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Research Article

Pharmacognostical, Phytochemical and Pharmacological Evaluation of selected Indian Medicinal Plant Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth) Benth and Capparis brevispina DC

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ABSTRACT

Indian healers employ the country's abundant natural resources and medicinal plants to cure a wide range of illnesses. Traditional herbal remedies are widely used worldwide and have been continuously studied to understand and take use of these cures' active ingredient. Tribal traditional practitioners have discovered that Argyreia cymosa (Roxb.) The phytochemical analysis of the medicinally significant liana Argyreia cymosa (Roxb.) Sweet is the focus of the current work. Its ethnomedical significance is confirmed by the phytochemical investigation, which revealed a notable concentration of alkaloids, flavonoids, phenolics, and other bioactive components. Cymosa argyreia (Roxb.) Sweet is a member of the Convolvulaceae family. The tropical Asian woody climber genus Argyreia grows to Australia is known for its striking purple blooms and silvery foliage. In traditional medicine, argyreia cymosa (Roxb.) sweet is employed. Its leaf extract is used to treat corneal opacity in sheep and goats' eyes and the paste made from its leaves is applied to wounds and cracks. A significant medicinal plant in India, Capparis brevispina DC is a member of the Capparaceae family. The plant, also called Indian caper, is a shrub with coriaceous leaves, thorny stipules, and white flowers. The berries are ellipsoid to ovoid in form. India uses the plant as a traditional medicine since it was discovered to have therapeutic qualities.

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KEYWORDS: Phytochemical, flavonoids, therapeutic, wounds, bioactive components.

1. INTRODUCTION

Organoleptic examination is the practice of examining herbal medications using the sense organs. Color, smell, taste, size, form, and distinctive qualities like touch and texture are among the analytical methods that are referenced. The extract or plants tend to identify itself as it has such a unique look at first. If this isn't enough, the plant or extraction could also smell or taste different. The organoleptic technique of analysis is the most fundamental but humane. Shah (2009). The current study sought to assess pharmacognostical characteristics by conducting macro, micro, and powder microscopy of a few Indian medicinal plants, including Argyreia cymosa, Limnophila repens, and Capparis, because there is little data on the comprehensive macroscopic and microscopic evaluation of chosen whole plants.

2. Materials and Method

2.1 Plant material

In this experiment, the dried leaf, stem, and root parts of a few chosen medicinal plants Argyreia cymosa (Roxb.) Sweet, Limnophila repens (Benth.) Benth, and Capparis brevispina DC were assessed separately.

2.2 Determination of the total ash

The powdered substance was burnt in a silicon crucible after being precisely weighed at 2.0 gm. at temperatures that don't exceed 450°C till carbon is eliminated. The ash was weighed once it had cooled. To get a consistent weight, the procedure was repeated. At last, it the proportion of the medication that was air-dried was determined. The powdered material of individual leaves, stems, and roots of particular Indian medicinal plants underwent the same process (Gautam et al., 2010).

2.3 Determination of the acid-insoluble ash

After collecting the ash as previously mentioned, it was boiled for five minutes in 25 milliliters of 2 M hydrochloric acid. The next step is to collect and filter insoluble materials using ashless filter paper. Following 15 minutes of lighting at a temperature of no more than 450 °C, it was weighed after being cleaned with hot water. The proportion of acid-insoluble

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ash in the air-dried medicine was determined. The leaves, stems, and roots of specific Indian medicinal plants were ground into powder using the same method. (Patel and others, 2006).

2.4 Determination of the water-soluble ash

The ash was made using the foregoing method, heated with 25 milliliters of 2 M hydrochloric acid for five minutes, filtered, and the insoluble material was gathered on ashless filter paper. After that, it was cleaned with hot water and heated for fifteen minutes at a temperature that didn't surpass at 450°C, it was weighed. The amount of acid-insoluble ash in the drug that was air-dried was calculated. For individual leaf, stem, and root powdered material of particular Indian medicinal plants, the same process was used (Gautam et al., 2010).

