Research Article

Phytochemical and pharmacological evaluation of *Sarcostemma acidium* methanolic extract for anti-acne and thrombolytic activities

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Abstract

Objective: The objective of present research work was the investigation of anti-acne and thrombolytic activity of *Sarcostemma acidium* methanolic extract. **Material and methods:** Anti-acne and thrombolytic activity of *Sarcostemma acidium* methanolic extract were determined using P. acne strain and human blood. **Results:** The methanolic extract of *Sarcostemma acidium* was found potential anti-acne and thrombolytic activity. Under the investigation of anti-thrombolytic activity at 5 mg/ml concentration of aerial part, extract of *Sarcostemma acidium* showed 16.2 % clot lysis activity which was highly significant as compared with standard drug 41.32% and 23.19% respectively. Under the investigation of anti-acne activity, methanolic extract of *Sarcostemma acidium* against *P. acnes* was 10±1.70 and 11±1.31. The zone of inhibition for standard drug measured 15±1.67 mm, respectively. **Conclusion:** The results of investigation of anti-thrombolytic and anti-acne activities confirmed that the herbal extract containing bioactive compounds could be used for the treatment of thrombolytic and acne disease.

***Keywords*:** Herbal extracts, thrombolytic and anti-acne activity

Introduction

A thrombosis disease is one of the main causes of morbidity and death in a wide range of venous and arterial diseases. Thrombosis can lead to chief clinical syndromes, some of which include: Acute myocardial infarction, deep vein thrombosis, pulmonary embolism, acute ischemic stroke and acute peripheral arterial occlusion. Arterial thrombosis causes 10 million deaths every year in the world. The investigations are being carried out to investigate for new bioactive antithrombolytic agents with limited adverse side effects. Which herbal medicines can be consider as choice remedies and have been noted by pharmaceutical industries. Increasing interest in antithrombolytic researches in current years is confirmed in many studies such as surveys on antiplatelet, fibrinolytic or anticoagulant properties of herbal extracts or natural products (Dessinioti et al., 2017; Kumar et al., 2005). These products can be used as alternative or complementary remedies, and also as a basis for the discovery of bioactive phytochemicals from which new antithrombolytic molecules would be identified. For example herbal constituent such as coumarins, flavonoids, alkaloids, xanthones, anthraquinones, stilbenes and naphthalenes have been shown to have some antithrombolytic and antiplatelet effects in several studies.

Acne vulgaris disease is a common inflammatory skin disorder relating the pilosebaceous follicles, characterized by comedones, papules, pustules, cysts, nodules, and often scars, mainly on the face, neck, and upper trunk. It is created by hyperkeratosis, which retains keratin and sebum, and caused by Propionibacterium acnes and Staphylococci bacteria (Dessinioti et al., 2017; Ray et al., 2013). Natural herbs have been widely investigated as a significant approach in encountering infections mainly those caused by multi-drug resistant pathogens. Several preparations of local herbs and oils, like olive oil and water extracts of pomegranate, were have been used for the treatment of skin infections. Several studies reported that phenolic extracts exhibited effective antibacterial and anti-inflammatory effects. These fractions contain high

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quantities of different phenolic acids including caffeic acid and ferulic acids which exhibited potent antimicrobial, antiseptic, preservative and anti-oxidant activities (Wash et al., 2016; Marcon et al., 1987; Daud et al., 2013).

According to literature survey work on polyphenolic extracts of herbs investigation, it possesses potent anti-microbial activity against Helicobacter pylori. However, their antimicrobial effects on P. acnes and anti-inflammatory effects were not well characterized. We are know very well, the excessive utilize of antibiotics for longer time develops resistance against P. acne and S. epidermidis, and consequences in undesirable effects like gram-negative folliculitis.

Sarcostemma acidium (Roxb) is a xerolytic plant of the family Asclepiadaceae. It is locally well-known as Khair, Khimp, Khurasni tanto, Art thor, Soma and somavalli. In English- Moon plant and moon creeper, in hindi-Somlata. It is very potent medicinal plant. It is classified as candidate of soma plants used to prepare Som ras. This plant is mainly found in India, Pakistan and Europ. It is a lot of branched, leafless, Straggling shrub, climbing on *Euphorbia caducifolia* haines on hils. It is bitter, acrid, cooling, narcotic, emetic, antiviral and rejuvenating. It is valuable in vitiated situation of pitta, dipsia, hydrophobia, psychopathy and general dibility (Dev et al., 2017). The objective of research work was to investigate thrombolytic and anti-acne activity of aerial parts of *Sarcostemma acidum*.

Material and methods

Collection of plant material

The aerial part of *Sarcostemma acidium* Linn was collected from National Botanical garden Jaipur, Rajasthan India. Selected medicinal plant parts were cut into small pieces, cleaned and shade dried at room temperature then subjected to physical evaluation with different parameters. The plant parts were subjected to size reduction to get coarse powder, by mechanical grinder and then passed through sieve no.40 and stored in well-closed container.

