

# Pharmacognostic, Physicochemical and Anti-Oxidant Screening of *Solenostemma Argel*'s Leaf Extract

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**Abstract:-** The present work deals with the identification of new approach towards the lead generation in medicinal chemistry and authenticating the reports present on the use of this Sudanese medicinal plant *Solenostemma argel* in the tribal region of Sudan. The pharmacognostic screening showed its physical and chemical composition with identification of active ingredients. The antioxidant activity was carried out by DPPH and nitric oxide scavenging method. In which ethanolic leaf extract showed prominent response which may be due to the promising presence of flavonoids and related poly phenolic compounds in it.

**Keywords:-** *Solenostemma Argel*, Antioxidant, Pharmacognostic, Flavonoids.

## I. INTRODUCTION

Nature is the most faithful and non-demanding friend to mankind. It provides all things which are essential for the healthy life of human being i.e. food, shelter, cloths, and medicine. The majority of things provided by a key component of nature that is plant kingdom. In terms of medicine, it is a very important and precious weapon against pathogens. There are a variety of plants with strong response against a number of disease like cough, cold, fever, diarrhea, dysentery, malaria, asthma[1], antibiotics, immunosuppressant, anticancer drugs[2], rheumatism[3], blood purifier[4], antioxidant[5], antileprotic[6] and many more reports on different diseases and their natural products treatments are present in literature[7]. Some plants also serve as probioactive ingredients for manufacturing of many crucial drugs[2]. Plants also presents themselves as precursors of model drugs production of new synthetic compounds[8] example erythroxyllum coca has served as model for the synthesis of procaine[9]. it is a well proven fact that due to the priceless importance of medicinal plants, i.e. their easy availability, cost effectiveness and less or zero side effects, a large population of the world dependent on them[10].

Widely diversified climates of Sudan showcase itself as a game player for its richness in vegetation and flora. It is estimated that more than 3156 plants species are present there and out of them many are used in traditional medicine. *Solenostemma Argel* is a desert plant of Sudan and used in traditional medicines and in agriculture as anti-inflammatory, Antimicrobial, Analgesic activity, anti-diabetic, Toxicity, Insecticidal, Nematicidal etc.[11]

Free radicals and reactive oxygen species (ROS) are responsible for many disorders in human which are generated due to imbalance in between formation and neutralization of peroxidants during oxidative stress. They cause oxidative damage to lipids, proteins and DNA leading to many chronic diseases i.e. cancer, aging etc. in human body, enzymes like superoxide dismutase, glutathioneperoxidase, catalase etc are generated to reduce the adverse effects of these free radicals. But due to less effectiveness of these enzymes and more generation of free radicals lead to cause diseases. Therefore it is very necessary to identify such species which serve as super antioxidant effect to support human to fight against such chronic diseases[12].

On this overview, we focused our search in this direction of finding new outcome and proving facts present on *S. argel* and use of leaf extract for evaluation of antioxidant activity at it is a rich source of saponin, flavonoids and glycosides.

## II. MATERIALS AND METHODS

### A. Plant Material:

The *S. argel* plant sample was collected from remote area of north Sudan and were authenticated by Dr. C. Prabhakar Raju, Taxonomist, Department of Botany, Faculty of Science, S.S.B.N. College Anantapur, AP (India) and herbarium voucher were also deposited in the departmental herbarium by reference no. S.S.B.N.-SA/PLA/2016/1014.

### B. Chemicals:

All chemicals and solvents (Lab Grade) are purchased from Sigma Aldrich Co USA, SD Fine Chem and used as such without further purification.

## III. PHARMACOGNOSTIC SCREENING

Preparation of Crude Drug for extraction: The Plant *S. Argel* leaves were washed thrice with distilled water and then dried under shade. After proper drying they were coarsely powdered by mechanical grinder. The powder was sieved through 40 mesh sieve and stored in an air tight container for further use.

### A. Physico-chemical screening:

The finely dried and sieved power (raw drug) was subjected to determine various parameter i.e. moisture, pH, total ash, water soluble ash, acid sulphated ash, sulphated

ash, solubility (in water and alcohol), crude fiber content, foaming index etc using a well-documented procedure[13-15]. The results were tabulated in table -1. Extractive value determination in either solvent (water / alcohol) was done by method mentioned in literature[13-15]. The percentage of soluble extractive value was also calculated and mention in table -1.

Determination of extractive Value: 50 gm of coarsely powdered and sieved air dried leaves were macerated with

100 ml of solvent (ethanol and water) in a closed flask for 24 hour, rapidly vibrating customarily for six hours and permitted to stand for eighteen hours. It was then sieved swiftly taking precaution against loss of alcohol and the strain was volatilized to dryness in tarred flat bottomed superficial dish, dried at 105° C and weighed.

The percentage of alcohol soluble extractive was measured with reference to the air dried drug[15].

