ISOLATION, CHARACTERIZATION, QUANTIFICATION OF SIDA SEEDS IN MARATHWADA REGION



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Submitted by

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DECLARATION

I hereby declare that this Minor Research Project (F.No. 47-335/12, Dated: 26.02.2013) report on the topic entitled "Isolation, Characterization, Quantification of Sida seeds in Marathwada region" is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which has been accepted for the award of my other degree or diploma of the University or other institutes of higher learning.

Place: Aurangabad Dr. Maqdoom Farooqui

Date: (Principal Investigator)

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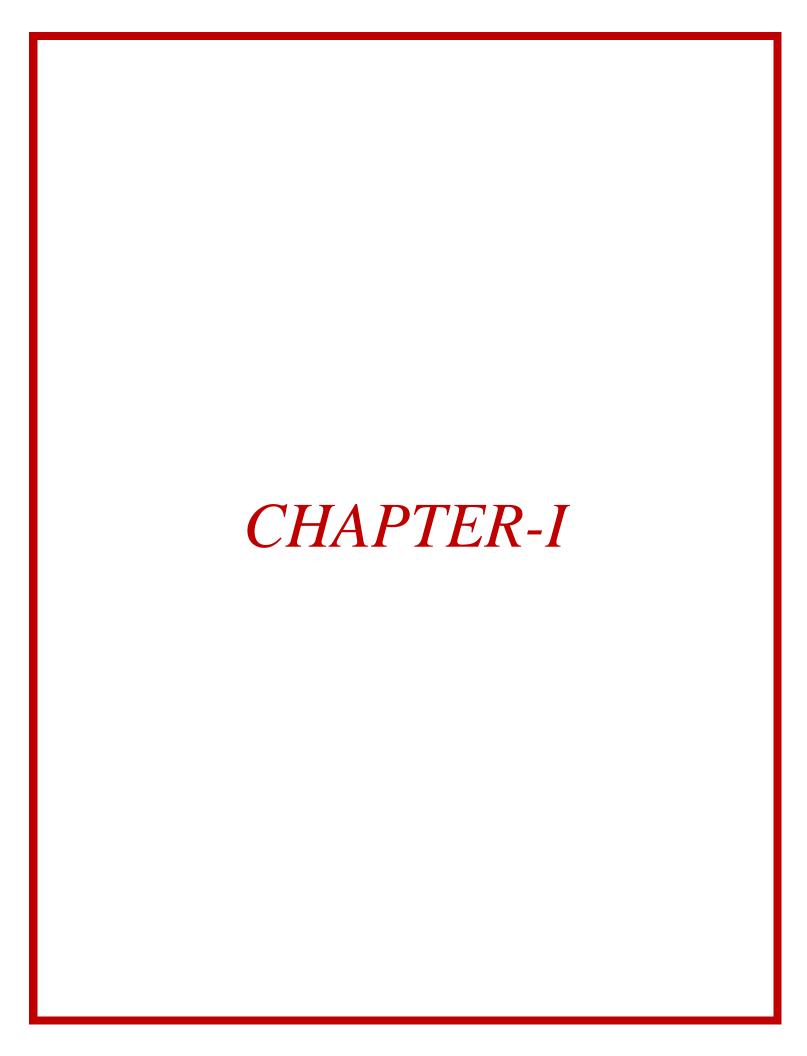
In my journey through MRP programme, there are many who deserve a place here. Some I name, and others, I thank in anonymity. I wish to make devotional supplication to the Almighty Allah for his blessing, without his inspiration this task would not have been accomplished.

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INDEX

SR. No.	Contents	Page No.
1	Introduction	
2	Experimental Procedure	
3	Experimental Results	
4	Discussion	





Sida Cordifolia Linn is a plant which belong to the family Malvaceae, is shrubby, branched, hairy and soft. Sida Cordifolia, is an erect sub shrubs 0.5-1.5 m tall, it is whitish to yellowish stellate tomentose. Flowers are usually auxiliary clusters, seeds are 1.8-2.3 mm long glabrous [1]. Sida veronicaefolia, family Malvaceae, is a straggling way side herb and found often growing in the shady places (India) [2]. This herb is growing in the forest and as weeds in grown grass of gardens and public places [3]. Sida Veronicaefolia is also called as Rajbala, Bhumibala, Farid buti, Shaktibala etc [4]. In India Taxa Sida, L. represented by 22 genera and 93 species. The members are predominantly annual or perennial herbs [5].

