responsible for their chemo preventive properties (eg. Antioxidant, carcinogenic antimutagenic and antiinflammatory effects) and also contributes their inducing apoptosis by arresting cell cycle, regulating carcinogen, metabolism, oncogenesis, inhibiting DNA binding and cell adhesion,migration ,proliferation or differentiation and blocking signaling pathways[14]. Phenolics were the main antioxidant compoenents and their total contents were directly proportional to their antioxidant activity[15]. In this present study, phenol quantification was done with UV spectrophotometric method. Folin –ciocalteu reagent and hydroxyl groups in the extract reacts to form a Prussian blue coloured complex. Phenols are better known for their antioxidant property, anthelmintic phytophenolics exert their inhibitory/cidal effect over helminthes due their prooxidant activity[16].

Helminthiasis is a serious macro parasite disease of human and other animals in which a part of body is infected with parasite worm called helminthes. Broad spectrum benzimidazoles are used in its treatment. Development of resistance in helminthes against conventional anthelmintics is the foremost problem in treatment of helminthes disease. Some serious side effects of albendazole is that it causes giddiness,decreased urination,fever, chills, or sore throat tiredness etc. hence it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelminthic activity[17].

Table 4 : Anthelminitic activity of aqueous extracts of *Sarcostemma acidum* against earthworm *Pheretima posthuma*

Treatment groups	Time Taken in minutes	
	Paralysis	Death
Ethanol	15	49
Methanol	13	40
Petroleum ether	21	42
Ethyl acetate	26	67
Distilled water	72	104
Albendazole	3	17

Anthelminthic property of crude extract of *Sarcostemma acidum* in various solvents is compared with albendazole using *Pheretima posthuma*. The paralysis and death of worms indicate its ability to be used as an anthelminthic. Anthelminthic property of crude methanolic extract of *Sarcostemma acidum* showed maximum response followed by ethanol, ethyl acetate, petroleum ether least by distilled water (Table 4).

The prime effect of albendazole is to cause a flaccid paralysis of the worm which results in expulsion of the worm by peristalsis. Earthworms are invertebrates composed of many segments. They moves by means of cilia. Outer slimy layer made up of mucopolysaccharides make this movement possible. Any damage to this layer restricts the movement and can cause paralysis. This may ultimately leads to the death of earthworms. Further investigations are needed for the isolation and identification of active compounds and to elucidate its mechanism of action as well as their potential role in biological activity.

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