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EVALUATION OF *IN-VIVO* ANALGESIC ACTIVITY OF *ARGYREIA CYMOSA* (ROXB.) SWEET

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ABSTRACT: The current research was accomplished to assess the analgesic activity of *Argyreia cymosa* (*A. cymosa*) methanolic extracts (MEAC) at 200 and 400 mg/kg. The entire herb was extracted with the methanol (MEAC). Acute oral toxicity of the extract was according to OECD-423 recommendations. Analgesic activity was explored by utilizing hot plate, tail immersion and acetic acid-evoked writhing models. In acute toxicity study, no mortality was observed when each extract was orally administered with 2000 mg/kg. At the doses (200 and 400 mg/kg) methanolic extract of *Argyreia cymosa* demonstrated considerable and dosed reliant analgesic effect. The conclusions from the present investigation confirmed the folkloric usage of *Argyreia cymosa* as an analgesic agent. This significant analgesic impact might be because of the presence of steroids, flavonoids, and glycosides within the extract. But, further phytochemical, as well as biological tests, are needed to determine the additional active chemical constituents responsible for the antinociceptive activity.

INTRODUCTION: Medicinal plants were often taking part in an important part in traditional medications intended for treatment of various health conditions. Alternatively, a crucial hurdle which has impeded the promotion in the usage of alternate medications in the developed countries is no evidence of documentation and then deficit of rigorous quality control measures. Additionally there is a requirement of the records of all of the research work meted out on traditional medicines by using documents.

On this issue, it is now necessary to help to make assurance about the standardization of the herb as well as parts to be utilised as a medication. Along the way of standardization, we are able to utilize different approaches and strategy to achieve the goal in a stepwise manner *e.g.* pharmacognostic and phytochemical analyses. These kinds of strategies and techniques are helpful on recognition and standardization of the plant material. The Appropriate depiction and quality assurance of starting materials is a crucial stage to ensure a reproducible quality of herbal medication to aid us to justify its safety and effectiveness. For that reason, we now have accomplished pharmacognostic research of *Argyreia cymosa* (*A. cymosa*) belongs to family Convolvulaceae ¹. This type of research is not going to help in authentication additionally, ensures reproducibility of herbal merchandise in marketing ².

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In the current study, we are emphasizing our investigation on one of the commonly available plant in India i.e., *A. cymosa*, belongs to family Convolvulaceae. The family Convolvulaceae contains nearly 1650 predominantly exotic species. The genus *Argyreia*, with around 135 species, some of the important species include *A. aggregate*, *A. cuneata*, *A. cymosa*, *A. daltoni*, *A. elliptica*, *A. fulgens*, *A. kleiniana*, *A. malabarica*, *A. nervosa*, *A. cymosa*, *A. setosa*, *A. strigosa* and *A. speciosa*³⁻⁵. *A. cymosa* is a stragglers on thickets; stem woody, terete, pubescent, leaves deltoid to cordiform, 6-8 × 4-6 cm, chartaceous, thinly pubescent on both sides, entire, acute or obtuse, base truncate or cordate; flowers pinkish in axillary, corymbose cymes; fruit globose, 1.7 × 1.4 cm, glabrous; seeds 2,3 or 4 ovate to elliptic, black^{6,7}.

A. cymosa is an ornamental, in addition to a medicinal plant. All parts of this plant are widely used as a folklore medicine for the treatment of various ailments by the Indian traditional healer. Its root is utilized to cure a various illness like sexually transmitted diseases viz., gonorrhoea and syphilis, blood diseases, cracks and wounds⁸⁻¹⁰. The leaf extracts of *Clausena dendata* and *Argyreia cymosa* is applied to the eyes of sheep and goat to cure corneal opacity¹¹.

The plant has been reported a few biological activities including Antibacterial⁹ and Antioxidant¹². Even though the drug has many uses, its pharmacological and phytochemistry is very poorly explored¹³. Consequently, this exploration was performed to evaluate the antinociceptive property of *A. cymosa*.

MATERIALS AND METHODS:

Plant Collection and Authentication: The herb, *Argyreia cymosa*, was collected at Tirupati during September 2017. The examined herb was recognized and verified by the botanist Dr. K. Madhava Chetty. A specimen of the herb, with the voucher number 1568, was deposited at A. M. Reddy memorial college of Pharmacy, Narsaraopet, Andhra Pradesh.