S.N	Parameters		Percentage (%w/w)
1	pH		7.5±0.5
2	Total ash		5.8±0.3
3	Acid insoluble ash		4.23±0.4
4	Water soluble ash		4.55±0.5
5	Sulphated ash		5.34±0.3
6	Solubility	Water soluble extractive	18.5±0.4
		Alcohol soluble extractive	9.6±0.4
7	Crude fiber content		4.56±0.2
8	Loss on drying		7.83±0.6
9	Foaming index		Less than 100

Table 1:- Determination of ash values of selected medicinal plants

#### B. Qualitative phyto-chemical evolution:

Phyto-chemical screening was carried out for identification of active ingredients present in various extracts. The extraction was carried out using literature method[12]. In this method the collected, cleaned and pulverized leaves of *Solenostemma argel* were used for the extraction purpose. 500gm of powdered leaves were evenly loaded in the soxhlet apparatus. It was then extracted with solvents with varying polarity order from non-polar to polar constantly. The extraction method used was incessant hot

percolation and carried out with various solvents, for 72 hrs. The extracts were concentrated by vacuum distillation to till complete dryness. Then they were cooled and placed in a desiccator for complete dryness. The dried extracts were packed in airtight containers and used for further studies. Then these fractions were evaluated for presence of different components by applying qualitative primary test methods followed by confirmatory tests for their particular fictional group. The results are tabulated in table-2.

S. No	Secondary metabolites	n-Hexane	Ethyl acetate	Ethanol	Aqueous
1	Sterols	+	-	+	+
2	Fixed oils and fats	+	+	++	-
3	Protein and amino acids	-	-	+	+
4	Tri terpenoids and saponins	+	-	++	-
5	Alkaloids	+	+	++	+
6	Carbohydrates	-	+	+	-
7	Flavonoids	+	+	++	+
8	Tannins	+	+	+	+
9	Glycosides	+	-	+	+
10	Phenolic comp and Polyphenols	+	+	++	+

+ : present; ++ : present at high level; - : absent

Table 2:- Phytochemical studies

The fluorescence analysis: the fluorescence analysis of the powdered raw drug was carried out under visible white and UV light (short  $\lambda$ - 254 nm and long  $\lambda$ - 365nm) in different solvent system which were used for extraction according to increasing polarity using well reported

method in literature[16-17]. In this procedure 0.5 gm of crude powder was mixed with 5 mL different solvent systems (acids or bases) and left for 12 Hr. then it was filtered and filtrate was examined for fluorescence analysis. The results are presented in table -3.

S. No	Reagents	Day light	Short UV	LongUV(365nm)
1	Powdered Leaf	Light green	Dark green	green
2	Powder + 1 N HCl	Green	Light green	Light green
3	Powder + 1 N NaOH	Light green	Red	Light green
4	Powder + 50% HCl	Light green	Fluorescent green	Fluorescent green
5	Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Light green	Dark green	Dark green
6	Powder +50% HNO <sub>3</sub>	Dark brown	Brown	Reddish brown
7	Powder + Methanol	Light green	green	Light green
8	Powder + Methanol + 1N NaOH	Light green	brown	Light green

Table 3:- Fluorescence analysis of powder

S. No	Extracts	Day light	UV light	
			Short (254nm)	Long (365 nm)
1	n-Hexane	Light green	green	Dark green
2	Chloroform	Reddish brown	Brown	Dark green
3	Ethyl acetate	Yellowish green	Light green	Light green
4	Ethanol	Brownish green	Orangish green	Light green

Table 4:- Fluorescence analysis of various extracts

#### IV. PHARMACOLOGICAL STUDY

##### A. In-Vitro Efficacy Evaluation:

Dried leaf's ethanolic extract was dissolved in the respective solvents at the stock of concentration of 1 mg/ml. The suitable preparation of the stock solutions were made and used for the *in vitro* antioxidant assays.

Diphenylpicrylhydrazyl radical assay method (DPPH method) : The protocol used for this assay is well reported by Brand-Williams W.[18] We used this method with some modifications as per requirement of protocol. In which 50 µL of leaf extract was taken in an universal bottle with varying concentration from 50 ppm to 400 ppm and 1 mL (W/V) (8 mg / 100 mL of ethanol) DPPH solution was added to sample and blank. It was kept at room temperature for 30 min. for incubation. During this time the DPPH radical is scavenged, the color of the reaction mixture changed to yellow from purple indicated the decrease in absorbance at wavelength 517 nm. So after incubation period the absorbance was taken at 517 nm by UV-1800 spectrophotometer, Shimadzu, Japan. Scavenging capacity was figuring by tracking the decrease in absorbance at 517 nm. The percentage inhibition was calculated against blank. The results were tabulated in table -4.