Sida Cordifolia, distributed in tropical and subtropical regions of both hemisphere [6]. This family contains about 75 genera and 100 species in the tropical and temperate regions. Usually the plant most found in the two regions of the country i.e. Garwal in Himalayas and Western Ghats' sweet tonic, astringent, emollient, aphrodisiac and administered in gonorrheoea [7]. Diabetes mellitus is one of the most common endocrine disorders, as it is the third killer disease of mankind after cancer and cardiovascular disease because of its Prevalence, mortality and morbidity [8]. To control the disease, several conventional drugs are there is market to use with insulin. But prolong use of insulin may lead to complications like blurred vision hypoglycemia and lingering condition such as coma [9-10]. The anti-diabetic drugs such as oral hypoglycemic agents, like Sulphonylurease (tolbutamide, glibenclamide) and insulin-sensitizer (trogglitazone) are associated with some side effect. To reduce this damaging effort, used of conventional drugs along with hypoglycemic herbs [11]. World wide more than 800 plants posses' anti-diabetic activity [12-13]. Sida Cordifolia (Linn), that improves the diabetic condition (14-15). It is found that, roots, leaves, stem and seeds are used in the folk medicines as anti-rheumatic [16], antipyretic [17], antiasthmatic [18], Laxative, diuretics [19-20], Vaso-relaxative [21], hypotensive CNS-depressant [22-23], antioxidant [24-26] and also shows hypoglycemic effect. Recent investigation shown that anti-oxidant property of various Sida Cordifolia plant, correlated with the oxidative stress defense and also various diseases in human being i.e. cancer, atherosclerosis, aging, inflammation and also CNS disorders. The antioxidants can interfere with the different oxidative process, by treating with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers [27]. The medicinal values of Sida species known from ancient times and are used for treatment of various

diseases. The species of Sida i.e. Sida acuta, Sida Cordifolia, Sida rhombifolia and Sida Spinosa, are used against externally healing of wounds, skin ulcer, inflammation and internally used against fever, liver disease, analgesic [28]. Since ancient time, the plants of family, Malvaceae are used as major source of medicine. WHO reported that 90 % of population of all over the world using those plants and its products as traditional medicine for primary health care [29]. Studies have undertaken to evaluate the extracts against P. falciparum. Sida acuta Burn f. has been shown to possess a wide spectrum of pharmacological activities. The study of the anti-material activity of Sida acuta Burn. F. extract was evaluated [30] using in vitro SYBR Green-I-based assay. The water extract of the leaves shows analgesic and anti-inflammatory activities in animals. The medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in many countries and are the source of powerful drugs.

Sida Cordifolia is a perennial subshurb of the mallow family Malvaceae, native of India. The name Cordifolia-refers to the heart shaped leaf.