Preparation of Extract: The freshly gathered herb was shade dried and pulverised. The powder (1 kg) was extracted by way of petroleum ether meant for removing fatty and waxy materials. It was air-dried and then macerated by way of methanol, strained

and then concentrated at 4 °C in Buchi rotavapor. Finally, the weight of methanolic extract acquired was 75g (7.5% w/w yield). The methanolic extract was reconstituted in distilled water within a separating funnel and then partitioned sequentially with the petroleum ether, chloroform, ethyl acetate and n-butanol to obtain fractions in these solvents. In due course, the remaining residual aqueous portion at the end was collected. The solvents were removed on a rotary evaporator at low pressure to obtain dried fractions. These extracts had been subjected to preliminary phytochemical screening, and these extracts had been kept in the refrigerator at 4 °C for additional make use of in future¹⁴.

Phytochemical Screening: The various extract of *A. cymosa* was subjected to qualitative chemical analysis by using standard procedures¹⁵⁻¹⁸.

Animals: Thirty adult albino rats weighing between 180 and 220 g and albino mice (25-30 g) of either sex procured from the Mahaveer Enterprises, Hyderabad, were used for this study. These were consequently housed in the Animal House of the Faculty of Basic Medical Sciences under standard environmental circumstances of the 12-h light/12-h dark cycle and allowed free access to clean drinking water and feed. The use of experimental animal complies with the OECD-423 guidelines¹⁹.

The Department Experimental Review Panel also evaluated the standard protocol before the beginning of the study. All the procedures involving the animals by the approved protocol of the Ethics Committee on Animal Experimentation of the A. M. Reddy Memorial College of Pharmacy, Narsaraopet, Andhra Pradesh with the IAEC NO: AMRMCP/ 10/19/PHD. After arrival, animals were acclimatized to the animal facility for ten days before the commencement of the experiments.

Acute Toxicity Study: To evaluate the degree of toxicity of *A. cymosa* methanolic extract, the acute toxicity study was worked based upon OECD (Organization for Economic Cooperation and Development) 423 recommendations to the dose of 2000 mg/kg. The experimental animals had been noticed for 1 h constantly after which hourly for 4 h and lastly every 24 h up to 14 days for any physical symptoms of the level of toxicity, including

writhing, gasping, palpitations and lowered respiratory rate or mortality. No animals died. Therefore the LD₅₀ is greater than 2000 mg/kg. Pre-screening investigation with 200 and 400mg per body weight was done²⁰.

Pharmacological Activities:

Analgesic Activities: The peripheral analgesic activity of *A. cymosa* methanolic extract was evaluated using the acetic acid-induced writhing test, while central analgesic activity was studied against thermal stimuli using the hot plate and tail immersion tests.

Hot Plate Method: The central analgesic activity of methanolic extract of *A. cymosa* was evaluated by Eddy's hot plate method²¹. The rat (n=5) were divided into four groups. Group I designated as control group received vehicle orally. Group II specified as reference group was given the standard drug tramadol 10 mg/kg p.o. While animals in groups III and IV received 200 and 400 mg/kg bodyweight of methanolic extract of *A. cymosa* respectively. Each animal was placed separately on the hot plate maintained at temperature 55 ± 2 °C. The reaction time (paw licking or jumping) was recorded for each mice at periods of 30 min, 60 min and 90 min following administration of medicine or vehicle with the cut-off time 15 sec to avoid injury. The rise in reaction time in plant extracts and standard treated groups were compared to those of the control group²².

$$\% \text{ Analgesic activity} = (T_a - T_b) \times 100 / T_b$$

T_a = Average of reaction time after the administration of the extract

T_b = Average of Initial reaction time

Tail Immersion Test: The lower two-thirds of the tail had been engrossed in a beaker comprising water kept at 50 ± 0.5 °C. The time in seconds before the tail was withdrawn from the water was defined as the reaction time. The animals had been pretreated 60 min before the tail immersion with the normal saline solution for the group I (negative control), 200 mg/kg acetylsalicylate acid for group II (positive control) and 200, 400 mg/kg of *A. cymosa* for groups III and IV respectively. The lower part of each tail was engrossed in hot water maintained at about 55 °C, leading to an

excruciating response. The time, in seconds, for tail withdrawal from the water was recorded as the response period, having a cut-off time for immersion set at 15 s. The latent period of the tail immersion response was determined at 0, 30, 60, 90, 120 and 180 min after the oral administration of standard and MELR. The percentage of inhibition of heat-induced pain was computed with the following formula²³.

$$\% \text{ Inhibition} = (L_n - L_o) \times 100 / L_n - L_o$$

Where L_o = Latent time before drug administration in seconds

L_n = Latent time after drug administration in seconds (n= 30 to 180 min)

