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Phytochemical and In-Vivo Biological Activities of Sarcostemma acidum Voigt in Albino Rats.

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KEYWORDS

Sarcostemma acidum, antipyretic, antipsychotic, antidiabetic, pharmacological, anti-inflammatory.

Abstract

Sarcostemma acidum Voigt, commonly known as Somlata, is a leafless plant from the Apocynaceae family, mainly found in Bihar, West Bengal, Odisha, and South India. Sarcostemma acidum Voigt, a medicinal plant traditionally employed in various folk medicine systems, has garnered significant attention due to its rich phytochemical composition and diverse pharmacological activities. This study aimed to comprehensively investigate the phytochemical constituents of Sarcostemma acidum Voigt and evaluate its pharmacological properties. It has traditional medicinal uses for conditions like otitis, dog bites, snake bites, rabies, emesis, arthritis, and leprosy. The plant extract exhibited significant antioxidant activity, as evidenced by its ability to scavenge free radicals. Furthermore, Sarcostemma acidum Voigt demonstrated anti-inflammatory properties by inhibiting key inflammatory mediators. The plant's juice is valued for its health-restorative properties. The stem extract inhibits spermatogenesis and reduces sperm count, showing an antipsychotic effect. Microscopic examination reveals its stem structure, and phytochemical analysis indicates various compounds present. Pharmacological studies on the aqueous stem extract show analgesic, antipyretic, and antidiabetic effects. The extract is found safe in acute toxicity studies, and the analgesic effect is confirmed by Eddy's hot plate method. Antipyretic effects are demonstrated in a Brewer's yeast-induced pyrexia rat model, while antidiabetic effects are shown in an adrenaline-induced diabetic rat model. Overall, the pharmacological study affirms the beneficial effects of Sarcostemma acidum.

1. Introduction

The interconnectedness between plants and humans is evident, as plants not only provide essential oxygen for survival but also offer food, shelter, and clothing. Certain plants, due to their therapeutic properties, play a role in treating various diseases. Different parts of plants contain chemicals with therapeutic value, leading to the use of isolated compounds or crude drugs in treating various conditions. Unlike synthetic compounds, herbal products generally have fewer adverse effects.[1] In modern medicine, plant-derived drugs are often used in conjunction with synthetic drugs. Herbal medicine has a rich history, with India being a pioneer in the development and utilization of herbal drugs. Ayurveda, Siddha, and Unani, traditional systems of medicine, heavily rely on herbal products for treatments.[2] Ayurveda, represented by works like Charaka Samhita and Sushruta Samhita, is grounded in the principles of Vata, Pitta, and Kapha (Tridosha). Siddha, an ancient system, bases its practices on Triguna, while Unani, developed during the Arabian civilization, follows the Hippocratic and Pythagorean theories.[3] Living plants are considered bio-synthetic laboratories for primary and secondary metabolites. Primary metabolites like sugars and amino acids serve as human food, while secondary metabolites, such as volatile oils and alkaloids, have pharmaceutical significance, earning them the name phytopharmaceuticals. These active constituents are isolated, purified, and structurally determined using modern techniques like HPTLC, HPLC, Gas chromatography, Infrared, NMR, and Mass spectroscopy. Sarcostemma acidum Voigt, locally

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known as Somlata, is a xerophytic leafless plant from the Apocynaceae family.[4] Traditionally used to prepare somras, it is found in dry rocky areas of Bihar, West Bengal, and Odisha. This plant has ethnomedicinal uses for conditions such as otitis, dog bites, snake bites, leprosy, and arthritis. Additionally, the stem juice exhibits psychopharmacological effects and reduces spermatogenesis. Ongoing research explores its therapeutic properties and aims to isolate compounds with medicinal value[5,6].

1.1. Plant profile

Sarcostemma acidum Voigt, also known as Somlata, is a shrub with branched stems that thrives in dry rocky environments. The stems, measuring 2 to 4 meters in length, are green and cylindrical, while the roots display numerous sub-root branches.[7] The plant's flowers are light yellowish-white in color. S. acidum is recognized for its ethnomedicinal uses, with its juice (somras) serving as a natural health restorative. Traditional applications include the treatment of otitis, dog bites, snake bites, rabies, emesis, leprosy, arthritis, and wounds. Extracts from the plant exhibit psychopharmacological effects such as anxiolytic and antipsychotic properties, and they also demonstrate a reduction in spermatogenesis.[8,9]

Botanical name: – *Sarcostemma acidum* Voigt

Family: - Apocynaceae

Taxonomic classification: Taxonomic classification shown[10]

Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Asterids
Family:	Apocynaceae
Genus:	Sarcostemma
Species:	*acidum*

1.2. Geographical distribution and collection:

The plant is found in India, Pakistan, Sri Lanka and European countries. In India it is mostly found in Odisha, Bihar, West Bengal and Tamil Nadu.[11]

The plant was collected from the Gandhamardan hill of balangir district of Odisha and It was collected in the month of September

Fig. 1 Plant *Sarcostemma acidum* voigt

1.3. Authentication

The plant was identified and authenticated by the scientist of Botanical survey of India, Central national herbarium, Howrah, Kolkata. **(CNH/Tech II/2016/34)**

2. Materials and Methods

2.1. Macroscopy

Sarcostemma acidum Voigt, commonly known as Somlata, is a leafless perennial shrub characterized by jointed stems. The green, cylindrical stems range from 2

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to 4 meters in length and 0.5 cm to 1 cm in diameter. Despite the presence of leaves arranged oppositely, they are reduced to scales, giving the plant its leafless nature. The actinomorphic flowers exhibit a light yellow or white color and consist of 5 sepals, 5 petals, 5 stamens, and 2 ovaries. The androecium and gynoecium are fused with 5 stigmas. The plant produces flat and ovate seeds within its fruit, and the roots are brownish, featuring 3 to 5 subroot branches.[12]