Kingdom : Plantae Order : Malvales Family : Malvaceae

Genus : Sida

Species : Sida Cordifolia

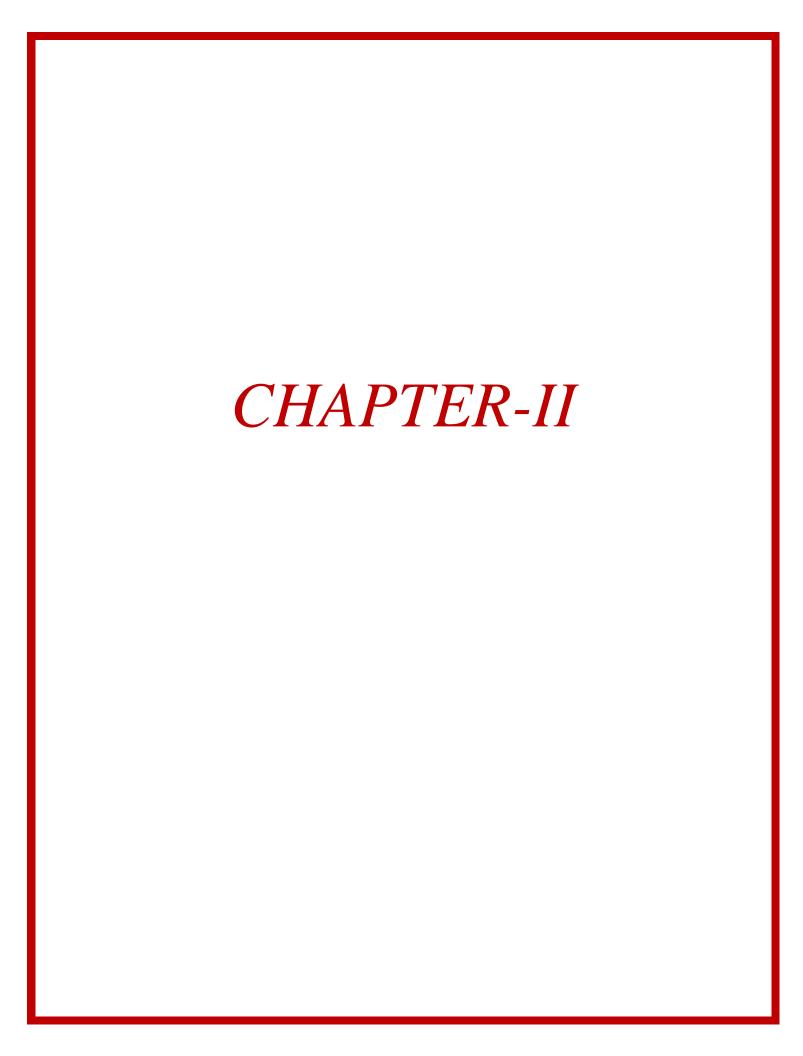
Sida Cordifolia is a shrub now it grows worldwide. It is used in preparation of many traditional medicine. People of India and world used S. Cordifolia against asthma, allergies, fatigue, common cold, but not scientific evidence is supports these uses. All the parts of Sida, i.e. roots, seeds, leafs, are used to make medicine.

As Sida Cordifolia contains ephedrine which is an amphetamine like stimulant that can causes harmful side effects. FDA banned ephedra, Sida Cordifolia and other products that contains ephedrine since April 2004. As far as serious safety concern, S. Cordelia is used for the treatment of many diseases like bronchial asthma, tuberculosis, cold, flu, swine flue, urinary infection etc. It also used for weight loss, erectile dys function, (ED), allergy, throat diseases.

Root of S. Cordifolia with ginger used in fever root of S. Cordifolia with milk used for urinary urgency and vaginal discharge. S. Cordifolia is likely unsafe. S. Cordifolia is banned in the U.S. It

causes dizziness, restlessness, irritability, insomnia, headache, nausea, vomiting, pounding, heartbeat. Don't used Sida Cordifolia with caffeine, it increases the chance of side effects.

Using Sida Cordifolia with other medications causes irregular heart beat (Causes heart attack) using Sida Cordifolia with methyl xanthene's, causes nervousness, a fast heat beat, high blood pressure and anxiety. S. Cordifolia is a highly reputed medicinal shrub, used in Ayurveda and other traditional medicines. In Ayurveda it is used as antirheumatic, analgesic, antipyretic, antiasthmatic, nasal anticongestant, antiviral, laxative, diuretics, aphrodisiac, hypoglycemic, heptaprotective and in the treatment of Parkinson's disease. The aim of study is the used of traditional plant in modern perspective, including taxonomy, in traditional medicines, geographical distribution, chemical constituent, pharmacological studies of plant extract. Bala i.e. also known as Indian Ephedra is plant drug used in various medicines in Ayurveda, Unani and Siddha system of medicine. Bala (Sida Cord) is described as Rasayan, vishaghana, Balya and pramehaghna in the Vedic literature. It is used in Ayurveda therapy internally and externally. It has good medicinal value and used in the treatment of diseases like fever, weight loss, asthma and nervous system diseases. The aim of providing the information regarding Sida plant for preparing new drugs to treat serious diseases. The use of medicinal plant is used is based on their traditional use in curing illness. Sida Cordifolia is having pharmacological activities against Nephroprotective, Antimicrobial, wound healing, anti-oxidant, cardio protective, Anticancer, Anti-ulcer etc. The alcoholic extract of S. Cordifolia at dosage level of 400 mg/kg produces antidiabetic effect in the straptozotocin-induced diabetes in wistar-rats. Literature survey reveals that all part of Sida-Cordifolia are useful in treatment of different disease. The alcoholic extract decreases the blood glucose level in diabetic rats. Modern research carried out on Malvaceae plants revealed that most of the plants are medicinally important and most of them contain biologically active compounds. These were used against ulcers, swelling etc. Phytochemical analysis of the ethanolic extract of the roots of S. Cordifolia indicates the presence of reducing sugar, alkaloids, steroids and saponine. The in-vitro antimicrobial activity of the ethanol extract of the roots of S. Cordifolia showed no antimicrobial activity i.e. inactive against micro-organisms. The isolation and purification of therapeutic potential compounds from S. Cordifolia could be used as an effective source against bacterial diseases in human and plants. Presently, synthetic drugs are more frequently used however compounds are danger, harmful. They may cause adverse effects for endless administration.