2.5 Determination of alcohol soluble extractive value

In a closed flask, 4 g of the coarsely ground, air-dried plant powder was macerated for 24 hours with 100 cc of ethanol. For the first 6 hours, the flask was constantly shaken, and after that, it was left to stand for 18 hours. After that, it was filtered and strained. method was used, and precautions were made to avoid ethanol loss; 25 milliliters of the filtrate were weighed and evaporated on a shallow, flat plate that was dried at 105 degrees Celsius. The air-dried medication's ethanol-soluble extractive value was computed as a percentage. For powdered leaf, stem, and root material from certain Indian medicinal plants, the same method was used (Gautam et al., 2010).

2.6 Determination of water-soluble extractive value

In a sealed flask, 4 g of the air-dried coarsely powdered medication had been macerated with 100 ml of water for 24 hours. It was shook frequently for the first 6 hours and then left to stand for 18 hours. After filtering, 25 milliliters of the filtrate were evaporated on a shallow, level dish and dried at 105 °C and measured. With reference to the medication that had been air-dried, the percentage of water-soluble extractive value was computed. For each powdered leaf, stem, and root material of certain Indian medicinal plants, the same process was used (Gautam et al., 2010).

2.7 Determination of loss of moisture content

About 10 g of the drug powder (without pre-drying) was weighed and allowed to dry at 105°C in a tared evaporating dish. At 1-hour intervals, the drying and weighing processes were repeated until the discrepancy between two consecutive weightings was less than 0.25%. It took two separate weighings to show a change of no more than 0.01 g following 30

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minutes of drying and chilling in a desiccator. For powdered leaf, stem, and root material from specific Indian medicinal plants, the same procedure was used. (Sagar and others, 2007).

2.8 Fluorescence analysis powder material

Following treatment with several substances, including distilled water, methanol, 1N NaOH, 1N HCl, 50% HNO3, FeCl3, CHCl3, picric acid, and ethyl acetate, the powdered sample was examined under daylight and UV light at short wavelengths of 254 nm and long wavelengths of 365 nm. Gautam and colleagues (2010).

3. Phytochemical Evaluation

3.1 Physicochemical evaluation Argyreia cymosa leaf

Table 1 displays the results of physico-chemical evaluations of the Argyreia cymosa leaf that were conducted in accordance with accepted guidelines and limits, such as drying misfortune, debris value, and extractive worth.

Parameters	Values(%w/w)
Total ash	6.7 ± 2.25
Acid in soluble ash	3.72 ± 0.32
Water soluble ash	3.63 ± 0.43
Alcohol soluble extractive value	8.95 ± 0.13
Water soluble extractive value	11.45 ± 0.15
Moisture content (Loss on drying)	10. 8 ± 1.25

Table 1 Physicochemical parameters of leaf powder of Argyreia cymosa

3.2 Fluorescence analysis of the leaf powder of Argyreia cymosa

The various physicochemical properties of Limnophila repens leaf powder, such as loss on drying, ash value, and extractive value, were determined and displayed in Table 2.

Table 2 Fluorescence analysis of Argyreia cymosa leaf powder

Solvent used	Visible light	UV light	
		At short (254nm)	At Long(365nm)
Distilled water	Dark Green	Pale green	Red

Methanol	d Greenih Green		Red	
1N NaOH	Light brown	Green	Brownish green	
1N HCl	Green	Black	Green	
50% HNO ₃	Pale green	Black	Green	
FeCl ₃	Pale yellow	Green	Orange	
CHCl ₃	Cl ₃ Green Green		Red	
Picric acid	Yellow	Fluorescent green	Orange	
Ethyl acetate	Green	Green	Black	

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3.3 Physicochemical evaluation of leaf powder of Limnophila repens

The various physicochemical properties of Limnophila repens leaf powder, such as loss on drying, ash value, and extractive value, were determined and displayed in Table 3.

Parameters	Values (%w/w)
Total ash	5.2 ± 0.23
Acid in soluble ash	1.25 ± 0.06
Water soluble ash	2.25 ± 0.34
Alcohol soluble extractive value	10.23 ± 0.34
Water soluble extractive value	12.25 ± 1.25
Moisture content (Loss on drying)	6.56 ± 0.56

Table 3 Physicochemical parameters of leaf powder of Limnophilarepens

3.4 Fluorescence analysis of leaf powder of Limnophila repens

The leaf powder fluorescence analysis of Limnophila repens was determined and displayed in 4.