Preparation of extracts by using soxhlet extracting methods

200g of whole plant material were taken into a soxhlet. The after complete set up assembly of extraction and performed the extraction process. Firstly defatted with petroleum ether and then extracted with methanol, after complete extraction of process, then the extract obtained is filtered by using ash less filter paper and evaporated under reduced pressure. Dried extracts were kept in refrigerator and used for further investigation.

Phytochemical screening

The prepared extract was tested by using qualitative test method, for the identification of phytochemical such as alkaloid, saponin, tannin, flavonoid, phenol, steroid and triterpenoids etc (Bajaj et al., 2012; Mourya et al., 2017; Gupta et al., 2015).

In vitro experiment of thrombolytic activity

Blood specimen

Venous blood samples were drawn from male healthy volunteers (age 20-22 years) without any recent history of oral contraceptive and anticoagulant therapy. About 500 µl of blood was taken into each pre-weighed microcentrifuge tube to form clots, and these were separated from each other by assigning a distinct number to each (Alageer et al., 2018; Milagros et al., 2015).

Streptokinase determination

To the commercially available lyophilized streptokinase (15, 00,000 IU) vial 5 ml phosphate buffered saline was added and mixed properly. The concentration of the streptokinase was suitably diluted to be 30,000 IU and 15,000 IU, which were used as the reference standard for thrombolytic activity since it has been used as standard thrombolytic drug, in earlier research work.

Study design

Venous blood drawn from healthy volunteers was immediately transferred in different pre-weighed sterile micro-centrifuge tubes (500 µl blood/tube, 10 tubes for plant extract). About 200 µl of 2% calcium chloride was added to each of these tubes, mixed well and incubated at 37°C for 45 min for clotting to occur.

After clot formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed to determine the clot weight (clot weight = weight of blood clot containing tube - weight of tube alone). Each microcentrifuge tube containing clot was properly labeled, and 500 µl of *Sarcostemma acidium* extract, normal saline (as a negative control), 30,000 IU and 15,000 IU of reference streptokinase were added to tubes with clots. All the tubes were incubated at 37°C for 90 min. The remaining fluid was then carefully separated, and the tubes were reweighed. The difference in weight before and after clot lysis was expressed as percentage clot lysis.

Anti-acne activity (in vitro)-was carried out by Paper disc diffusion method.

a) Micro-organism and media used

1. Propionibacterium acne bacteria (MTCC1951) and brain heart infusion media (BHI)

2) Sterile Disc: The Whatmann paper No.1 (diameter = 5.42 mm)

3) Samples: *Sarcostemma acidium* methanolic extract and Clindamycin gel (Standard)

The petri plates were washed carefully and sterilized using hot air oven at 160 °C for 2 hours. 20 ml of sterile molten antibiotic assay medium was seeded by micro-organism; it was transferred aseptically in sterile plate and permitted to solidify at room temperature. After the complete solidification of soft agar, sterile disc impregnated with the gel sample were placed over the solidified agar plate. The plates were left for 10 min's at room temperature to allow the diffusion of gel and incubated at 37 °C for 24 hours for *Propionbacterium acnes* suitable conditions. The plant extracts of various concentrations (50µg/ml and 100µg/ml) were taken. The plates were then observed for the zone of inhibition (mm). The zone of inhibition was estimated by measuring the diameter of the zone including the well diameter. The readings were taken in triplicates (in three different directions) form and the average values were calculated (Dessinioti et al., 2017; Davd et al., 2017). Measurement of zone of inhibition of micro-organism and the zone of inhibition for each sample was observed, measured and represented in mm. From this the activity index (A. I.) and Percent Inhibition (P. I.) were measured by using the following formula. A. I = Mean zone of inhibition of each formulation Zone of inhibition obtained for standard P. I. = Activity index x 100.

Statistical analysis

The significance of percentage clot lysis between plants extracts and negative control (normal saline) by means of the weight difference was tested by the Dunnett t-test analysis. Significance was set at both $P < 0.001$ and $P < 0.05$ levels. Data are expressed as mean ± standard error mean. Percentage blood clot lysis = (weight of the clot after lysis by sample and removal of serum/weight of the clot before lysis by sample) ×100. Own.

Results and discussion

The objective of research work was to investigate the anti-acne and anti-thrambolytic activity of *Sarcostemma acidium* plant. Where the newly developed test model, validated, sensitive, reliable, and simple technique was used that can be performed with limited facilities available.

The *Sarcostemma acidium* extractive value was found to be 4.66%. The phytochemical analysis of methanolic extract of *Sarcostemma acidium* showed the presence of flavonoids, tannins, saponins, steroids, naphthoquinones, resins, and alkaloids, etc. These secondary plant metabolites specially polyphenolics are well known to produce various pharmacological effects and may be responsible for therapeutic activity of *Sarcostemma acidium*.