Collection of samples: - Samples were collected from the field of Taluka Ajanta 100 km away from Aurangabad and Taluka Guatala 60 km. Samples sorted and dried in shadow area. After complete dry the samples were grind to powder. The powder was passed through 30 mesh size sieve, and packed in a closed and ambered glass container to avoid from moisture and sunlight.

Chemicals and reagents:

All the chemicals used are of analytical reagent grade. Mercuric iodide, potassium iodide, copper sulphate, sodium potassium tartrate, sodium hydroxide, cupric sulphate, sodium citrate, anhydrous sodium carbonate, naphthol, ferric chloride, lead acetate were obtained from Sigma Chemical Co., USA and concentrated hydrochloric acid, sulfuric acid was obtained from S.d. Fine chemicals.

Preparation of extract: -

1. Ethanol Extract of seeds of Sida acuta were prepared by Soxhlet apparatus.

The seeds of Sida acuta were dried in shade and powdered. The powdered seed of plants was subjected to Soxhlet extraction by using Ethanol (70-80 °C). Before each extraction the powdered material was dried in hot air oven below 50 °C. The extract collected was concentrated by distilling off the solvent and then evaporating to dryness on the water bath. Extract were weighed and percentage was calculated.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis was done for the screening of different kinds of secondary metabolites. The phytochemical investigation was performed by doing various qualitative chemical tests including alkaloids, carbohydrates, glycosides, tannins, saponins, resins and flavonoids in different extracts of Sida.

Test for Alkaloids

At the beginning 2 mL solution of the extract and 0.2 mL of dilute hydrochloric acid were taken in a test tube. Then 1 mL of Mayer's reagent was added. Yellow color precipitate was formed that indicated the presence of alkaloids or 1 mL of Dragendroff's reagent was added. Orange brown precipitate was formed that indicated the presence of alkaloids.

Test for Carbohydrate

Molish's Test

A few drops of Molish reagent were applied on 5 ml sample, and the tube was tilted and 1 ml of sulphuric acid was gradually added through one side at the bottom of the test tube. Reddish violet ring formed in the presence of sugars, at the junction of the two layers.

Fehling's Test

1 ml of filtrate was boiled on a water bath with 1 ml of each Fehling's solutions A and B. A red precipitate indicated the presence of reducing sugar.

Test for Flavonoids

Few magnesium turns and few drops of concentrated hydrochloric acid were added and boiled in red or orange-red color for 5 min. gives the existence of flavonoids.

Killar-Killani Test

1 ml of glacial acetic acid containing traces of ferric chloride and 1 ml of concentrated sulphuric acid were added to the test solution. Glycoside was indicated by the formation of reddish-brown color at the junction of two layers and the upper layer turned bluish-green.

Legal Test

A few drops of pyridine and sodium nitroprusside solution was added to the test solution and made alkaline with NaOH solution, the presence of glycoside was indicated by a pink or red color.

Resin Test

Few drops of acetic anhydride solution and 1 ml of sulphuric acid were applied to 1 ml of the test solution. The presence of resin suggests orange to yellow color.

Saponin Test

1 ml of the test solution was diluted to 20 ml with distilled water and shows saponins attendance when shaken in a graduated flask for 15 minutes 2 cm foam sheets.