Table 4. Fluorescence analysis of *Limnophilarepens* leaf powder

Solvent used	Visible light	UV light		
		At short (254nm)	At Long (365nm)	
Distilled water	Green	Green	Black	
Methanol	Pale green	Brownish black	Yellowish green	
1 N NaOH	Brownish red	Brownish green	Brownish Black	

1N HCl	Blackish green	Dark grey	Dark green
50% HNO ₃	Crimson yellowish	Brownish black	Green
FeCl ₃	Blackish green	Brownish green	Dark green
CHCl ₃	Pale green	Brownish black	Dark grey
Picricacid	Picricacid Yellowish white		Black
Ethylacetate	Green	Buff	Greenish black

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3.5 Physicochemical evaluation of leaf powder of Capparis brevispina.

The various physicochemical parameters of stem powder of Capparis brevispina i.e., ash values, loss on drying, and extractive value were determined and displayed in table 5.

Parameters	Values %w/w
Total ash	4.28 ± 0.85
Acid insoluble ash	1.54 ± 0.66
Water soluble ash	1.32 ± 0.12
Alcohol soluble extractive value	6.32 ± 0.1
Water soluble extractive value	7.88 ± 0.55
Moisture content	6.37 ± 0.44

Table 5 Physicochemical parameters of leaf powder of Capparis brevispina.

3.6 Fluorescence analysis of leaf powder of Capparis brevispina

The fluorescence evaluation of the leaf powder of Capparis brevispina was determined and displayed in Table 6

Table 6 Fluorescence analysis of powdered leaf of Capparis brevispina

		UV light	
Solvent used	Visible light	254 nm	366 nm
Distilled water	Pale Buff	Brown	Dark Brown
Methanol	Light brown	Green Brownish gr	
1 N NaOH	Light brown	Green	Brownish green
1N HCl	Brown	Greenish brown	Pale green
50%HNO3	Brown	Green	Pale yellow
FeCl3	Buff	Bluish yellow	Bluish green
CHCl ₃	Light Buff	Dark brown	Black

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Picric acid	Yellowish brown	Green	Bark brown	
Ethyl acetate	Buff	Black	Light green	

4. Preparation of extracts

After thoroughly washing the Argyreia cymosa, Limnophila repens, and Capparis brevispina plants with water, the materials were shade-dried and ground into a powder using a pulverizer. Finely ground, homogenized Argyreia cymosa and Limnophila repens powder as well as Capparis brevispina were extracted independently. petroleum ether, chloroform, ethyl acetate, and methanol, in order of polarity. Following the loading of the air-dried powder into a Soxhlet extractor, ethylacetate and methanol were added one after the other. For future phytochemical and pharmacological studies, the excess solvents from each extract were ultimately rotary flash and hot plate evaporated and kept in desiccators.

4.1 Phytochemical evaluation

A preliminary analysis of petroleum ether extractethyl acetate extract, chloroform extract, methanolic extract, and water from powdered Argyreia cymosa, Limnophila repens, and Capparis brevispina was conducted in accordance with conventional laboratory guidelines (Tiwari et al. 2011).

Phyto-constituents	Test	Hexane	Petroleum			Methanolic
		extract	ether	extract	acetate	extract
			Extract		extract	
Flavonoids	Shinoda's Test	+	-	-	+	+
	Zn. HCL test	+	-	-	+	+
	Lead acetate	+	-	-	+	+
	Test					
Volatile oil	Stain test	-	-	-	-	-
Alkaloids	Wagner Test	-	-	+	-	+
	Hager'sTest	-	-	+	-	+
Tannins & phenols	FeCl3Test	+	-	-	+	+
	Potassium	-	-	-	-	+
	dichromate					
	test					
Saponins	Foaming Test	-	-	-	-	-
Steroids	Salkowski test	+	+	+	+	+
Sterols	Libermann's	+	-	+	+	+
	test					
	Salkowski	+	-	-	-	-
	Conc.	+				
	Sulphuricacid					

 Table 7 Phytochemical analysis of various extracts of Argyreia cymosa

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Fixed oils and fats	Spot test	-	+	-	-	-
Carbohydrates	Molish test	-	-	-	-	-
Acid compounds	Litmus test	-	-	-	-	+
Glycoside	Keller-Killani	-	-	-	-	+
	Test					
Amino acids	Ninhydrin test	-	-	-	-	+
Proteins	Biuret	-	-	-	-	+

