Section A-Research paper

PHYTO-CHEMICAL POTENTIAL OF *SIDA CARDIFOLIA* LEAVES FOR ANTINOCICEPTIVE, ANTI-INFLAMMATORY AND ANTIOXIDANT ACTIVITY

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Abstract

Background: To investigate the phytochemical potential of Anti-inflammatory, Antinociceptive, and Antioxidant activity of different leaves extracts of *sida cordifolia*. **Materials and Methods:** The air-dried powder materials of the *sida cordifolia* were allowed for successive extraction with the help of soxhlet apparatus by using petroleum. ether, chloroform, acetone, ethanol & water as a solvent. Anti-inflammatory activity was performed by using carrageenin-induced edema in the rat paw method. Antinociceptive activity was performed by using Mouse Writhing test and Hot Plate test. Antioxidant activity was determined through the ability of hydrogen peroxide scavenging. **Result:** The ethanolic extract (200 mg/kg) oral showed maximum anti-inflammatory activity 51.10 (maximum, % inhibition) after 2hr. The ethanolic extract (200 mg/kg) oral showed maximum % inhibition of writhing 54.94 for Writhing test and 2.00±0.02 time (sec) of jumping for Hot Plat test. The ethanolic extract of the drug showed high scavenging (57.30%) of hydrogen peroxide. **Conclusion:** On successive extraction process of aerial parts of *sida cordifolia* reported that different ethanolic extracts are more effective as Anti-inflammatory, Antinociceptive, and Antioxidant activity respectively.

Keywords: *Sida cordifolia,* Anti-inflammatory, Antinociceptive, Antioxidant.

INTRODUCTION:

Sida cordifolia is an annual or perennial plant (family- Malvaceae) native to tropical and subtropical countries[1]. Common names are countary mallow or heart-leaf (English), huang hua mu (china), bala, (Hindi), atibala (Sanskrit), chittamadi (Srilanka), petoria bassie (Africa)[2]. This plant contains chemical constituents like Alkaloids, ascorbic acid, beta-carotene, beta-phenethylamine, calcium, carbohydrates, ephedrine, gums, ephedrine, indole alkaloids, saponin, mucilage, phenolic compounds, protein, pseudoephedrine, saponin, steroids, tannins, triterpenoids, vascin, vasicine, flavonoids.

The traditional uses of *Sida cordifolia* are reported like fever, ulcers, boils, urinary diseases, asthma[3], toothaches, relive constipation, applied in the vagina as an antiseptic, and treatment of gout[4]. The pharmacological uses of *Sida cordifolia* show hepatoprotctive, cytotoxic, antibacterial, antifungal, anti-arthritic, anti-gout, anthelmintic, and hypoglycemic activities[5].

Fig.1: Flower of *Sida cordifolia*

MATERIAL AND METHODS

Collection, authentication of plants material

The leaf part of *Sida cordifolia* was obtained from F.R.I. Dehradun, UK, India. Plant material was authenticated by Dr. Shiddamallayya N (senior scientist) at N.A.D.R.I (Ay), Bangalore, India. Drug Authentication/SMPU/NADRI/BAG/2011-12/540.

Extraction

The air-dried powder materials of the *Sida cordifolia* were allowed for successive extraction with the help of soxhlet apparatus by using pet. ether, chloroform, acetone, ethanol & water as a solvent.[6,7,8]

Anti-inflammatory activity

Anti-inflammatory activity was performed by using carrageenin-induced edema in the rat paw method. After 16 hr. fasting, 42 rates equally divided into seven groups. Group first received 0.5% carboxy methyl cellulose at a dose of 1 ml/100 gm, and served as a control group. Group second to six, animals received a suspension of 0.5% w/v CMC of pet ether, chloroform, acetone, ethanol, and water extract respectively, with a 200 mg/kg dose orally. Group seven received a standard drug (indomethacin) with a dose of 10 mg/kg orally. After one hour of administration, 0.1 ml (l% w/v carrageenin in normal saline) was injected into the right hind paw to induced edema in animals. Plethymometer was used to measurement of the paw volume of animals.[4,9,10,11]

Detail Study Plan: For Anti-inflammatory activity

The study was conducted consisting of 7 groups each containing six animals.

1st Group – 0.5 w/v CMC, at a dose of 1ml/100 gm.

2nd To 6th Group – Suspension of 0.5% w/v CMC of pet ether, chloroform, acetone, ethanol, and water extract respectively, with 200 mg/kg dose, orally.