2.2. Powder microscopy:

Powder microscopy stands as a vital technique in quality control, delving into specific microscopic characteristics through the application of staining agents like hydrochloric acid (1:1), iodine, chloral hydrate, and phloroglucinol. This method proves highly effective in evaluating potential adulteration in crude medications. In the context of this study, fresh *Sarcostemma acidum* Voigt steam were meticulously collected, left to dry in the shade, and subsequently coarsely ground using a pulverizer.[13] The freshly powdered *Sarcostemma acidum* Voigt steam underwent a soaking process in 20% nitric acid throughout the night, followed by a thorough cleansing with distilled water the next day. The resulting powder was meticulously transferred onto a slide, and staining reagents such as hydrochloric acid, safranin, iodine, and phloroglucinol were methodically introduced to facilitate the examination process.[14]

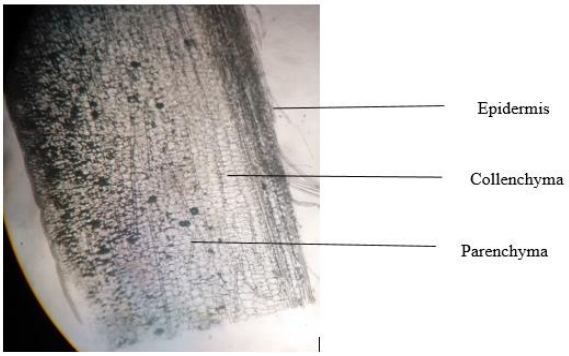

Fig.2- Microscopy of stem

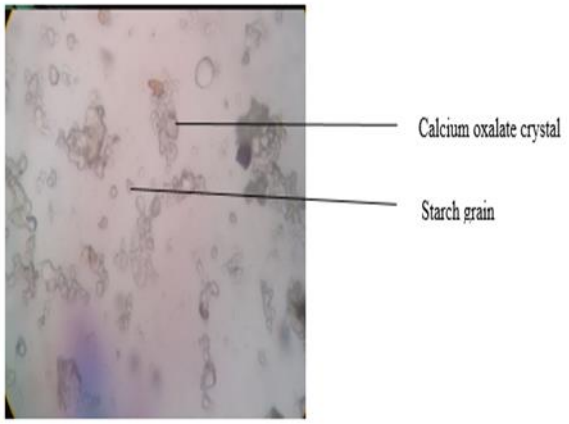

Fig. 3 - Microscopy of powder

2.3. Powder drug with chemical reagents[15]

The powder drug with different chemical reagents shows different colour when seen on naked eye. (Table No.1)

2.4. Reaction of chemicals with powdered crude drugs & fluorescence analysis:

Fluorescence, also referred to as luminescence, describes the phenomenon wherein light is absorbed and subsequently re-emitted. Certain materials exhibit longer wavelengths with lower energy than the absorbed radiation, making the emitted light visible, while the absorbed radiation remains invisible to the naked eye. When subjected to ultraviolet (UV) radiation, these materials emit a distinctive color characteristic of fluorescence. Fluorescence analysis is a crucial factor in determining the quality of medicinal plants .In this study, the dried steam were first powdered and treated with various solvents before undergoing fluorescence analysis under UV light.[16]The examination involved

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observing the dried, roughly ground steam in daylight and under two specific UV light wavelengths (254 and 365 nm). A gram of the powdered substance was placed in a test tube and exposed to two to five milliliters of various bases, acids, and solvents, as detailed in tables no. 1 and 2. This process aimed to assess the fluorescence properties of the medicinal plant material and contribute to the overall quality analysis. (Table No.2).[17,18]

2.5. PH of powdered drug 1% and 10% solution

A beaker containing 1 gram of powdered drug and another beaker containing 10 gram of powder drugs was utilized in the experiment. To this, 100 ml of distilled water was added in each beaker ensuring thorough mixing of the powder with the water. Subsequently, the mixture was filtered to obtain a clear filtrate. The pH of the resulting filtrate was then measured using a pH meter. This procedure aimed to assess and record the acidity or alkalinity of the solution, providing valuable information about the chemical nature of the powdered drug when dissolved in water. [19](Table No. 3)

3. Physical evaluation

3.1. Loss on Drying

Initially, a weighing bottle was weighed to establish its baseline weight. Subsequently, 5 mg of the powdered drug was carefully measured and placed into the weighing bottle. The bottle, along with its contents, was then reweighed to determine the combined weight accurately. Next, the bottle containing the powdered drug was placed inside a hot air oven set at a temperature of 100°C for a duration of 30 minutes to facilitate the drying process.[20] After the allotted time, the bottle was removed from the oven and allowed to cool to room temperature. Once cooled, the bottle, along with its contents, was weighed again to measure any changes in weight resulting from the drying process. The difference in weight between the bottle with its contents before and after drying was noted, providing the loss on drying or moisture content.[21] (Table No. 4.)

