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Journal homepage: www.ijpbr.in**Original Research Article****Development of quality control parameters for the standardization of Leaves and bark of *Sida acuta* Burm.f**Alok Semwal^{1*}, M. Senthil Kumar²¹Department of Pharmacy, Shri. Venkateshwara University, Gajraula, U.P (India)²Department of Pharmacy, Himachal Institute of Pharmacy, Paonta Sahib-173025, H.P (India)**ARTICLE INFO:****Article history:**

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ABSTRACT

Sidha acuta Burm. f belonging to family Malvaceae is a terrestrial, erect shrub which is up to 155 cm tall. The plant is native to Mexico, Central America and Himalayan region of India but has spread throughout the tropics and subtropics. *Sida acuta* Burm. f is widely distributed in pantropical areas and is widely used as traditional medicine in many cases. Among illnesses the plant is may be used alone or in combinations with other plants to cure fever, skin diseases, snake bites, Hemorrhoids, impotency and for boils and eye cataracts. Although the plant has been screened and suggested for various therapeutic activities its photocomposition remained unrevealed because of very restricted amount of research work carried out. Thus it was thought worthwhile to explore this endangered plant on the basis of various standardization parameters. The present research work deals with the collection, identification, extraction, pharmacognosical and phytochemical investigation of Leaves of *Sidha acuta* Burm. f.

Introduction

Nature has been a source of medicinal agents since the beginning of human civilization. During the last few decades, there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world[1-3]. Documenting indigenous knowledge through ethnomedicinal studies is important for the conservation and utilization of biological resources. The World Health Organization (WHO) suggested that as many as 80% of the worlds people depend on traditional medicine for their primary healthcare needs[4]. *Sida* is one of such ethnomedicinally important genus of plants with about 200 species distributed throughout the world and 17 are reported to occur in India[5,6]. *Sida acuta* Burm. f. (Family: Malvaceae) is used in Siddha system of medicine and in folk medical practice in Uttarakhand under the Folk names Bala or Karenti. Uttarakhand is a hill state in north India which is spread over 58,484 square kilometers (Figure 1). The state is located between 30° 33' N and 78°06' E[6].

The drug Bala is mentioned in Charak Samhita under Brihaniya Kasaya, by the name of Vatyayani, under Prajasthapan Mahakasaya by the name of Vatyapushpi[7]. *Sida acuta* Burm. f. is widely distributed in Tropical and subtropical areas and is widely used as traditional medicine in many cases[4]. The plant is also used for spiritual practices. Among illnesses the plant is used to cure, fever is the most cited. The administration may be application of the paste directly on the skin for skin diseases or

snake bites[8]. The plant may be used alone or in combination with other plants according to the diseases or to the healers.

In Sri Lanka plant is used in the treatment of Hemorrhoids, fevers, impotency, root extract is given in leucorrhoea gonorrhoea, and rheumatism. In mixture *Sida acuta* Burm.f. is used as aphrodisiac and for boils and eye cataracts. Various scientific studies revealed that *Sida acuta* Burm. f. not only used as traditional medicine but also contain various useful activities such as Antibacterial[9,10], Anticancer[11], Antiplasmodial[12], Antioxidant[13], Analgesic[14,15], Antimalarial[16], Hepatoprotective[17], Demulcent, Diuretic[4], Larvicidal and Repellent activities[18] which are scientifically validated.

Material and methods**Processing of Plant material**

The Plant material (*Sidha acuta* Burm. f Leaves) was collected from Srinagar Garhwal, Uttarakhand, India and identified by the Botanist Dr. R. M Painuli, Incharge GUH, Harbarium Department of Botany, H. N. B. Garhwal University (A Central University) (U.K.) India. The Leaves are separately dried in shade and preserved in air tight container. The dried Leaves are than powdered in mixture grinder.

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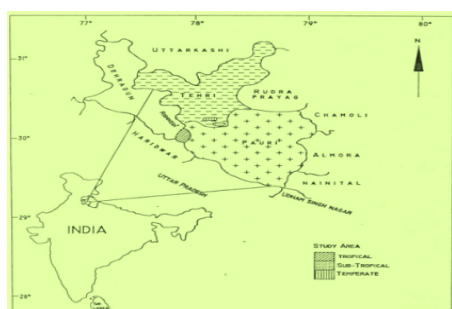


Figure 1: Tropical and subtropical regions of Uttarakhand[4]

The air dried leaves of *Sida acuta* Burm.f. were reduced to coarse powder. The dry powder of plant leaves (500 gm) was subjected to successive solvent extraction procedure using various solvents such as; petroleum ether, chloroform, acetone and methanol in the increasing order of polarity (Figure 2). The solvents were evaporated under reduced pressure to obtain a semisolid mass and then vacuum dried to yield solid residues. The dried extracts were stored in air tight container until the time of use[19,20].