7th Group **–** received standard drug (indomethacin), with dose (10 mg/kg), orally.[12, 13,14,15,16]

Antinociceptive activity

Hot Plate test

The animals were placed on a hot plate for a maximum time of 30 sec., at a temperature of 55^0C. Reaction time was measured on time i.e 30, 45, 60, and 90 min. by animal licking paws and jumping responses after I.P administration of 200 mg/kg of different extracts to different seven groups. Reference drug morphine was used with a dose of 10 mg/kg.

Detail Study Plan: Hot Plate test

The study was conducted consisting contain seven groups each containing eight animals.

1st Group- Dis. water (10 ml/kg), treated as control.

2nd to 6th Group- Administration of 200 mg/kg each group Pet. ether, chloroform, acetone, ethanol, and water extract respectively.

7th Group- Reference drug Morphine was used at a dose of 10 mg/kg.[14, 15, 16]

Mouse writhing test

The rates were divided into seven groups, every group containing eight animal doses (100 mg/kg) of *Sida cordifolia* were administered 1 to 5 groups, while groups 6 and 7 administered a 10 ml/kg dose of distilled water and 5 mg/kg dose of Indomethacin respectively. After 30 min., 0.6% acetic acid solution in normal saline was injected with I.P (10 ml/kg). After acetic acid injection, the writhe numbers were counted for 15 minutes.

(N - Nt/N)100

Where

Nt= Average number of writhes (test group).

N= Average number of writhes (control group).[17, 18, 19]

Detail Study Plan: Mouse writhing test

The study was conducted consisting contain seven groups, each containing eight animals.

1st To 5th Group- Administration of 200 mg/kg each group, Pet. ether, chloroform, acetone, ethanol, and water extract, respectively.
6th and 7th Group- Received dis. water (10 ml/kg) and Indomethacin (5 mg/kg) respectively.
. (Ahmed *et al*., 2001) [20, 21, 27-43]

Antioxidant activity

Scavenging of Hydrogen Peroxide

The different extracts of *Sida cordifolia* were used to determine antioxidant activity on the behalf of the ability of hydrogen peroxide scavenging. The different extracts, standard and ascorbic acid were prepared in phosphate buffers (pH 7.4). 0.5ml standard was taken in the different test tubes and each test tube contain a different extract sample with 0.6 ml hydrogen peroxide solution (2mM hydrogen peroxide in phosphate buffer, pH 7.4). Phosphate buffers were taken in the control group test tube. These solutions were standing for 10 minutes at room temperature. The absorbance of the blank solution was measured by ultraviolet-visible spectroscopy at 230 nm. The % inhibition was calculated by:

$$\frac{\text{Absorbance}_{(\text{Control})} - \text{Absorbance}_{(\text{Sample})}}{\text{Absorbance}_{(\text{Control})}} \times 100$$

Where

Sample - Standard and extract solution.

Control – Hydrogen peroxide in phosphate buffer. [8]

The result of antioxidant activity was expressed as IC_{50}. (Narendhirakannan *et al.*, 2010) [11,24,25,26]

RESULT AND DISCUSSION

Anti-inflammatory activity of extracts

Animal activities were conducted as per CPCSEA guideline.

Table No.1: Anti-inflammatory activity of *Sida cordifolia* by Carrageenan - induced hind paw edema.

Sr. No.	Groups	Dose (mg/kg)	Mean Differences in paw volume (ml) ± S.E.M			
			1 hour	2 hour	3 hour	4 hour
1.	Vehicle (control)		1.59±0.02	1.71±0.03	1.25±0.01	1.16±0.87
2.	Pet. Ether	200	1.25±0.03	1.21±0.02	1.15±0.02	1.03±0.04
3.	Chloroform	200	1.33±0.04	1.31±0.04	1.23±0.03	1.18±0.03
4.	Acetone	200	1.31±0.04	1.28±0.04	1.11±0.02	1.11±0.03
5.	Ethanol	200	1.21±0.03	1.16±0.03	1.05±0.02	1.01±0.02
6.	Water	200	1.35±0.03	1.28±0.02	1.15±0.02	1.10±0.16

7.	Standard (Indomethacin)	10	1.10±0.12	0.80±0.08	0.60±0.05	0.56±0.04

The volume of hind paw oedema was expressed as mean ± S.E.M, *Data differs significantly ($P \leq 0.01$) when compared against treated group (normal saline). Data differs significantly ($P \leq 0.05$) when compared Indomethacine treated group.