Test for Phenols

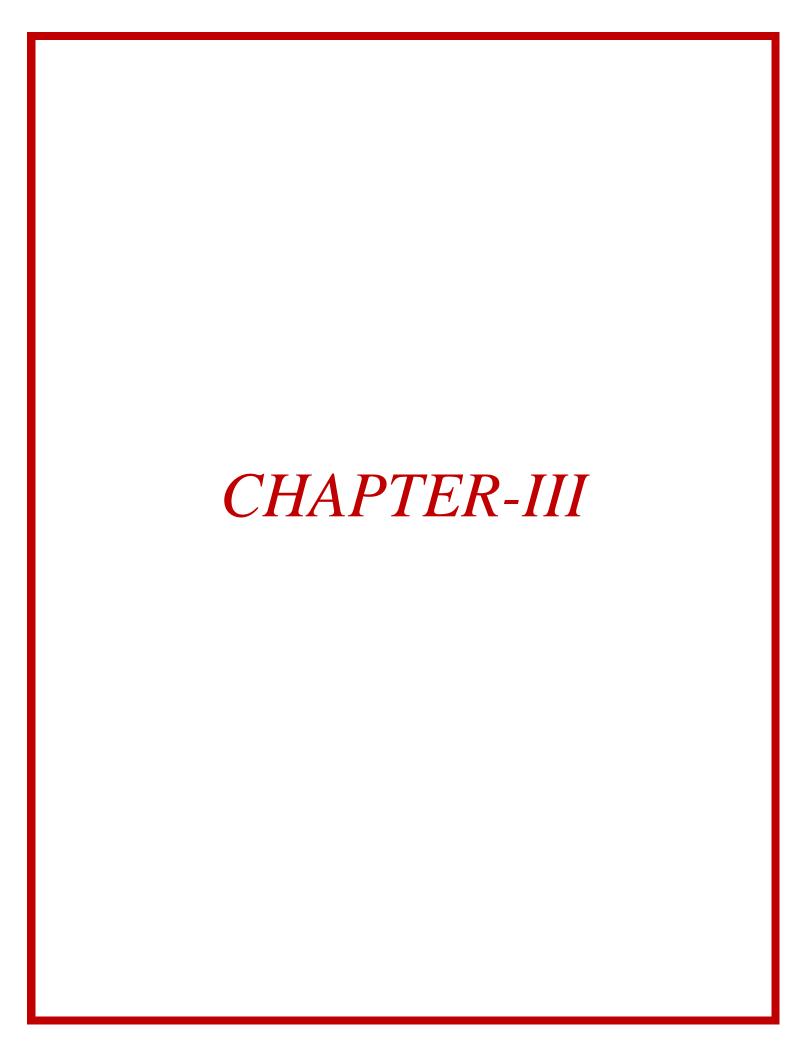
Ferric chloride test: In 5 ml of distilled water, few drops of 5 percent ferric chloride solution was added to 50 mg of extract dissolved in. The presence of a phenolic compound indicates a dark green color.

Determination of tannins

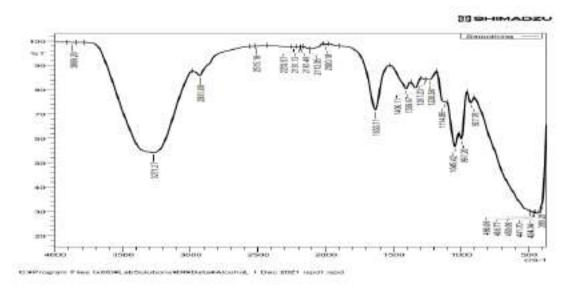
At the beginning 5 mL of the extract was taken in a test tube. Then 1 mL of 5% ferric chloride solution was added or 1 mL of 10% potassium dichromate solution was added

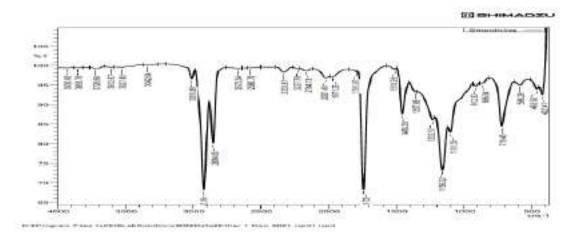
Determination of steroids

At first 1 mL solution of extract was taken and then added 1 mL sulphuric acid. Red color indicated the presence of steroid.



Comparative Analysis of FTIR: -





Ether Extract		Alcohol Extract			
Wave no. cm ⁻¹	Possible functional	Wave no. cm ⁻¹	Possible functional Group		
	Group				
3342.64	N-H stretching	3271.27	-NH ₂ Stretching		
2854.65	C-H Stretching	2931.8	-CH ₂ in phase stretching		
1456.26	N-CH ₃ out of plane	1406.11	(N)-CH ₃ in phase stretching		
	bending				
1357.89	Aryl-CH ₃ in phase	1336.67	-CH ₃ in phase bending		
	stretching				
1101.42	C-O stretching	1267.23	Aryl-N-Stretching		
866.04	N-O stretching	1045.42	C-O stretching		
		1633.71	C=N		