Table 8 Phytochemical analysis of various extracts of Limnophila repens

Phyto constituents	Test	Hexane	Petroleum	Chloroform		Methanolic
		extract	ether	extract	acetate	extract
			Extract		extract	
Flavonoids	Shinoda Test	+	-	-	+	+
	Zn+HCltest	+	-	-	+	+
	Lead acetate Test	+	-	-	+	+
Volatileoil	Stain test	-	+	-	_	+
Alkaloids	Wagner Test	-	-	+	-	+
	Hager's Test	-	-	+	-	+
Tannins &	Fecl3 Test	+	-	-	+	-
Phenols	Potassium	-	-	-	+	-
	Dichromate test					
Saponins	FoamTest	-	-	-	-	+
Phytosterols	Libermann'stest	-	+	+	+	+
	Salkowski	-	-	-	-	-
	Conc. Sulphuric	-	-	-	-	-
	acid					
Carbohydrates	Molish test	-	-	-	-	+
Acid compounds	Litmus test	-	-	-	-	-
Glycoside	Bontrager's test	-	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	-	+
Proteins	Biuret test	_	-	-	-	-
Fixed oils & fats	Spot test	-	+	-	-	-

Table 9 Phytochemical analysis of various extracts of Capparis brevispina

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Phyto constituents	Test	Hexane extract	Petroleum ether Extract	Chloroform extract	Ethyl acetate extract	Methanolic extract
Flavonoids	Shinoda Test	+	-	-	+	+
	Zn.HCL test		-	-	+	+
	Lead acetate Test	+	-	-	+	+
Volatile oil	Stain test	-	+	-	-	+
Alkaloids	Wagner Test	-	-	+	-	+
	Hager's Test	-	-	+	-	+
Tannins& phenols	FeCl ₃ Test	+	-	-	+	+
	Potassium dichromate test	-	-	-	-	+
Saponins	Foaming Test	-	-	-	+	+
Phyto sterols	Libermann's test	-	+	+	+	+
	Salkowski	-	-	-	-	-
	Conc. Sulphuric acid	-	-	-	-	-
Fixed oils and fats	Spot test	-	+	-	-	-
Carbohydrates	Molish test	-	-	-	-	+
Acid compounds	Litmus test	_	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	-	+
Proteins	Biuret	-	-	-	-	+

5. Pharmacological Evaluation

Wistar strain Albino rats of either sex (200-250 g) was used for the hot plate method, Swiss albino mice used for tail immersion and the acetic acid writhing method was acquired from Ghosh Enterprises. The animals were cared for under typical lab circumstances. They were given a typical pellet diet and unlimited access to water. The Institutional Animal Ethical Committee (IAEC) examined and approved the study protocol, and experiments were carried out in accordance with CPCSEA rules.

5.1 Antinociceptive activity by using the hot plate method

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Central analgesics may be measured using the hot plate paradigm. In order to determine if MEAC, MELR, and MECB had a core analgesic effect, the hot plate test was conducted. In this trial, significant outcomes were seen at 200 and 400 mg/kg with MECB, MELR, MEAC. The MEAC significantly improved (p<0.05) at 200 and 400 mg/kg, going from an 8.9 ± 0.45 latency time to 12.21 ± 0.96 , and from 8.6 ± 0.96 to 14 ± 0.33 after 90 minutes while Tramadol (2 mg/kg, p.o.) rose dramatically from a latency period of 8.2 ± 0.25 to 16.3 ± 0.74 at 30 minutes. At 200 and 400 mg/kg, the MELR significantly (p < 0.01) rose from 9.62 ± 0.22 to 12.95 ± 0.62 and 9.49 ± 0.22 , respectively. After 120 minutes, 14.95 ± 0.85 . Table 9.1 shows a small increase in the MECB delay at 200 and 400 mg/kg (p < 0.01) from 8.5 ± 0.33 to 14.28 ± 0.12 and 8.7 ± 0.97 to 15.55 ± 0.68 at 90 minutes. Thus, it is possible to believe that MEAC, MELR, and MECB have an impact on the central nervous system.