Preliminary phytochemical screening of different extracts

The different plant extracts were subjected to qualitative chemical tests for the identification of various constituents such as alkaloids, carbohydrates, glycosides, proteins, tannins, sterols, saponins, amino acids etc[21].

Preparation of plant extracts

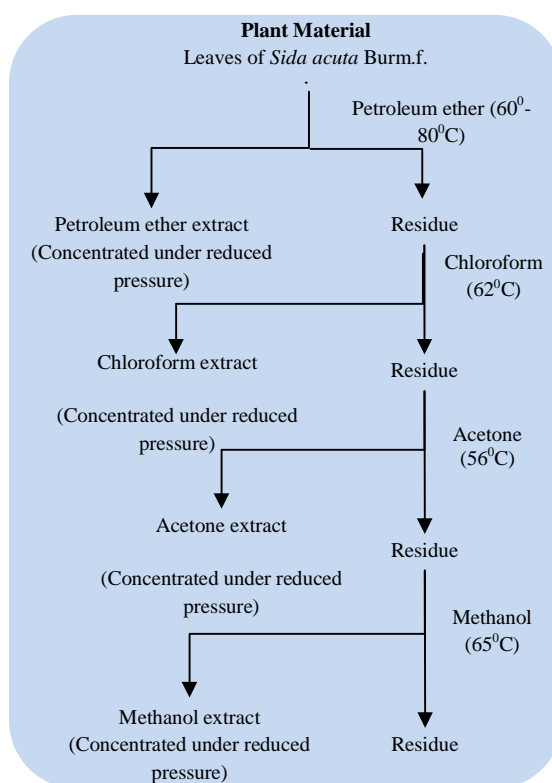


Figure 2: The scheme for extraction of leaves of *Sida acuta* Burm.f

Development of Standard analytical parameters[22]

Macroscopical evaluation, physical parameters such as foreign matter, ash values, fluorescence analysis, extractive value, moisture content, chromatographic and other analysis were performed according to the standard official methods[23,24]. Thin layer chromatography analysis of petroleum ether, chloroform, acetone, ethanol and aqueous extracts were carried out in various solvents according to the standard protocols[25, 26].

Result and discussion

Organoleptic evaluation

It is a technique of qualitative evaluation based on study of morphological and sensory profiles of whole drugs. Organoleptic evaluation means conclusions drawn from studies resulted due to impression on organ of senses.

Table 1: Organoleptic evaluation of leaves of *Sida acuta* Burm. f

Leaves	
Colour	Dark green
Odour	Odourless
Taste	Bitter

Foreign Organic Matter

Foreign organic matter means the material consisting of material not coming from the original plant source or not covered by definition of the herbal drug. It also includes insects, moulds and other animal contamination, parts of the organ or organs from which the drug is derived. The results of foreign matter were recorded in the form of % w/w (Table 2).

Table 2: Foreign organic matter

Foreign matter %
0.8

Extractive Values

This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists. The air dried, accurately weighed drug was treated with solvents: petroleum ether, chloroform, acetone, ethanol and water (Table 3).

Table 3: Extractive values

Water soluble extractive value (%)	Alcohol soluble extractive value (%)
3.00	1.40

Ash Value

Ash value is used to determine quality and purity of a crude drug. It contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc (Table 4).

Table 4: Ash Value

Total (%)	Ash (%)	Water soluble (%)	Ash Acid insoluble (%)	ash
8.53	2.90		0.53	

Determination of moisture content

The most common method for the determination of moisture is to heat the drug till one gets constant weight at 100°C. For the substances which undergo change with consequent loss of weight at a temperature of 100°C, other methods are used. A result of the total moisture content of the crude drug is given in Table 5.

Table 5: Loss on drying

Loss on drying %
9.40

Fluorescence analysis

Behaviour of powdered leaves of *Sida acuta* Burm.f. with different chemical reagents is detected. The colour changes were observed under day light and fluorescence UV-light and results are presented in the Table 6.

The drug powder was taken and treated with various chemical reagents like glacial acetic acid, 10% NaOH, Conc. HCl, Conc. HCl + H₂O, Conc. HNO₃, Conc. H₂SO₄, Ethanol, Distilled water, 5 % Iodine, Picric acid, Ferric chloride solution, Ammonia solution and the color obtained was visualized under visible light and short UV light (254 nm) in UV chamber.

Table 6: Fluorescence studies

S. no	Treatment	Visible Light	UV 254 nm
1.	Powder + As such	Light green	Light green
2.	Powder + Glacial acetic acid	Green	Light green
3.	Powder + 10 % NaOH	Light green	Dark green
4.	Powder + Conc. HCl	Dark green	Dark green
5.	Powder + Conc. HCl + H ₂ O	Light green	Dark green
6.	Powder + Conc. HNO ₃	Light brown	Yellowish green
7.	Powder + Conc. H ₂ SO ₄	Light green	Light yellow
8.	Powder + Ethanol	Light green	Green
9.	Powder + Distilled water	Light green	Dark green
10.	Powder + 5 % Iodine	Dark blackish	Dark blackish
11.	Powder + Picric acid	Yellowish green	Light green
12.	Powder + Ferric chloride solution	Dark brown	Dark brown
13.	Powder + Ammonia solution + HNO ₃	Light brown	Light brown

Thin Layer Chromatography

Identification by TLC of different extract which was run in different solvents, visualized with different visualizing agents with R_f value and solvent system (Table 7).