Phytochemical Analysis:

Plant constituents	Ether Extract	Ethanol Extract
Carbohydrate	-	+
Tannins		+
Steroids	+	+
Amino Acids	+	-
Glycosides	-	+
Phenols	+	+
Saponins	+	-
Flavonoids	+	+









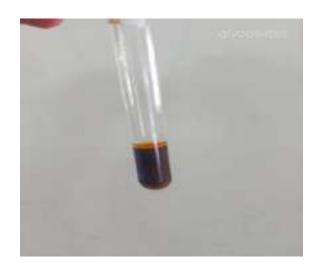


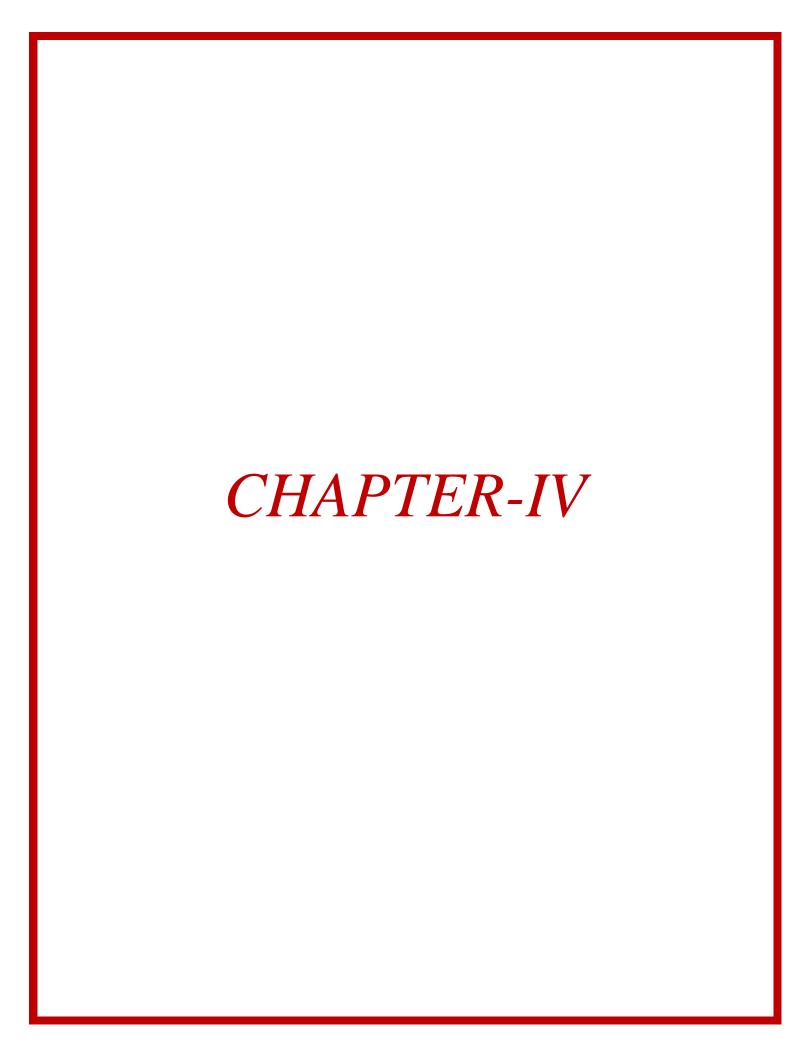












The percent alcoholic extract ability of S. Cordifolia was 19.06 % (w/w) and extract revealed the presence of alkaloids, glycosides saponins, sterols, resins, fixed oil and flavonoids. Diabetes is characterized with the loss in weight of body as protein and fats are used as energy generation through gluconeogenesis [1]. Many diseases are primarily due to different factors such as imbalance between pro-oxidant antioxidant homeostasis.

The results are expressed as changes in blood glucose level. As alloxan induced diabetes indicated that alcohol, butanol, chloroform water and butanone showed more significant, as well as anti-diabetic activity in acute and prolonged treatment compared to diabetic control. The single dose of alcoholic extract reduces the blood glucose level at 3rd hour and hypoglycemia [4] was significantly maintained for another 4 hour. The work carried out revealed that the hydroalcohol extract roots of Sida acuta, Sida Cordifolia, Sida rhombifolia and Sida Spinosa were found to be non-toxic, as high dose as 2000 mg/kg body weight. The extract of Sida species shows positive results for alkaloids, flavonoids, tannins, steroids, resins and triterpenoids. Sida cordifolia was found to be show superior in analgesic activity than sida acuta and sida rhombifolia, sida spinosa was very least effective. Sida acuta and sida cordifolia shows significant reduction in oedema dosage of 200 mg/kg body weight after every 4th hour, comparable to the standard diclofenac sodium. Whereas S. rhombifolia and S. Spinosa shows anti-inflammatory activity in paw oedema method [3].