Treatment	Reaction Time(s)						
	Time after Treatment (min)						
	0	30	60	90	120		
Control	8.25 ±0.15	8.33 ±0.03	8.30 ±0.04	8.4 ±0.08	8.2 ±0.04		
Tramadol (2mg/kg)	8.2 ±0.25	$16.3 \pm 0.74*$	14.1 ±0.2*	$11.4 \pm 0.680*$	9.6 ±0.526*		
MEAC(200mg/Kg)	8.9 ±0.45	8.3 ±0.20a	9.8 ±0.241a	12.21±0.96a	9.52 ±1.237a		
MEAC(400mg/Kg)	8.6 ±0.96	9.2 ±0.78b	$10.3 \pm 0.26b$	14±0.33b	12.22±0.53b		
MELR (200mg/kg)	9.62 ±0.22	9.46 ±0.1#	10.95±0.58#	12.95±0.62#	10.09±0.56#		
MELR (400mg/kg)	9.49 ±0.22	10.72±0.38	12.15±0.31	14.95±0.85	10.64±0.54		
MECB (200mg/Kg)	8.5 ±0.33	9.2 ±0.25a	10.8 ±0.84 a	14.28±0.12a	9.56±0.38a		
MECB (400mg/Kg)	8.7 ±0.97	9.8 ±0.78	11.52±0.91	15.55±0.68	12.22±0.22		

 Table 10 Effect of MEAC, MELR and MECB by using the hot plate method



Figure 1 Effect of MEAC, MELR and MECB on hot the plate method

5.2 Antinociceptive activity by using the tail immersion method

At 200 and 400 mg/kg, MEAC, MELR, and MECB all exhibit substantially longer response times during the tail immersion investigation as compared to control animals. The latency

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time rose considerably (p < 0.05) from 3.8 ± 0.21 to 400 mg/kg of MEAC. At 90 minutes, they were $4.3.21 \pm 0.13$ and 3.8 ± 0.96 to 7.3 ± 0.53 , respectively. The latency period rose considerably (p < 0.05) from 2.03 ± 0.07 to $5.4.39 \pm 0.16$ and 2.44 ± 0.11 to 6.4 ± 0.18 at 120 minutes for the MELR with 200 and 400 mg/kg, respectively. The latency period rose considerably (p < 0.01) from 3.6 ± 0.12 to 9.21 ± 0.33 and from 3.9 ± 0.15 to 12.21 ± 0.44 at 90 minutes for the MECB at 200 and 400 mg/kg, respectively. However, as seen in the data, tramadol (2 mg/kg) markedly extended the latency period from 3.6 ± 0.23 to 14.08 ± 0.17 at 120 minutes, respectively.

Table 10 Effect of MEAC, MELR and MECB by using tail withdrawal reflexes induced					
by tail immersion method in rats.					

Treatment		Reaction Time(s)						
	Time after Treatment (min)							
	0	30	60	90	120			
Control	3.6 ±0.12	3.7 ±0.20	3.9 ±0.17	3.6 ±0.13	3.4 ±0.22			
Tramadol (2mg/kg)	3.4 ±0.23	16±0.63*	$11.4 \pm 1.55*$	6.7 ±1.45*	14.08±0.17*			
MEAC(200mg/Kg)	3.8 ±0.21	3.6 ±0.11*	4.5 ±0.27*	7.3 ±0.53*	5.3 ±0.23*			
MEAC(400mg/Kg)	3.7 ±0.51	4.5 ±0.44 ^a	6.3 ±0.74 ^a	8.2 ± 0.44^{a}	6.6 ±0.43 ^a			
MELR(200 mg/kg)	2.03 ±0.07	2.8 ±0.15	3.6 ±0.22	4.46 ±0.22	5.4 ±0.16			
MELR(400 mg/kg)	2.44 ±0.11	3.89 ±0.23	4.89 ±0.18	5.78 ±0.14	6.4 ±0.18			
MECB (200mg/Kg)	3.6 ±0.12	3.9 ±0.22*	5.4 ±0.72*	9.21 ± 0.33*	6.5 ±0.43*			
MECB (400mg/Kg)	3.9 ±0.15	5.4 ±0.44 ^a	8.3 ±0.74 ^a	12.21±0.44 ^a	9.23 ± 0.43^a			