Phytochemical screening

The various extracts of stem bark of *Sida acuta* Burm.f were subjected to qualitative chemical examination for the presence or absence of alkaloids, carbohydrates, flavanoids, proteins, saponins and tannins, phenolic compounds and glycosides (Table 8).

Conclusion

Sida acuta Burm. f is a erect, branched small shrub which is up to 155 cm tall. Leaves are sharp in shape, dark green in colour, odorless and Bitter in taste. In preliminary

phytochemical screening, the petroleum ether extract showed positive results for steroids and fixed oil, the chloroform extract showed positive results for alkaloids, steroids, the

acetone extract showed positive result for phenolic compounds and tannins.

Table 7: Phytochemical investigation of various extracts of *Sida acuta* Burm.f. leaves

S.No.	Plant Constituent	Extracts				
		PEE	CE	AE	ME	AE
1.	Alkaloids					
a)	Hager's reagent	-ve	+ve	-ve	-ve	-ve
b)	Wagner's reagent	-ve	+ve	-ve	-ve	-ve
c)	Mayer's reagent	-ve	-ve	-ve	-ve	-ve
d)	Dragendorff's reagent	-ve	+ve	-ve	+ve	-ve
2.	Phenolic compounds and tannins					
a)	Ferric Chloride solution	-ve	+ve	-ve	+ve	+ve
b)	Lead acetate test	-ve	-ve	-ve	+ve	-ve
c)	Acetic Acid Solution	-ve	-ve	-ve	-ve	-ve
d)	Dil. Nitric acid	-ve	-ve	-ve	+ve	-ve
e)	Bromine Water	-ve	+ve	-ve	+ve	-ve
f)	Dil. Iodine	-ve	-ve	-ve	-ve	-ve
g)	Pot. Permanganate	-ve	-ve	-ve	+ve	-ve
h)	Gelatin Solution	-ve	-ve	-ve	-ve	-ve
i)	Pot. Dichromate	-ve	+ve	-ve	-ve	-ve
3.	Flavonoids					
a)	Shinoda test	-ve	-ve	-ve	+ve	+ve
b)	Lead acetate test	-ve	-ve	-ve	+ve	-ve
c)	Alkaline test	-ve	-ve	-ve	+ve	-ve
4.	Saponins					
d)	Biuret test	-ve	-ve	-ve	-ve	-ve
e)	Million's test	+ve	-ve	-ve	-ve	-ve
f)	Test proteins containing sulphur	-ve	-ve	-ve	-ve	-ve
g)	Precipitation test	-ve	-ve	-ve	-ve	-ve
5.	Amino acids					
a)	Ninhydrin test	-ve	-ve	-ve	-ve	-ve
6.	Fats and oils					
a)	Solubility test	+ve	+ve	-ve	+ve	+ve
b)	Filter paper test	+ve	+ve	+ve	+ve	-ve
7.	Steroids					
a)	Salkowski reaction	+ve	+ve	+ve	+ve	+ve
b)	Tannins	+ve	-ve	-ve	+ve	+ve
c)	Saponins	+ve	+ve	-ve	+ve	+ve
10.	Carbohydrates	-ve	+ve	-ve	+ve	-ve

Table 8: TLC data of various extracts of *Sida acuta* Burm.f. leaves

S.No.	Extract	Developer	Rf. value
1.	PPE	5 % concentrated sulphuric acid in methanol	0.07, 0.4, 0.3, 0.55, 0.74
2.	CE	5 % concentrated sulphuric acid in methanol	0.62, 0.64
3.	AE	5 % concentrated sulphuric acid in methanol	0.14, 0.20, 0.31
4.	ME	5 % concentrated sulphuric acid in methanol	0.44, 0.36
5.	AE	5 % concentrated sulphuric acid in methanol	0.06, 0.07, 0.30, 0.55, 0.67, 0.80

The methanolic extract showed positive results for phenolic compounds, tannins and flavanoids. This investigation also reported the total ash values (8.53 % w/w), the acid insoluble ash values (0.53 % w/w), the moisture content (9.40 % w/w), the water soluble extractive values (3.00 % w/w) and the alcohol soluble extractive value (1.40 % w/w) for the leaves of *Sida acuta* Burm. f. This study thus provides a monograph on the plant for its proper identification and preliminary detection of phytochemicals responsible for its various activities.

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