3.2. Extractive values

These extracts are crucial in the field of pharmacognosy, providing researchers and scientists with essential tools to analyze and quantify the chemical compounds present in various crude drugs. The use of different solvents for extraction allows for a comprehensive analysis of diverse constituents, including water-soluble compounds like glycosides, tannins, and mucilage, alcohol-soluble compounds such as tannins, glycosides, and resin, and ether-soluble compounds found in drugs with volatile constituents and fats [22]. This analytical approach enhances understanding of the chemical composition of crude drugs, opening avenues for potential applications in pharmaceutical and medicinal formulations.The extractive value plays a pivotal role in identifying exhausted or adulterated drugs, making it a crucial tool for quality control and purity assessment of crude drugs. Evaluating the extractive value helps determine the overall quality and purity of the drug, ensuring adherence to required standards for safe and effective use in various medicinal applications. Information obtained from these solvent extracts not only reveals the nature of chemical constituents in crude drugs but also proves valuable for estimating the quantity of these constituents extracted with the chosen solvent. This extraction method is particularly advantageous when dealing with materials lacking suitable chemical or biological assays, providing a reliable means to assess the composition and potential properties of the crude drug.[23]

3.2.1. Water Soluble Extractive

4 grams of powdered drug were mixed with 100 ml of distilled water in a conical flask. The container was sealed and shaken continuously for 6 hours, followed by allowing it to stand for an additional 18 hours. After a total maceration period of 24 hours, the mixture was filtered. Subsequently, 25 ml of the filtrate was taken and dried at 100°C in a previously weighed beaker. The beaker was then reweighed, and the difference in weight was used to determine the amount of water-soluble extractive. The percentage of water-soluble extractive was calculated with reference to the weight of the powdered drug[24] (Table No 4).

3.2.2. Methanol Soluble Extractive

In this process, 4 grams of powdered drug were combined with 100 ml of methanol in a conical flask. The container was sealed and vigorously shaken continuously for 6 hours, followed by allowing it to stand for an additional 18 hours. After a total maceration period of 24 hours, the mixture was filtered. Subsequently, 25 ml of the filtrate was carefully measured and placed in a previously weighed beaker. The filtrate was then dried at 100°C, and the beaker was reweighed. The difference in the weight of the beaker before and after drying provided the amount of methanol-soluble extractive. The percentage of methanol-soluble extractive was then calculated with reference to the initial weight of the powdered drug.[25] (Table No. 4.)

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3.2.3. Ethanol Soluble Extractive

In this procedure, 4 grams of powdered drug were introduced into a conical flask along with 100 ml of ethanol. The container was sealed, and the mixture was continuously shaken for 6 hours, followed by allowing it to stand for an additional 18 hours. After a total maceration period of 24 hours, the mixture underwent filtration. Next, 25 ml of the filtrate was carefully measured and transferred to a previously weighed beaker. The filtrate was then dried at 100°C, and the beaker was reweighed. The difference in the weight of the beaker before and after drying provided the amount of ethanol-soluble extractive. The percentage of ethanol-soluble extractive was subsequently calculated with reference to the initial weight of the powdered drug.[26] (Table No. 4.)

3.2.4. Ether Soluble Extractive

In this procedure, 4 grams of powdered drug were combined with 100 ml of petroleum ether in a conical flask. The container was sealed, and the mixture was continuously shaken for 6 hours, followed by allowing it to stand for an additional 18 hours. After a total maceration period of 24 hours, the mixture underwent filtration. Subsequently, 25 ml of the filtrate was carefully measured and transferred to a previously weighed beaker. The filtrate was then dried at 100°C, and the beaker was reweighed. The difference in the weight of the beaker before and after drying provided the amount of ether-soluble extractive. The percentage of ether-soluble extractive was then calculated with reference to the initial weight of the powdered drug. [27](Table No.4)

3.2.5. Ethyl acetate Soluble Extractive

In this process, 4 grams of powdered drug were mixed with 100 ml of ethyl acetate in a conical flask. The container was sealed, and the mixture was continuously shaken for 6 hours, followed by allowing it to stand for an additional 18 hours. After a total maceration period of 24 hours, the mixture underwent filtration. Next, 25 ml of the filtrate was carefully measured and transferred to a previously weighed beaker. The filtrate was then dried at 100°C, and the beaker was reweighed. The difference in the weight of the beaker before and after drying provided the amount of ethyl acetate-soluble extractive. The percentage of ethyl acetate-soluble extractive was subsequently calculated with reference to the initial weight of the powdered drug.[28,29] (Table No. 4.)

3.3. Ash value

The examination of ash values in herbal drugs primarily involves quantifying the inorganic residue, which may include phosphates, carbonates, and silicates. These values serve as crucial indicators, offering insights into both the quality and purity of the herbal medicine under examination. By eliminating all traces of organic matter during the ashing process, potential interference in subsequent analytical evaluations is effectively minimized.[30] This ensures the generation of accurate and reliable results in the analysis of herbal medicines. Ash can be categorized into two main types: physiological ash and non-physiological ash. Physiological ash is produced by the plant itself through biochemical reactions, while non-physiological ash does not originate from biochemical reactions within the plant. Understanding and distinguishing between these types of ash is important for interpreting the results of ash value analysis and drawing conclusions about the herbal medicine's composition and quality.[31]

3.3.1. Total Ash Value

The analytical process began with the measurement of the empty crucible's weight. Subsequently, 1 gram of powdered drug was carefully placed into the crucible. The crucible, containing the powdered drug, was then subjected to incineration in a muffle furnace, maintained at a temperature of 450°C, until complete carbon elimination was achieved. The resulting ash was collected, and its weight was measured. With reference to the initial weight of the powdered drug, the percentage of ash was calculated.[32] (Table No. 4.)

3.3.2. Water Soluble Ash

In the subsequent step, the obtained ash was mixed with 25 ml of distilled water, followed by boiling the mixture for 5 minutes. Afterward, the mixture underwent filtration, collecting the insoluble matter on filter paper. The filter paper, now containing the insoluble matter, was carefully transferred into a crucible. The crucible, along with the filter paper, was incinerated in a muffle furnace at 450°C for 15 minutes. The weight of the resulting insoluble matter was determined. Subsequently, the difference between the total ash weight and the weight of the insoluble matter was calculated to obtain the water-soluble ash. With reference to the initial weight of the powdered drug, the percentage of water-soluble ash was then calculated.[33] (Table No. 4.)