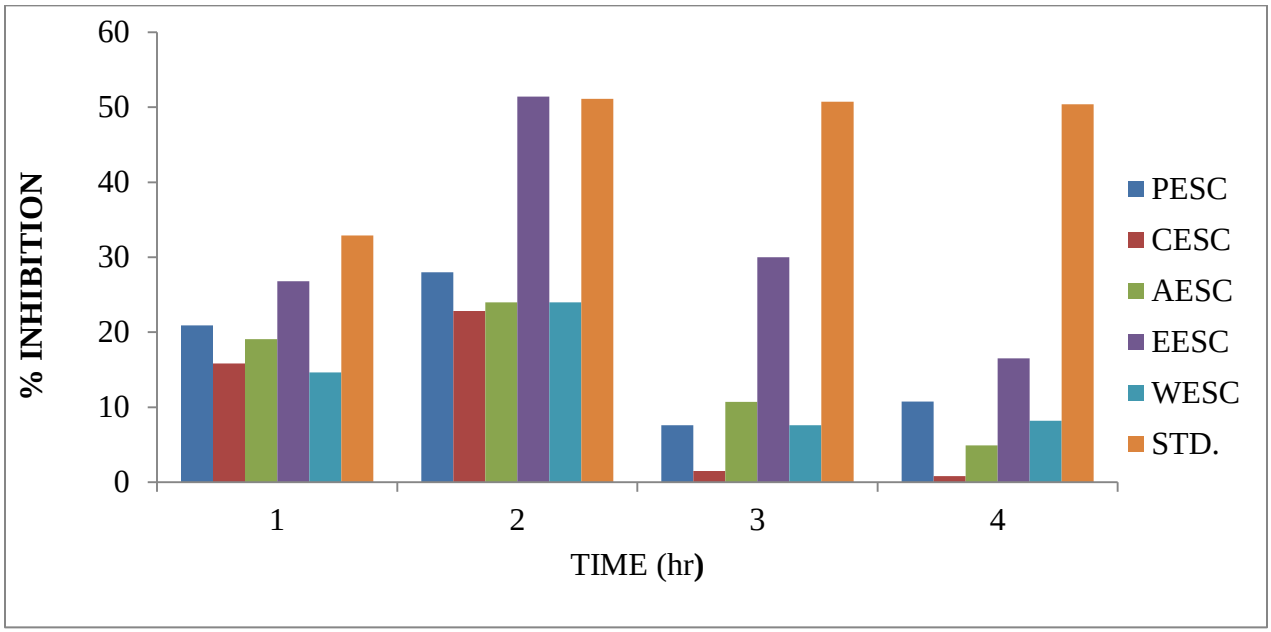

Fig.2: Graphical representation of % inhibition.

Anti-inflammatory activity was performed by pet. ether, chloroform, acetone, ethanol and water extracts. The ethanolic extract (200 mg/kg. oral) showed maximum % inhibition 51.10 after 2 hr while pet.ether, chloroform, acetone, and water extracts showed maximum % inhibition 27, 21.85, 23, and 23.10 respectively but standard drug Indomethacine (10 mg/kg) showed 51.15. From the above result we can conclude that ethanolic extract is more comparable to standard drug so ethanolic extract is more effectiveness as Anti-inflammatory.

Antinociceptive activity of extracts

Writhing test

Table No. 2: Effect of different extracts of *Sida cordifolia* by writhing tests.

Animal Group	Dose (mg/kg)	Writhes Response	% Inhibition
Control		36.40±0.86	36.4
Pet. Ether extract	200	22.15±0.59	39.14
Chloroform extract	200	21.20±0.53	41.75
Acetone extract.	200	19.52±0.32	46.37
Ethanol extract.	200	16.40±0.42	54.94
Water	200	25.30±0.56	30.49
Standard	5	14.35±6.30	60.57

The writhing response was expressed as mean ± S.E.M, *Data differs significantly ($P \leq 0.01$) when compared against the normal saline. Data differs significantly ($P \leq 0.001$) when compared with Indomethacine treated group