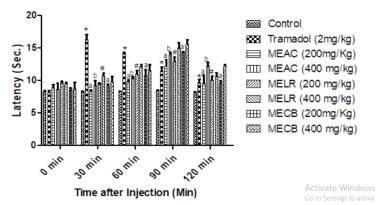


Figure 2 Effect of MEAC, MELR and MECB on tail withdrawal reflexes caused by the tail immersion treatment in rats

5.3 Antinociceptive activity by using the acetic acid-induced writhing method

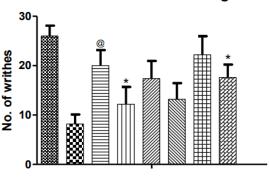
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MELR (400 mg/kg) greatly reduced the number of wriths at 49.23%, MEAC (400 mg/kg) considerably reduced the number of wriths at 51.78%, MEAC (200 mg/kg) significantly reduced the number of writhes at 29.68%, and MELR (200 mg/kg) also significantly reduced the number of wriths at greatly reduced the number of writhes at 33.07%, while MECB (200 mg/kg) continued to significantly reduce writhes at 25.14%. The MECB vehicle control group (400 mg/kg) also significantly reduced writhes at 31.88%. Maximum activity was seen by diclofenac (5 mg/kg), which inhibited the wrighting response by 68.46% (p<0.001). It was discovered that the 400 mg/kg dosage was more effective than the 200 mg/kg dose in all analgesic formulations. This might suggest that the 400 mg/kg dose of the extract was the ideal amount to produce both central and peripheral analgesic effects. The findings were displayed in Table.

Treatment		Wrig	hting (Count		Wrightings	% of	
Treatment	M-1	M-2	M-2 M-3 M-4 M-5		M-5	(Mean±SEM)	Wrighting	Inhibition
Control	28	26	25	23	28	26 ± 2.12	100	0
Diclofenac Sodium (5 mg/kg)	7	9	11	6	8	8.2 ± 1.92	31.54	68.46
MEAC (200 mg/kg)	18	24	16	20	22	$20 \pm 3.16*$	70.32	29.68
MEAC (400 mg/kg)	8	12	10	14	17	$12.2 \pm 3.49*$	48.22	51.78
MELR(200 mg/kg)	16	20	12	18	21	17.4 ± 3.57	66.93	33.07
MELR(400mg/kg)	12	15	8	16	15	$13.2 \pm 3.27*$	50.77	49.23
MECB(200 mg/kg)	28	20	22	23	18	22.2 ± 3.76	74.86	25.14
MECB(400 mg/kg)	20	16	18	20	14	$17.6 \pm 2.60*$	68.12	31.88

Table 11 Effect of MELR, MEAC, MEB on acetic acid-induced writhing behaviour in mice.

M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5.), \$, p < 0.001 versus control, *, p < 0.001 versus Diclofenac sodium and @, p < 0.001 versus MELR (200 mg/kg).



Treatment

Acetic Acid-Induced Writhing test

Control
 Diclofenac Sodium (5 mg/kg)
 MEAC (200mg/Kg)
 MEAC (400 mg/kg)
 MELR (200 mg/kg)
 MELR (400 mg/kg)
 MECB (200mg/Kg)
 MECB (400 mg/kg)

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Figure 3 Protective Effect of MELR, MEAC, and MECB on acetic acid-induced writhing behaviour in mice.

Conclusion

Standardization of herbs becomes one of the most indispensable steps to determine the plant material's quality. In the present study, pharmacognostic standardization of Argyreia cymosa, Limnophila repens and Capparis brevispina was carried out as per standards. The remarkable aspects of herbal extracts were that they act on human systems with an internal synergy. Argyreia cymosa, Limnophila repens, and Capparis brevispina all contain several medicinally significant secondary metabolites in all of their extracts, according to the study's findings. Phytoconstituents found in organic extracts were further supported by research using thin-layer chromatography. Certain plants, including Argyreia cymosa, Limnophila repens, and Cappparis brevispina, have methanolic extracts that may have dose-dependent anti-nociceptive effects as both central and peripheral analgesics.

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