Acetic Acid-Induced Writhing Method: For this study acetic acid induced writhing method was acquired. Mice (n = 5) were divided into two groups for each extract of *A. cymosa*, in addition to that two more groups (n = 5) of mice were used for control and standard study. Control group received the vehicle; the standard group was treated with acetylsalicylic acid 100 mg/kg, p.o. Groups assigned for extracts were treated with 100 and 200 mg/kg, p.o. After 30 min, writhings were induced in mice by intra-peritoneal injection of 0.6 % v/v acetic acid. The number of writhing was counted throughout 30 min. The percentage inhibition of writhing count of the treated group was calculated from the mean writhing count of the control group by applying the formula^{24,25}.

$$\% \text{ Inhibition} = \frac{\text{Mean no. of writhes (Control)} - \text{Mean no. of writhes (Treated)}}{\text{Mean no. of writhes (Control)}} \times 100$$

Anti-inflammatory Activity:

Statistical Analysis: Results were expressed as mean ± SEM (standard error of the mean). The statistical analysis of the results was carried out with the ANOVA application (GraphPad Prism 5). Values of p<0.05 were considered statistically significant.

RESULTS:

Phytochemical Screening: The phytochemical screening for various extracts viz., petroleum ether, chloroform, ethyl acetate, methanol, n-butanol, and water was carried out, and results were displayed in **Table 1**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF VARIOUS EXTRACTS OF ARGYREIA CYMOSA WHOLE PLANT

Phytoconstituents	Method	Pet. ether extract	Ethyl acetate extract	Chloroform extract	Methanol extract
Flavonoids	Shinoda Test	-	+	-	+
	Zn. Hydrochloride test	-	+	-	+
	Lead acetate Test	-	+	-	+
Volatile oil	Stain test	-	-	-	-
	Alkaloids	Wagner Test	-	+	+
Tannins & phenols	Hager's Test	-	-	+	+
	FeCl ₃ Test	-	+	-	+
	Potassium dichromate test	-	-	-	+
Saponins	Foaming Test	-	-	-	-
Steroids	Salkowski test	+	-	+	+
Fixed oils and fats	Spot test	+	-	-	-
Carbohydrates	Molish test	-	-	-	+
Acid compounds	Litmus test	-	-	-	+
Glycoside	Keller-Killani Test	-	-	-	+
Amino acids	Ninhydrin test	-	-	-	+
Proteins	Biuret	-	-	-	+

“+”- Present; “-” – Absent

Acute Toxicity Studies: Oral administration of the highest dose of 2000 mg/kg of the methanolic extract of *Argyrea cymosa* did not produce any acute toxic symptoms. Moreover, no mice and rats mortality occurred during the observation period of 24 h. The extracts were found to be safe at the highest dose of 2000 mg/kg; fifth and ten-fold dilutions of the highest dose were selected for the analgesic and anti-inflammatory activities, *i.e.*, 200 and 400 mg/kg.

Analgesic Activity:

Hot Plate Method: The results (mean \pm SEM) of hot plate showed that the MEAC (200 and 400 mg/kg) exhibited an increase in basal reaction time from 8.5 ± 0.52 and 8.6 ± 0.52 at 0 min to 14.88 ± 0.43 and 15.12 ± 0.32 at 120 min respectively **Fig. 1, Table 2**.

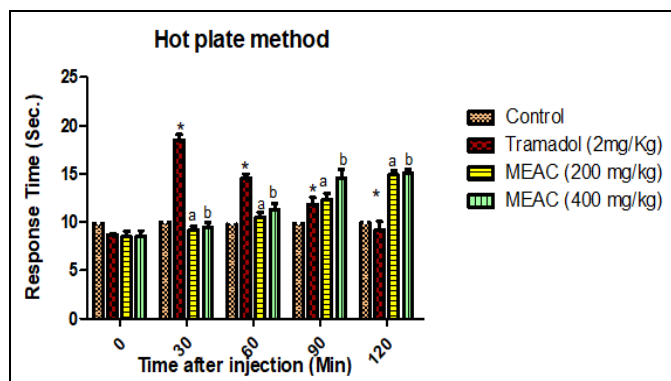


FIG. 1: EFFECT OF METHANOLIC EXTRACTS OF ARGYREIA CYMOSA ON HOT-PLATE METHOD. All the values are expressed as mean \pm SEM; n = 5 mice in each group, by one way ANOVA followed by Tukey's Multiple Comparison Test. *, p<0.01 versus control; ^a, p<0.01 versus tramadol; ^b<0.05 versus tramadol.