Thrombolytic activity

Under the investigation of thrombolytic activity, were found addition of 500 µl of streptokinase of 30,000 IU and 15,000 IU concentrations to tubes showed highly significant ($P < 0.001$) clot lysis of 41.32%, 23.19% and 5.10% clot lysis of normal saline exhibited which was taken as a negative control.

At 5 mg/ml concentration of aerial extract of *Sarcostemma acidium* showed 16.21% clot lysis activity respectively, which were highly considerable ($P < 0.001$) comparing with negative control (normal saline). The results are shown in table 1.

Anti-acne activity

As *Sarcostemma acidium* extract show prominent result against P. acnes, consequently *Sarcostemma acidium* extract could be a good source for the anti-acne medicine. Herbal extracts have negligible adverse effects compared

Table 1. Potentiality of methanolic extracts of *Sarcostemma acidium* on human blood clot lysis in vitro

Concentration of blood extract, control and standard	% of blood clot lysis
NC - 0.9% NS	5.10±0.11
SK 30,000 IU (SK30K)	41.32±1.03
SK 15,000 IU (SK15K)	23.19±0.50
Sarcostemma acidium (MESA)	16.21 ±1.60

Table 2. Anti-acne activity against Propionbacterium acnes

Plant extract	Zone of inhibition (mm) Mean±SD
Methanolic extract of *Sarcostemma acidium* (50D[ )	10±1.70
Methanolic extract of *Sarcostemma acidium* (100D[ )	11±1.31
Propionbacterium acnes (100D[ )	15±1.67

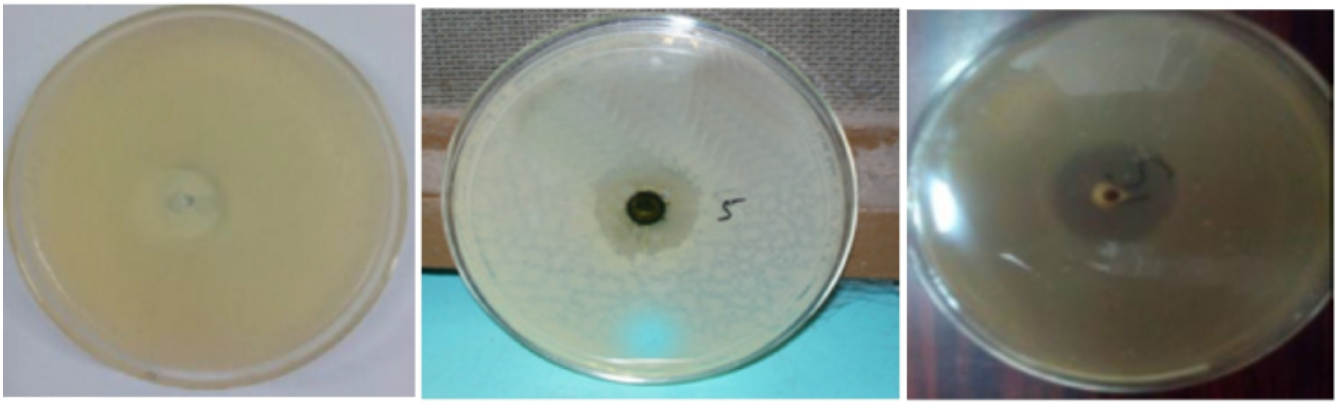

Figure 1. Anti-acne activity against *P. acne* bacterial strain **(a).** Plant extracts (50µl) **(b).** Plant extracts (100µl) **(c).** Standard drug (100µl)

with modern medicine. It has been commonly used in the treatment of skin disease. The efficacy of these herbal agents in acne treatment is not only based on anti-microbial activity. *Sarcostemma acidium* is used in acne due to their skin detoxification property but until it is not reported, hence, under the investigation of our research work. We were found the zone of inhibition for methanolic extracts of *Sarcostemma acidium* were found against P. acnes was 10±1.70 and 11±1.31 respectively. The zone of inhibition for standard drug measured 15±1.67 mm (Bhaskar et al., 2009). The results are shown in Table 2. The result of anti-thrambolytic and ant-acne activity of plant extract is showed very near to standard drug. This represented the extract of *Sarcostemma acidium* have potential anti-thrombolytic and ant-acne activity.

Conclusion

In this study, we evaluated thrombolytic and anti-acne activity of methanolic extract of *Sarcostemma acidium* commonly used traditional medicinal plants from India. Various research works are undertaken in quest of thrombolytic and anti-acne drugs. Herbal drugs can be a source to address this concern. This is only a preliminary study and to make the final statement about the potentiality of these herbs as anti-acne and thrombolytic drugs may require further study. Studies may be undertaken to identify the chemical structure of the active ingredients of the root extracts and to elucidate the exact mechanism of action. Methanolic extract displayed a potent antibacterial activity in the dose-dependent manner. Further studies are needed for these potent plant extracts to evaluate the other parameters of anti-acne effectiveness (e.g. in vivo efficacy and toxicity).

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Conflicts of interest: Not declared.

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