Percentage Inhibition was measured by using formula; Inhibition (%)

$$= \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

##### B. Nitric oxide radical scavenging activity:

In this method sodium nitroprusside (SNP) solution is used which generate nitric oxide spontaneously at physiological pH and when this oxide interacts with oxygen produces nitrite ions, which are quantified and determined according to Griess reaction[19]. The reaction mixture (final volume-3 ml) of various concentration of drug containing sodium nitroprusside (10 mM) in 0.5 M phosphate-buffer (pH- 7.4) saline were incubated at 25° C for 150 min. then 0.5 mL Griess reagent was added. The absorbance of purple chromophore formed by the diazotization of nitrite ion with sulfanilamide and subsequent coupling with α-naphthyl-ethylenediamine were measured by spectrophotometrically at 546 nm. Ascorbic acid was used as control sample. Percent inhibition was calculated by comparing the absorbance values of test samples as per the formula:

$$\text{Inhibition}(\%) = \frac{(\text{Control} - \text{Test})}{\text{Control}} \times 10$$

Extracts	Conc. Of extracts in ppm	% of DPPH free radical activity	% of Nitric oxide radical activity
n-Hexane	50	45%	39%
	100	58%	48%
	150	60%	52%
	200	65%	57%
	400	68%	63%
Ethyl acetate	50	55%	47%
	100	58%	52%
	150	65%	59%
	200	70%	63%
	400	75%	68%
ethanol	50	65%	60%
	100	68%	63%
	150	70%	68%
	200	75%	71%
	400	82%	79%
Aqueous	50	55%	52%
	100	58%	57%
	150	65%	61%
	200	68%	69%
	400	70%	72%
Ascorbic acid Standard	50	-	68%
	100	-	73%
	150	-	77%
	200	-	83%
	400	-	89%

Table 5:- Antioxidant activity of bark solvent extracts based on their polarity

## V. RESULT AND DISCUSSION

In this present study, relatively all physical, chemical and biological parameters were studied to find a concentrated conclusion. All the results mentioned in different tables showed specific results which are briefed below.

Physico chemical screening: this analysis of the powdered leaves of *S. argel* showed that the moisture content was 7.8% and total solid was 92.20%, total ash was 5.8%, in which water soluble ash was 4.5% and acid insoluble ash was 4.2%, and sulphated ash was 5.3%. The extractive value of crude drug was as water soluble 18.5 % and alcohol soluble was 9.6 %. The crude fiber content was 4.5 %. Its foaming index was <100 recorded.

Qualitative screening of extracts revealed the presence of alkaloids and carbohydrates in all extracts. Spot test was found to be positive revealing the presence of fixed oils. Phenolic compounds and Tannins were found to be present in all extracts. Tests for proteins were also found to be positive indicating the presence of proteins and amino acids. Tests for Alkaloids were found to be positive in

extracts of *Solenostemma argel* whereas tests for carbohydrates and glycosides were found to be positive for all other three extracts. Tests for tannins and phenolic compounds were found to be positive for all extracts.

The screening of *Solenostemma argel* extracts of different solvents was performed to test for the presence of steroids, triterpenes, saponins, alkaloids, carbohydrates, flavonoids, tannins, glycosides and results were tabulated.

The fluorescence results were very specific with raw drug as well as with different extracts as mentioned in table – 3 & 4. The results showed very specific color change pattern with strong dark colors after treatment with various reagents or solvent extractions. It may be due to characteristic salts or complexes formed with alkaloids or flavonoids present in that particular fraction. This technique may be used in the identification of authentic samples and recognizing adulterants.

### A. Evaluation of In Vitro Efficacy:

#### ➤ DPPH Radical Scavenging Activity of Extracts:

Different fractions of plant extract for free radicals of 1, 1-diphenyl 1-2-picryl-hydrazyl (DPPH) showed remarkable scavenging activities in Table 5. Ethanolic extract showed the highest scavenging activity followed by Ethyl acetate. DPPH scavenging activity was significantly correlated with phenolic compounds and flavonoids present in different extracts.

#### ➤ Nitric oxide radical scavenging activity:

As results presented in table-5 the different extracts caused a low dose dependent inhibition of nitric oxide. The best results were showed by ethanolic extract with followed by ethyl acetate extract. It may be due to the solvents polarity which resulted in extraction of majority of polyphenolic compounds or flavonoids and terpenoids or active polar entity. The results of both of solvent extractions were quite comparative with slandered dose of ascorbic acid.

## VI. CONCLUSION

Phytochemicals present in the different extracts of Leaves of *Solenostemma argel* was identified. Among the extracts ethanolic extract has highest anti-oxidant property when compared to other extracts. In the present study it was found that *Solenostemma argel* leaves ethanolic extract has an excellent antioxidant activity. Therefore the future studies should be aimed to exploit this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria. However further investigations are needed for isolation and purification of key ingredients of this plant for prominent use of it as drug candidate for human being.

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