Tail Immersion Test: The tail immersion method revealed a well-marked increase in basal reaction time of 7.89 ± 0.48 in MEAC (200 mg/kg) and 1032 ± 0.37 in MEAC (400 mg/kg) at 120 min **Fig. 2**. The inhibition was the highest at 120 min at 400 mg/kg dose which was lower than standard.

Writhing Test: The peripheral analgesic activities of *Argyrea cymosa* on acetic acid-induced abdominal writhing in mice were shown in **Table 4**. Control group showed maximum writhing (25.4 ± 1.14), while MEAC at a dose of 200 and 400 mg/kg demonstrated a significant antinociceptive effect against acetic acid-induced writhing, inhibiting pain by 31.14% and 58.19% as compared to the control respectively **Table 4, Fig. 3**. Diclofenac at 10 mg/kg had 63.11% (p<0.001) inhibition of writhing response.

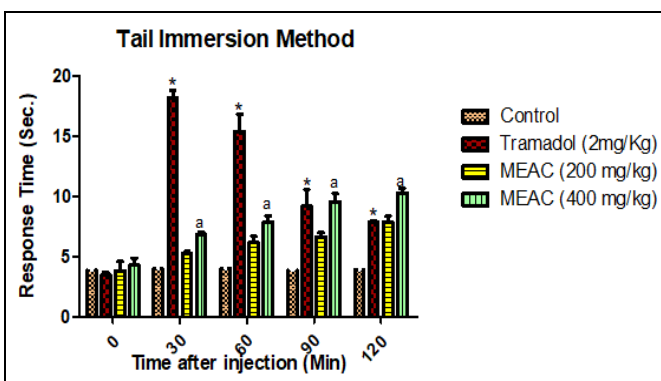


FIG. 2: EFFECT OF METHANOLIC EXTRACTS OF ARGYREIA CYMOSA ON TAIL IMMERSION METHOD. All the values are expressed as mean \pm SEM; n = 5 rats in each group, by one way ANOVA followed by Tukey's Multiple Comparison Test. Results are presented as mean \pm SEM, (n=5), *p<0.05 versus Control; ^a, p<0.05 versus Tramadol.

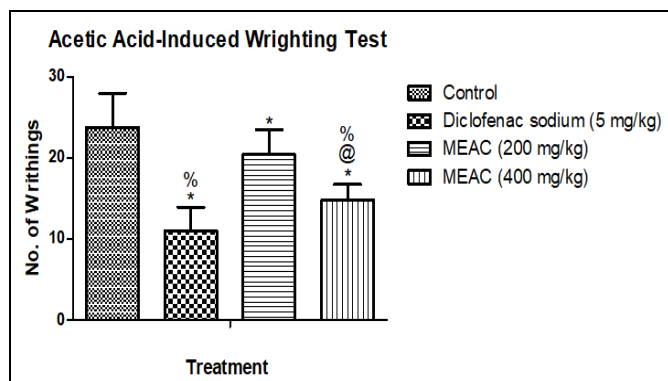


FIG. 3: EFFECT OF MEAC ON ACETIC ACID-INDUCED WRITHING BEHAVIOR IN MICE.

*, p<0.05 versus control, @, p<0.05 versus Aspirin and %, p<0.01 versus MELR (200 mg/kg)

TABLE 2: EFFECT OF METHANOLIC EXTRACTS OF *ARGYREIA CYMOSA* ON HOT-PLATE METHOD

Treatment	Reaction Time (s)				
	Time after Treatment (min)				
	0	30	60	90	120
Control	9.87 ± 0.32	10.02 ± 0.58	9.85 ± 0.22	9.88 ± 0.63	9.91 ± 0.45
Tramadol (2 mg/kg)	8.7 ± 0.11	18.52 ± 0.56	14.56 ± 0.42	11.86 ± 0.70	9.22 ± 0.85
MEAC (200 mg/Kg)	8.5 ± 0.58	9.2 ± 0.36	10.48 ± 0.54	12.36 ± 0.66	14.88 ± 0.43
MEAC (400 mg/Kg)	8.6 ± 0.52	9.5 ± 0.43	11.32 ± 0.63	14.58 ± 0.88	15.12 ± 0.32

TABLE 3: PROTECTIVE EFFECT OF *ARGYREIA CYMOSA* METHANOLIC EXTRACT ON TAIL WITHDRAWAL REFLEXES INDUCED BY TAIL IMMERSION METHOD IN RATS