3.3.3. Acid Insoluble Ash

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In the subsequent procedure, the ash was combined with 25 ml of diluted hydrochloric acid (dil. HCl). The mixture was then boiled for 5 minutes and filtered, with the insoluble matter collected on filter paper. The filter paper, containing the insoluble matter, was transferred to a crucible. The crucible, along with the filter paper, was incinerated in a muffle furnace at 450°C for 15 minutes, resulting in the determination of the weight of the insoluble matter.Subsequently, with reference to the initial weight of the powdered drug, the percentage of acid-insoluble ash was calculated. [34](Table No. 4.)

3.4. Swelling Index

In this process, 1 gram of powdered drug was placed in a stoppered measuring cylinder. Distilled water was then added to reach the 20 ml mark. The mixture was thoroughly shaken at intervals of 10 minutes for a duration of one hour. Subsequently, the mixture was allowed to stand for an additional 24 hours. After this period, the volume occupied by the plant material was measured in milliliters. [35](Table No. 4.)

4. Preparation of extracts[36,37,38]

4.1. Preparation of Aqueous extract

In this extraction process, 500 grams of drug powder were placed in a round bottom flask. Distilled water was added to the flask, and the mixture was thoroughly shaken to ensure proper mixing of the drug powder with water. The opening of the flask was covered with aluminum foil to facilitate maceration, and the entire setup was left undisturbed for 24 hours. After the maceration period, the mixture was filtered to obtain the filtrate. The obtained filtrate was then subjected to evaporation on a hot plate at 80°C to obtain the aqueous extract. This process aimed to concentrate the active components of the drug into the aqueous solution. The resulting aqueous extract could be further analyzed or used for various purposes in the context of the study or application.

4.1.1. Preparation of Methanolic extract

The preparation of the methanolic extract was conducted through the Soxhlet extraction process. In this procedure, 500 grams of drug powder were placed in the extractor. Initially, the drug powder was subjected to extraction with petroleum ether to remove any fatty substances that might be present. Subsequently, the drug was extracted with methanol, with the methanol placed in the round bottom flask. The extraction process was carried out at 40°C for a duration of 72 hours. After the completion of the 72-hour extraction, the resulting extract was collected and filtered using a cotton cloth. The filtrate obtained from this filtration was then taken for evaporation, aiming to concentrate and obtain the methanolic extract. This extract could be further analyzed or utilized for various applications as needed in the study or research.

5. Preliminary phytochemical screening[39,40,41,42,43]

The chemical tests or preliminary phytochemical screening for various phytoconstituents in the dried powder and extracts of Sarcostemma acidum Voigt steam were conducted as outlined below, and the outcomes were documented in table no..

5.1. Test for Carbohydrates

A small amount of methanolic extract, aqueous extract, and powder were individually dissolved in distilled water and then filtered. The resulting filtrates were used for conducting various tests to detect carbohydrates.

a. **Molisch's Test:** To the filtrates, 2 to 3 drops of 1% alcoholic α-naphthol solution were introduced. Subsequently, 2 ml of concentrated H_2SO_4 was carefully added along the sides of the test tube. The appearance of a purple color serves as confirmation for the presence of sugar.
b. **Benedict' s Test**: A small portion of the filtrates was mixed with an equal volume of Benedict's reagent and then heated on a water bath. The emergence of an orange precipitate serves as confirmation for the presence of reducing sugar.
c. **Fehling's Test:** Equal volumes of Fehling's solutions A and B were added to the filtrate, followed by boiling on a water bath. The appearance of a brick-red precipitate serves as confirmation for the presence of sugar.

5.2. Test for Alkaloids

The extracts were dissolved in concentrated hydrochloric acid, and the resulting filtrate was used for additional tests for alkaloids:

a. Dragendorff's test: The filtrate was mixed with a few drops of Dragendorff reagent (potassium bismuth iodide). The presence of a reddish-brown color indicated the presence of alkaloids.

b. Mayer's test: An acidic solution of the filtrate was placed in a test tube, and a few drops of Mayer's reagent (potassium mercuric iodide) were introduced. The formation of a yellowish precipitate indicated the presence of alkaloids.

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c. Wagner's test: A few drops of Wagner's reagent (iodine in potassium iodide) were added to the filtrate. The presence of a reddish color confirmed the presence of alkaloids.

d. Hager's test: The filtrates were treated with Hager's reagent (saturated picric acid solution). The confirmation of alkaloids was indicated by the formation of a yellow-colored precipitate.

5.3. Test for Protein

A small quantity of methanolic extract, aqueous extract, and powder was initially dissolved in distilled water, mixed, and then filtered. The resulting filtrates were subjected to various tests for the detection of protein:

(a) Ninhydrin Test: The Ninhydrin reagent was combined with the different extracts and boiled. The appearance of a purple color confirmed the presence of protein.

(b) Biuret Test: The extracts were treated with an equal volume of 5% w/v NaOH solution, followed by the addition of a few drops of 1% copper sulfate solution. The development of a violet color confirmed the presence of protein.

(c) Millon's Test: Millon's reagent was mixed with the different extracts.The formation of a white precipitate confirmed the presence of protein.

(d) Test with Tannic Acid: A 10% tannic acid solution was combined with different extracts. The occurrence of a white precipitate confirmed the presence of protein.