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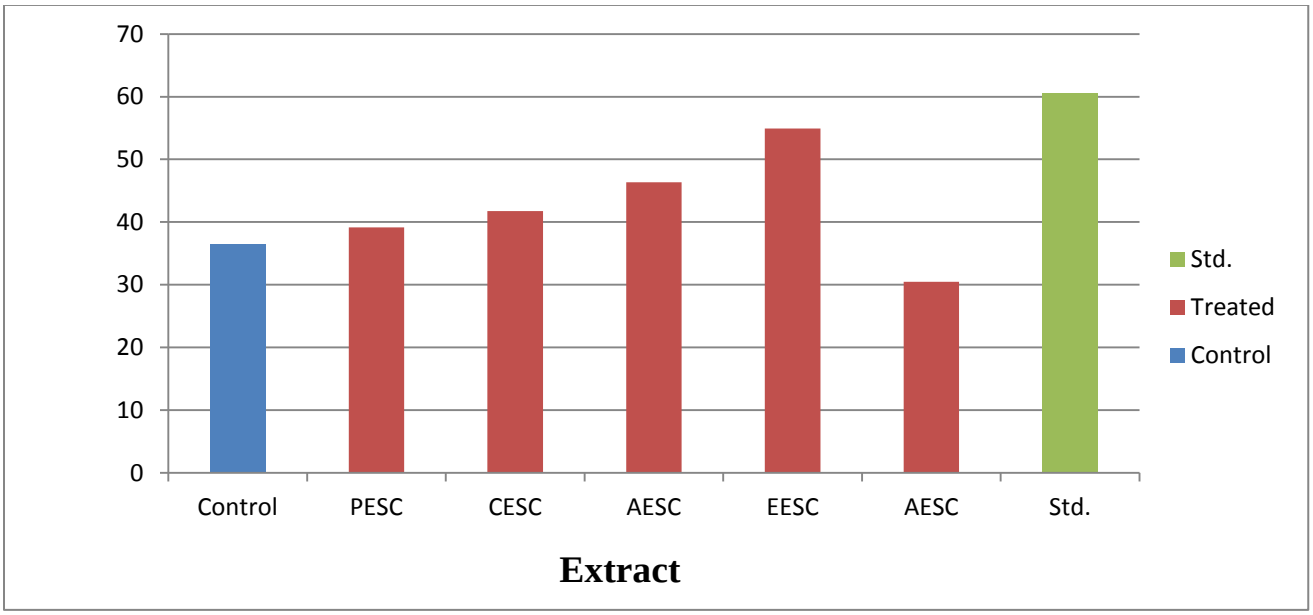

Fig. 3: Graphical representation of % inhibition in Mouse Writhing test.

Antinociceptive activity was performed by using different extracts i.e. pet. ether, chloroform, acetone, ethanol, and water extracts. The ethanolic (200 mg/kg. oral) showed maximum % inhibition of writhing 54.94 after 15 min. while pet. ether, chloroform, acetone, and water extracts showed 39.14, 41.75, 46.37, and 30.49 respectively in the Writhing test but the standard drug Indomethacin (5mg/kg) showed 60.57, from the above result we can conclude that ethanolic and acetonic extracts are more comparable to the standard drug so they have more effectiveness as Antinociceptive activity.

Hot Plate test

Table No. 3: Effect of different extract of *Sida cordifolia* by using hot plate.

Animal Group	Dose (mg/kg)	Time (sec.)			
		0	30	45	60
Control		3.25±0.06	3.1±0.10	2.80±0.03	2.4±0.06
Pet. Ether	200	2.0±0.21	3.25±0.26	2.0±0.20	1.10±0.09
Chloroform	200	3.50±0.14	4.61±0.03	4.24±0.08	3.60±0.04
Acetone	200	3.12±0.11	4.13±0.04	3.90±0.02	3.27±0.01
Ethanol	200	2.10±0.6	4.0±0.03	2.30±0.07	2.00±0.02
Water	200	2.50±0.05	3.68±0.03	1.9±0.06	1.10±0.09
Standard	10	2.10±0.20	5.15±0.08	2.06±0.07	2.20±0.80

The frequency of rat paw licking, jumping or shaking off from the surface was expressed as mean ± S.E.M, *Data differs significantly ($P \leq 0.01$) when compared against the normal saline with treated group. Data differs significantly ($P \leq 0.05$) when compared with Morphine treated group.