Treatment	Reaction Time (s)				
	Time after Treatment (min)				
	0	30	60	90	120
Control	3.86 ± 0.22	3.98 ± 0.31	4.01 ± 0.74	3.87 ± 0.13	3.94 ± 0.25
Tramadol (2mg/kg)	3.45 ± 0.25	18.2 ± 0.63	15.45 ± 1.42	9.23 ± 1.37	7.86 ± 0.10
MEAR (200mg/Kg)	3.85 ± 0.74	5.26 ± 0.16	6.15 ± 0.54	6.65 ± 0.35	7.89 ± 0.48
MEAR (400mg/Kg)	4.31 ± 0.55	6.85 ± 0.23	7.89 ± 0.48	9.55 ± 0.73	10.32 ± 0.37

All the values are expressed as mean ± SEM; n = 5 rats in each group, by one way ANOVA followed by Tukey's Multiple Comparison Test. Results are presented as mean ± SEM, (n=5), * p<0.05 versus Control; ^a, p<0.05 versus Tramadol.

TABLE 4: EFFECT OF *ARGYREIA CYMOSA* METHANOLIC EXTRACT ON ACETIC ACID-INDUCED WRITHING BEHAVIOR IN MICE

Treatment	Writhing Count					Writhings (Mean ± SEM)	% of Writhings	% of Inhibition
	M-1	M-2	M-3	M-4	M-5			
Control	25	24	26	27	25	25.4 ± 1.14	100	0
Diclofenac Sodium	6	11	12	8	8	9 ± 2.4*	36.88	63.11
MEAC (200 mg/Kg)	16	18	15	21	14	16.8 ± 2.77 ^b	68.85	31.14
MEAC (400 mg/Kg)	10	11	9	13	8	10.2 ± 1.92*, ^a	41.80	58.19

Here, MEAC: methanolic crude extract of *Argyrea cymosa*. M-1= Mice 1, M-2 = Mice 2, M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5. Results are presented as mean ± SEM, (n=5), * p<0.001 versus Control, ^a, p<0.001 versus Diclofenac sodium; ^b, p<0.05 versus Diclofenac Sodium and ^{bbb}, p<0.001 versus MEAR (200 mg/Kg).

DISCUSSION: The preliminary phytochemical analysis of the *A. cymosa* herb shows the presence of several compounds such as flavonoids, alkaloids, tannins, phenols, steroids, acid compounds, glycosides, amino acids, and proteins.

It is well known that inflammation and pain are the most common diseases in human and animals, and the current treatment is to use steroidal and nonsteroidal anti-inflammatory drugs which have

several side effects^{26, 27}. *Argyrea cymosa*, a well-known traditional Indian medicine, has a long history of being used for treating a lot of diseases. But its analgesic feature has never been reported. With this investigation, we demonstrated the potent analgesic action of *Argyrea cymosa* in various animal models.

The hot-plate test is astonishing in elucidating centrally mediated anti-nociceptive responses,

which works mainly on changes over a spinal-cord level. The significant upsurge in pain tolerance produced by methanolic extract of *A. cymosa* implies the involvement of central pain pathways. Pain is centrally controlled *via* some intricate processes consisting of opiate, dopaminergic, descending noradrenergic and serotonergic systems. The analgesic result caused by the extract might be *via* central mechanisms including these types of receptor systems or through peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances which can be crucial players in swelling and pain²⁸.

The abdominal constriction response evoked by acetic acid is a sensitive process to assess peripherally acting analgesics. Generally, acetic acid triggers pain by releasing endogenous substances such as serotonin, histamine, prostaglandins, bradykinins and substance P, which in turn activate nerve endings. Local peritoneal receptors will be postulated to be involved in the abdominal constrictions impulse. The technique is involving prostanoids in general, that is, elevated levels of prostaglandin-E2 (PGE2) and PGF2 in peritoneal fluids and also lipoxygenase products²⁹. The significant lowering of acetic acid-induced writhes through the methanolic extract of *A. cymosa* shows that the analgesic impact might be peripherally mediated with the inhibition of synthesis and release of prostaglandins along with endogenous substances.

This significant analgesic effect may be due to the inhibition of any inflammatory mediators by the steroids³⁰, flavonoids³¹ and glycosides present in the extract.

CONCLUSION: The current result indicates the efficacy of *Argyrea cymosa* as an effective therapeutic agent in the treatment of acute inflammations. The result also authenticates the folklore information on the analgesic property of the *A. cymosa* extract. But, further phytochemical, as well as biological tests, required to determine the other active chemical constituents accountable for the antinociceptive activity.

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