5.4. Test for Glycosides

The methanolic extract, aqueous extract, and powder were combined with distilled water and subsequently filtered. The resulting filtrates underwent hydrolysis with HCl for one hour on a water bath. The resulting solutions were then used for the Keller Kiliani test, Legal test, and Baljet test for the detection of glycosides.

(a) Keller Kiliani Test: Glacial acetic acid with a few drops of ferric chloride was blended with the filtrates. Sulphuric acid was added from the side of the test tubes. The formation of a brown ring confirmed the presence of cardiac glycosides.

(b) Legal Test: Pyridine with a few drops of sodium nitroprusside solution was mixed with the different filtrates. Sodium hydroxide solution was then added, and the development of a pink color confirmed the presence of glycosides.

(c) Baljet Test: Sodium picrate solution was mixed with the different filtrates. The formation of a yellowish-orange color confirmed the presence of glycosides.

5.5. Test for Flavonoids

The extracts and powder were separately combined with ethanol and subsequently filtered. The resulting filtrates underwent various tests for the detection of flavonoids:

(a) Ferric Chloride Test: 1 to 2 drops of ferric chloride were mixed with the different filtrates. The formation of a blackish-red color confirmed the presence of flavonoids.

(b) Shinoda's Test: A piece of magnesium ribbon was added to concentrated hydrochloric acid. This solution was then mixed with different filtrates, and the appearance of a red color confirmed the presence of flavonoids.

(c) Acid Test: 2 to 3 drops of concentrated sulfuric acid were added to the different filtrates. A yellow-orange color indicated the presence of flavonoids.

(d) Alkali Test: 10% aqueous sodium hydroxide solution was mixed with the different filtrates. A yellowish-green color indicated the presence of flavonoids.

5.6. Test for Phenol

Individually, all the extracts and powder were mixed with distilled water and subsequently filtered. To the resulting filtrates, potassium ferricyanide along with ammonia was added. The formation of a deep red color confirmed the presence of phenolic compounds.

5.7. Test for Tannins

Individually, all the extracts and powder were mixed with distilled water and filtered. Subsequently, 10% ferric chloride solution was added to the filtrates. The formation of a bluish-black color confirmed the presence of tannins.

5.8. Test for Saponins

Individually, all the extracts and powder were mixed with distilled water. The resulting solutions were shaken, and the formation of foam confirmed the presence of saponins.

5.9. Test for Volatile Oil

The powdered drug was initially placed in a Clevenger apparatus. Subsequently, it was distilled for 6 hours to extract the volatile oil.

5.10. Test for Fixed Oils and Fats

A small quantity of powder was placed between two filter papers and pressed. Stains on the paper indicated the presence of fixed oils and fats.

6. Chromatographic evaluation

Thin Layer Chromatographic Separation [44,45,46]

The separation of compounds or a mixture of compounds based on adsorption is effectively achieved

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through thin-layer chromatography (TLC). This method is particularly useful for separating non-volatile mixtures. In this technique, a thin glass plate coated with aluminum or silica gel serves as the stationary phase. The choice of the mobile phase, usually a solvent, is determined by the properties of the components in the mixture. Upon applying the sample on the plate, capillary action draws it up through the solvent mixture. The underlying principle relies on the solid stationary phase, applied as a coating on the glass or plastic plate, while the mobile phase moves over it. The sample is typically applied at the bottom of the TLC plate, just above 2 cm from the bottom. TLC can be performed on an analytical scale to monitor reaction progress or on a preparative scale for small-scale purification of compounds. Components in the mixture move toward the stationary phase based on their affinity. Those with a stronger affinity towards the adsorbent travel more slowly, while components with weaker affinity move faster. This differential migration helps in the separation and identification of the individual compounds in the mixture.

Plate preparation:

To boost reproducibility, TLC (Thin-Layer Chromatography) plates usually incorporate standardized particle size ranges. The production involves mixing silica gel and water to create a dense slurry. This blend of the stationary phase is uniformly applied to a glass plate. Following this, the plates are moved to a heating chamber, commonly a hot air oven, and subjected to temperatures between 105 and 110 °C for a duration of 30 minutes.

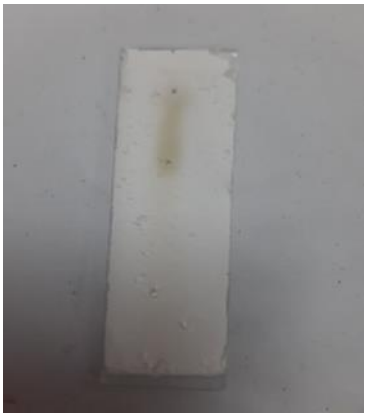

Figure No. - 4 TLC plate with spots

Selection of mobile phase:

Stationary phase - Silica gel g

Mobile phase – Acetic acid: ethanol (1:3) & Chloroform: Methanol: Water 7:4:1

Detecting agent – 10% Sulphuric acid in methanol

The Rf values were calculated using the formula =

$$\frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

EXTRACT	Rf VALUE
Aqueous extract	1 spot Rf value – 0.72
Methanolic extract	2 spots Rf value - 0.69 Rf value - 0.92

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7. Pharmacological study

Herbal remedies have been employed for various ailments since ancient times, with their usage persisting alongside synthetic drugs due to the latter's propensity for adverse effects. Therefore, the current study aims to explore additional therapeutic properties of the ethnomedicinal plant Sarcostemma acidum beyond its traditional uses. Ethnomedicinally, S. acidum has been applied in treating conditions such as otitis, dog bites, snake bites, leprosy, emesis, and arthritis . In addition to its ethnomedicinal applications, this study investigates other pharmacological activities of S. acidum. Specifically, the evaluation focuses on its analgesic, antipyretic, and antidiabetic effects using the aqueous extract of the stem of S. acidum. Prior to conducting these pharmacological assessments, an acute toxicity study of S. acidum was performed.[47]

All scientific investigations were conducted in the pharmacology laboratory of Gayatri College of Pharmacy, Sambalpur, Odisha, with the approval of the Institutional Animal Ethics Committee (IAEC) under Registration No. 1339/PO/Re/s/10/CCSEA.