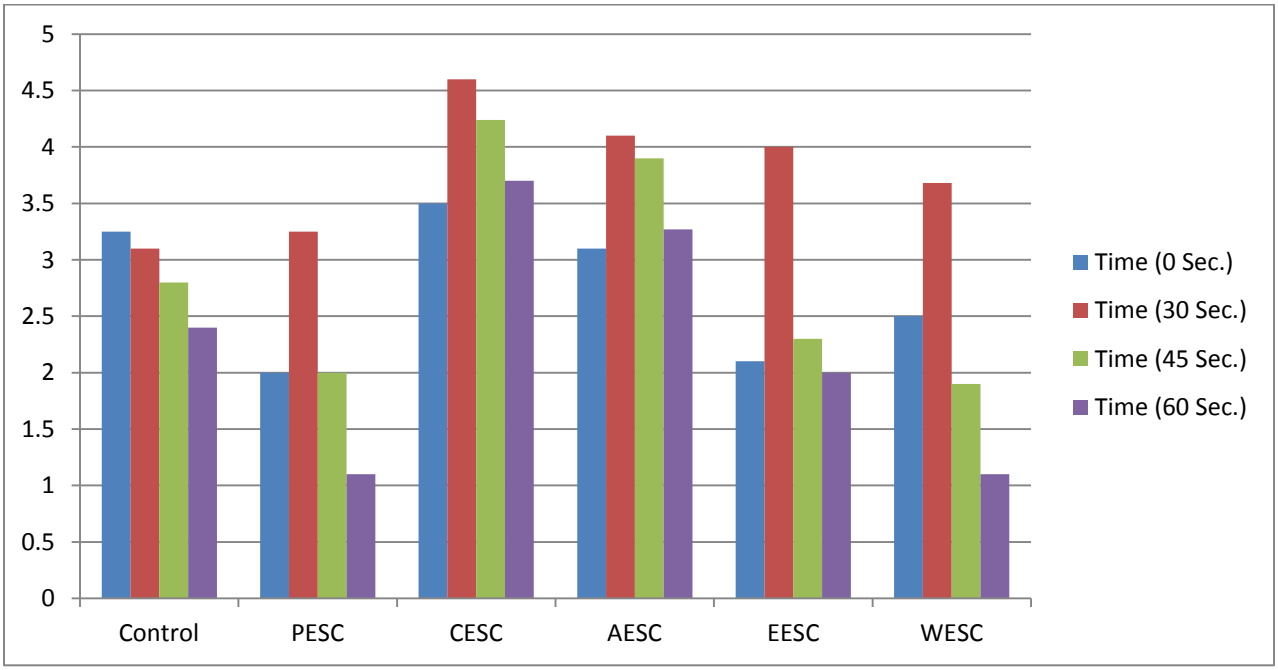

Fig. 4: Graphical representation of frequency of rat paw licking, jumping or shaking off from the surface of hot plate.

Antinociceptive activity was performed by per. ether, chloroform, acetone, ethanol and water extracts with respect to standard drug. The ethanolic extract (200 mg/kg. oral) and acetone extract showed 2.00±0.02 and 3.27±0.01 time (sec) for jumping after 60 min. while pet. ether, chloroform, water extracts showed 1.10±0.09, 3.60±0.04, 1.10±0.09 respectively in Hot plate test but standard drug Morphine (10 mg/kg) showed 2.20±0.80 sec, from the above result we can conclude that ethanolic and acetonic extracts are more comparable to standard drug so they have more effectiveness as Antinociceptive activity

Antioxidant activity of extracts

Table No. 4: Antioxidant activity of *Sida cordifolia* using Hydrogen peroxide-scavenging model

Conc. (µg/ml)	% Antioxidant activity					
	Ascorbic acid	*Sida cordifolia*				
		Pet. ether	Chloroform	Acetone	Ethanol extract	Aqueous extract
10	46.88	24.10	27.30	31.64	41.93	11.49
20	47.60	26.95	32.75	33.59	46.38	15.18

40	49.80	35.47	36.58	36.68	48.49	19.84
60	51.72	37.80	38.20	39.70	50.41	24.87
80	54.60	41.72	40	41.82	54.60	26.75
100	61.85	43.85	40.20	46.62	57.30	31.90