Phytochemical Screening: - The research extract was subjected to the preliminary phytochemical testing to detect the presence of different group of compounds such as Saponins, tannins, alkaloids, flavonoids, glycosides, carbohydrates oil and fats, amino acids.

Ether Extract		Alcohol Extract		
Wave no. cm ⁻¹	Possible functional	Wave no. cm ⁻¹	Possible functional Group	
	Group			
3342.64	N-H stretching	3271.27	-NH ₂ Stretching	
2854.65	C-H Stretching	2931.8	-CH ₂ in phase stretching	
1456.26	N-CH ₃ out of plane	1406.11	(N)-CH ₃ in phase stretching	
	bending			
1357.89	Aryl-CH ₃ in phase	1336.67	-CH ₃ in phase bending	
	stretching			
1101.42	C-O stretching	1267.23	Aryl-N-Stretching	
866.04	N-O stretching	1045.42	C-O stretching	
		1633.71	C=N	

The result collected of ether extract and alcohol extract shows different characteristic band corresponds to various functional groups like N-H stretching, γ C-, aryl CH₃ in phase stretching, NH₂ & CH₃, C-O stretching, N-O etc. It shows 1232.51-1159.35 broad band for ester carbohydrates, sharp peak at 1101.35, 912.33, 866.04 and 719.45 in the sida poll. Whereas 1620-1710 cm⁻¹ amid band is absent, 1633.71 for C=N

FTIR spectral data of specific absorption peaks for test species, peaks at 3400.3, 2955.1,2850.9,1629.5, 1442.9, 1324.5, 1245.4, 1107.1, 1047.6, 928.8, 897.1, 783.1, 716.6, 634.3 & 586.2 cm⁻¹ were characteristic of Hibiscus pollen and 3418.3, 2955.1, 2920.9, 2850.7, 1732, 1651.1, 1538.5, 1455.3, 1159.9, 1023.7, 723 and 465.5 cm⁻¹

Phenol & flavonoids:

Plant constituents	Ether Extract	Ethanol Extract
Carbohydrate	-	+
Tannins		+
Steroids	+	+
Amino Acids	+	-
Glycosides	-	+
Phenols	+	+
Saponins	+	-
Flavonoids	+	+

S. Cordifolia contains many phytochemicals with alkaloids and flavonoids. The Biological activity of a medicinal plant is influenced by presence of richness of phytochemical. The seed extracts were found to cause an increase in blood pressure in rats. There are many uses of the roots, including treatment of facial paralysis neuralgia, neurosis and cervical spondylosis. A systematic examination of S. Cord. a drug plant by extraction with different solvents system showed the presence of alkaloids in whole plant including leaves, stem, roots, seeds. Seed contains larger quantity of alkaloid as compare to other parts of plant. The aqueous extract showed excellent antibacterial activity against selected gram –ve organisms which are the main causative agents of urinary tract infections & secondary infections⁴. The in-vitro anti-inflammatory test was performed for the test, showing effect of S-Cordifolia against denaturation of egg albumin, plant extracts have an important role in modern medicine as chemical and medicinal constituents are found in natural form. Aqueous extract of S. Cardifolia, showing maximum antibacterial and anti-inflammatory

activity in water and alcohol extract⁵. Flavonoid compound isolated from S. Cordifolia leaves extract and asses their anti-inflammatory activity. The alcohol extract shows anti-oxidant activity. The anti-inflammatory activity of Sida Cordifolia is due to the presence of flavonoids in the extract. Alcoholic extract of S. Cordifolia showed a significant increase in anti-oxidant activity. It has potency to act as anti-diabetic, hypoglycemic and anti-oxidant properties. The photochemical screening of various extracts i.e. chloroform, petroleum ether, ethanol and water; showed presence of alkaloid & flavonoids. The presence of flavoinds in ethanol extract of S. Cordifolia show anti-anxiety activity.

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