7.1. Acute toxicity study[48,]

Prior to any pharmacological evaluation of a drug or chemical, it is imperative to consider the safety of the agent on tested animals or human subjects. Therefore, from a safety perspective, the initial step involved conducting the acute toxicity study of *Sarcostemma acidum*. This study adhered to the guidelines outlined in the Organization for Economic Co-operation and Development (OECD) guideline number 423.

Method[49]

Male albino rats, with a healthy weight ranging from 200 to 220 grams, were selected for the study. The rats were organized into four groups, each consisting of 6 animals. Subsequently, the animals underwent an overnight fasting period. Following the fasting period, varying doses of the extract were administered to the respective groups of animals.

Group-I (Control):

The animals assigned to group-I were orally administered 1ml/100gm of body weight of a 1% w/v CMC solution, serving as the control group.

Group-II, III, IV (Test):

Animals in test groups II, III, IV received a single oral dose of S. acidum extract at concentrations of 300 mg, 1000 mg, and 2000 mg per kg body weight, respectively, dissolved in a 1% CMC solution. Subsequently, these tested animals were subjected to continuous observation for 24 hours to monitor any physiological changes or mortality. This observation period was sustained for a duration of 14 days.

7.2. Analgesic activity study of *Sarcostemma acidum*[50,51]

These agents are designed to alleviate pain. Pain manifests as a symptom resulting from cell destruction or tissue damage. When cells undergo destruction, various mediators such as prostaglandin, histamine, bradykinin, and 5-HT are produced from the damaged cells. These mediators induce pain by stimulating pain receptors located at the sensory nerve endings of somatosensory nerves. Subsequently, these impulses travel to the somatosensory area in the parietal lobe of the cerebral cortex. Consequently, the central nervous system is activated, leading to the perception of pain.

Method[52,53,54,55]

Male albino rats, with a healthy weight ranging from 200 to 220 grams, were selected for the experiment. These rats were then divided into four groups, each comprising six animals. Prior to the commencement of the experiment, all animals underwent a fasting period of 2 hours. The different groups of animals were administered control, standard, and test substances as part of the experimental protocol.

Group-I (Control):

Animals in group-I received an oral administration of 1ml/100gm of body weight of a 1% w/v CMC solution, serving as the control group.

Group-II (Standard):

Animals in group-II were intraperitoneally injected with 9mg/kg of diclofenac sodium in a 1% w/v CMC solution, serving as the standard group.

Group-III and IV (Test):

Animals in test groups III and IV were orally given the aqueous extract of S. acidum at doses of 200mg/kg and 400mg/kg body weight, respectively, in a 1% w/v CMC solution [33,34,35]. Subsequently, individual animals were placed on the hot Eddy's plate maintained at 55°C. The latency period of each animal on the hot plate was recorded, with the observation intervals set at 30, 60, 120, and 180 minutes. If an animal jumped due to pain, it was promptly removed from the hot surface, and the latency period was recorded, with a cutoff time of 15 seconds.

7.3. Antipyretic activity study of *Sarcostemma acidum*[56,57]

These are substances that alleviate pyrexia or fever. Pyrexia or fever represents an elevation in body

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temperature above the normal range. It commonly occurs during infections but can also be a result of trauma, inflammatory conditions, or autoimmune diseases. During such instances, prostaglandins are formed from damaged tissues, stimulating the temperature-regulating center in the hypothalamus of the brain, leading to an increase in body temperature or the onset of fever. To mitigate fever or pyrexia, antipyretic drugs are administered. Antipyretics act by blocking the cyclo-oxygenase (COX) enzyme, inhibiting prostaglandin synthesis, and consequently reducing fever. Some Analgesic-Antipyretic drugs block both COX-1 and COX-2 enzymes, while others selectively target COX-2 enzyme.

Method [58,59,60,61,62]

Healthy male albino rats, weighing approximately 200-220 grams, were selected for the experiment. The rats were then divided into four groups, each consisting of six animals. Before the initiation of the experiment, all animals underwent an overnight fasting period. To assess the baseline temperature, the rectal temperature of all rats was measured by inserting the lubricated bulb of a thermometer into the rectum. This initial temperature served as a reference point. Subsequently, pyrexia was induced in all rats by subcutaneously injecting a 15% suspension of Brewer's yeast (10ml/kg body weight) in 0.9% saline at the neck side. After 24 hours from the yeast injection, the rectal temperature of all rats was recorded to determine the pyretic response induced by yeast, and this temperature was considered the zero-hour reading. Following this, control, standard, and test substances were administered orally through a feeding tube to the respective groups of animals.

Group-I: (Control)

Animals in group-I received an oral administration of 1ml/100gm of body weight of a 1% w/v CMC solution, serving as the control group.

Group-II: (Standard)

Animals in group-II were given paracetamol at a dose of 150mg/kg body weight in a 1% w/v CMC solution, serving as the standard group.

Group-III and IV (Test):

Animals in groups III and IV were orally administered the aqueous extract of S. acidum at doses of 200mg/kg and 400mg/kg body weight, respectively, in a 1% w/v CMC solution.

After drug administration, the temperature of the rats in different groups was recorded at 1, 2, 3, and 4 hours. Subsequently, the mean temperature of the test groups was compared with the standard group to evaluate the antipyretic effect of the test substance.