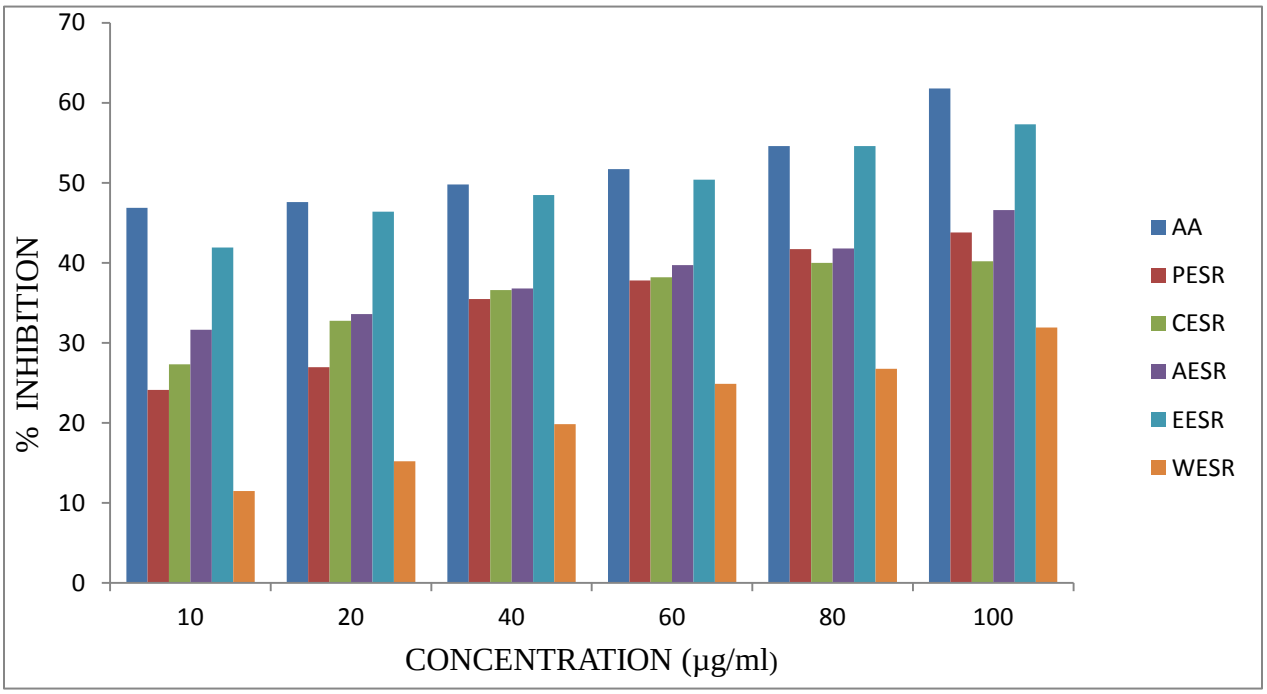

Fig. 4: Graphical representation of Antioxidant activity Hydrogen peroxide-scavenging model.

Antioxidant activity was performed by using different extracts i.e. pet ether, chloroform, acetone, ethanol, and aqueous *Sida cordifolia* with the help of the Hydrogen peroxide-scavenging model. Ethanolic extract of the drug showed high scavenging (57.30%) of hydrogen peroxide. The aqueous extract showed the least scavenging (31`.90%) while the standard drug (Ascorbic acid) showed 61.85. After comparison, ethanolic extract showed the comparative result with the standard drug (Ascorbic acid). From the above result, it was observed that ethanolic extract of *Sida cordifolia* showed maximum scavenging activity.

CONCLUSION

In anti-inflammatory activity, the ethanolic extract (200 mg/kg, orally) showed maximum % inhibition of 51.10 after 2 hrs while petroleum ether, chloroform, acetone, and water extracts showed maximum % inhibition of 27, 21.85, 23, and 23.10 respectively, whereas Indomethacin (10 mg/kg) showed 51.15%. From the above result, we can conclude that ethanolic extract is more effective when compared to standard drugs.

In antinociceptive activity, the ethanolic (200 mg/kg, orally) showed maximum % inhibition of writhing 54.94 after 15 min while petroleum ether, chloroform, acetone and water extracts showed 39.14, 41.75, 46.37 respectively but standard drug Indomethacin (5mg/kg) showed 60.57 in the Writhing test while in Hot plate test the ethanolic and acetone extract (200 mg/kg, orally) showed

more comparable result with standard drug Morphine (10 mg/kg), from the above result we can conclude that ethanolic and acetonic extracts are more comparable to the standard drug so they have more effectiveness as antinociceptive activity.

In antioxidant activity, the free radical scavenging activity was done by the H_2O_2 scavenging model. It was observe that the ethanolic extract (200 mg/kg) of *Sida cordifolia* showed the maximum scavenging (57.30%) of hydrogen peroxide and aqueous extract show the least scavenging (31.90%) while the standard Ascorbic acid showed scavenging (61.85%).

After comparison with a standard, ethanolic extract of *Sida cordifolia* showed the comparative result so ethanolic extract is more effective as antioxidant activity.

Conclusively, it revealed from the present study that *Sida cordifolia* leaves has anti-inflammatory, antinociceptive & antioxidant activities that may be due to the presence of alkaloids, flavonoids and phenolic constituents respectively present in the drug.

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