7.4. Antidiabetic activity study of *sarcostemma acidum*[63,64]

Diabetes mellitus is a metabolic disorder characterized by elevated blood glucose levels resulting from insufficient insulin production or reduced insulin receptor sensitivity. Insulin, a hormone produced by the β-cells of the islets of the pancreas, plays a crucial role in facilitating the transport of glucose from the blood into cells. In conditions of reduced insulin or decreased insulin receptor activity, glucose is unable to enter the cells, leading to an increase in blood glucose levels and the development of hyperglycemia. Diabetes mellitus is primarily categorized into two types:

(1) Type-1

(2) Type-2

Method[65,66,67,68]

Healthy male albino rats, weighing approximately 200-220 grams, were selected for the experiment. These rats were then divided into four groups, each comprising six animals. Prior to the commencement of the experiment, all animals underwent an overnight fasting period. To establish the baseline, the blood glucose level of each rat was measured by obtaining blood from the tail vein and using a glucometer. This initial blood glucose level served as the reference point. Following this, control, standard, and test substances were administered orally through a feeding tube to the respective groups of animals.

Group-I: (Control)

Animals in group-I received an oral administration of 1ml/100gm of body weight of a 1% w/v CMC solution, serving as the control group.

Group-II: (Standard)

Animals in group-II were given glipizide at a dose of 0.5mg/kg body weight in a 1% w/v CMC solution, serving as the standard group.

Group-III and IV: (Test)

Animals in groups III and IV were orally administered the aqueous extract of S. acidum at doses of 200mg/kg and 400mg/kg body weight, respectively, in a 1% w/v CMC solution.

One hour after drug administration, intraperitoneal injections of adrenaline at a dose of 0.8mg/kg body weight were given to each group of animals to induce diabetes. Following the adrenaline administration, the blood glucose levels of rats in different groups were recorded at 30, 60, 120, and 180 minutes. Subsequently,

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the mean glucose levels of the test groups were compared with the standard group to evaluate the antidiabetic effect of the test substance.

8. Result and discussion

2.3. Powder drug with chemical reagents

The powder drug with different chemical reagents shows different colour when seen on naked eye. (Table No.1)

Reagents	**Colour observed**
Powder as such	Yellowish brown
Powder + 5% Ferric chloride	Light brownish yellow
Powder + Ethanol	Yellow
Powder + Conc. H_2SO_4	Black
Powder + Glacial acetic acid	Light yellow
Powder + Chloroform	Greenish yellow
Powder + Pet. ether	No colour
Powder + Ammonia	Deep red
Powder + Conc. HCl	Brown
Powder + Conc. HNO_3	Yellow
Powder + Picric acid	Yellow
Powder + NaOH solution	Light yellow
Powder + KOH solution	Light yellow
Power + Methanol	Light yellow

2.4. **Reaction of chemicals with powdered crude drugs & fluorescence analysis**

Reagents	**Fluorescence observed**
Powder as such	Light green
Powder + 5% Ferric chloride	Green
Powder + Ethanol	Deep green
Powder + Conc H_2SO_4	Black
Powder + Glacial acetic acid	Light green
Powder + Chloroform	Light green
Powder + Pet. ether	No colour
Powder + Ammonia	Light green
Powder + Conc. HNO_3	Green
Powder + Picric acid	Green
Powder + NaOH solution	Green
Powder + KOH	Green
Powder + Methanol	Light green

2.5. PH of powdered drug 1% and 10% solution

pH of 1% solution	**pH of 10% solution**
6.26	**5.74**

3. Physical evaluation

Sl. No.	**Parameter**	**Values (% w/w)**
1	**Loss on Drying**	0.4%
2	**Extractive Value**	
	Water Soluble Extractive	12%
	Methanol Soluble Extractive	8%

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	Ethanol Soluble Extractive	6.23%
	Ether soluble Extractive	5.17%
	Ethyl acetate Soluble Extractive	7.21%
3	**Ash Value**	
	Total Ash	9%
	Water Soluble Ash	4.12%
	Acid insoluble Ash	1.7%
4	**Swelling Index**	0%

4. Preliminary Phytochemical Screening

In the phytochemical study of *Sarcostemma acidum*, the different phytochemical tests were taken on powdered drug, aqueous and methanolic extract of *S.acidum*. The results of these tests were obtained as-

Test	Power drug	Aqueous extract	Methanolic extract
Test for carbohydrates			
Molisch's Test	+	+	+
Benedict's Test	+	+	+
Fehling's Test	+	+	+
Test for Starch	+	+	+
Test for alkaloids			
Mayer's Test	+	+	+
Dragendroff's Test	+	-	+
Wagner's Test	+	+	+
Hager's Test	+	+	+
Test for protein			
Ninhydrin Test	+	+	+
Biuret Test	+	+	+
Millon's Test	+	+	+
Test with Tannic acid	+	+	+
Test for glycosides			
Keller-kiliani Test	+	+	+
Legal Test	+	+	+
Baljet Test	+	+	+
Test for flavonoids			
Ferric chloride Test	+	+	+
Shinoda's Test	+	+	+
Acid Test	+	+	+
Alkali Test	+	+	+
Test for phenol	-	-	-
Test for tannins	+	+	+
Test for saponins	+	+	+
Test for volatile oil	+	-	-
Test for fixed oils and fats	+	-	-

From this phytochemical study, it was shown that the powdered drug, aqueous and methanolic extracts of

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S.acidum contain the different phytochemicals like carbohydrates, alkaloids, proteins, glycosides, flavonoids, tannins and saponins**.**

7.1. Acute toxicity study

The result of acute toxicity study of *Sarcostemma.acidum* shown that, on giving the single dose of 2000mg/kg of extract orally, there was no mortality or toxicity seen on tested rats. So, it estimated that the approximate lethal dose (LD_{50}) of *S.acidum* extract must be higher than 2000mg./kg in rats. And the aqueous extract of *S.acidum* is safe for pharmacological study in rat.

7.2. Analgesic acitivity study of *Sarcostemma acidum*

In the analgesic activity study of *Sarcostemma acidum*, the latency period of rats in hot plate were expressed as mean±SEM.

Sl.No.	Group	Dose (mg/kg)	latency time in sec.				
			0 min	30 min	60 min	120 min	180 min
1	Group-I (Control)		3.10±0.52	2.62±0.85	2.94±0.42	3.12±0.63	2.82±0.53
2	Group-II Standard)	9mg/kg	3.16±0.34	4.64±0.27	6.22±0.48	8.25±0.61	7.67±0.63
3	Group-III (Test)	200mg/kg	3.23±0.24	3.48±0.49	5.62±0.41	7.56±0.36	6.71±0.25
4	Group-IV (Test)	400mg/kg	3.17±0.17	3.92±0.65	6.16±0.31	8.17±0.13	7.56±0.35

The results were expressed as mean±SEM.

This result of Eddy's hot plate shown that the latency period of rat on hot plate was increased at 120 minutes after giving the extract or the reduction of pain occur at 120 minutes. The extract of 200mg/kg and 400mg/kg showed high mean reaction time at 120 minutes (7.56±0.36, 8.17±0.13)sec. respectively as compared to control (3.12±0.63) sec.

So, the aqueous extract of *sarcostemma acidum* show marked analgesic effect.

7.3 Antipyretic activity study of *sarcostemma acidum*

In the antipyretic activity study of *Sarcostemma acidum*, the rectal temperature of rats was expressed as mean±SEM.

Group	Dose (mg/kg)	Initial Temp. in ^{0}C.	Rectal Temperature in ^{0}C.				
			0 Hour	1 Hour	2 Hour	3 Hour	4 Hour
Group-I (Control)		37.06±0.28	39.10±0.15	39.26±0.32	39.40±0.24	39.26±0.16	39.12±0.13
Group-II (Standard)	150mg/kg	37.13±0.34	39.16±0.31	38.85±0.15	38.13±0.42	37.36±0.21	37.10±0.27
Group-III (Test)	200mg/kg	37.16±0.13	39.23±0.34	39.12±0.42	38.72±0.27	38.23±0.15	37.43±0.21
Group-IV (Test)	400mg/kg	37.02±0.16	39.17±0.45	38.92±0.31	38.32±0.16	37.45±0.14	37.16±0.17

The Brewer's yeast induced pyrexia model result shown that the aqueous extract of *S.acidum* reduce the pyrexia in tested group of animals. The aqueous extract of 200mg/kg and 400mg/kg significantly reduced the pyrexia from 39.23±0.34, 39.17±0.45 to 37.43±0.21, 37.16±0.17 respectively as compared to control.

So, it confirmed that the aqueous extract of *Sacostemma acidum* has antipyretic effect.

7.4. Antidiabetic activity study of sarcostemma acidum

In the antidiabetic activity study of *Sarcostemma acidum*, the blood glucose level of rats was expressed as mean±SEM

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Group	Drug	Dose (mg/kg)	Initial Blood Glucose level mg/dl	Final Blood Glucose level mg/dl			
				30 min	60 min	120min	180 min
Group-I (Control)	Adr	0.8mg/kg	76±0.23	132±0.56	175±0.45	190±0.24	106±0.42
Group-II (Standard)	Adr +Gli	0.5mg/kg	75±0.34	76±0.52	78±0.63	73±0.25	72±0.36
Group-III (Test)	Adr +AE	200mg/kg	76±0.46	78±0.43	85±0.53	81±0.47	78±0.31
Group-IV (Test)	Adr +AE	400mg/kg	74±0.52	79±0.24	82±0.27	76±0.41	76±0.32

Adr- Adrenaline, Gli- Glipizide, AE- Aqueous extract of *Sarcostemma acidum*

From the result of antidiabetic effect of *Sarcostemma acidum* in adrenaline induced diabetic rat model, it was shown that the aqueous extract of *S.acidum* control the blood sugar level in tested group of animals as compared to control group after the injection of adrenaline. So, it confirmed that aqueous extract of *Sarcostemma acidum* has significant antidiabetic effect.

9. Conclusion

The study titled "Phytochemical and Pharmacological Activity Study of Sarcostemma acidum Voigt" focuses on exploring the phytochemical composition and pharmacological activities, specifically analgesic, antipyretic, and antidiabetic effects of the plant. Sarcostemma acidum Voigt, also known as somlata, has a traditional medicinal history and has been used in conditions such as otitis, dog bites, rabies, emesis, arthritis, and leprosy. Recent studies indicate psychopharmacological effects of S. acidum. Collected from Gandhamardan hill in Odisha, the plant is a leafless shrub with green cylindrical stems, light yellowish-white flowers, and branched roots. Microscopic and phytochemical analyses reveal its structural and chemical characteristics, with a high water-soluble extractive value. The pharmacological evaluation on albino rats involves analgesic, antipyretic, and antidiabetic studies using aqueous stem extract. Before these studies, an acute toxicity test establishes the safety of the extract. Results show significant analgesic, antipyretic, and antidiabetic effects, making S. acidum a promising candidate for further isolation of active constituents. Future research aims to explore the effectiveness and potential benefits of these isolated products in human applications, potentially offering improved efficacy with fewer side effects compared to conventional drugs.

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