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Evaluation of the Anti-Inflammatory and Analgesic Activity of *Allamanda blanchetii (Apocynaceae)*

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ABSTRACT: Background: *Allamanda blanchetii* commonly known as purple Allamanda, is an ornamental plant of the Allamanda genus and it belongs to *Apocynaceae* family. All parts of the plant are poisonous if ingested. *A. blanchetii* is commonly used as an ornamental plant. Only a few reported that *A. blanchetii* shown to have antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. **Aim:** The study aimed to evaluate the effect of anti-inflammatory and analgesic activity of *A. Blanchetti*. **Methods:** The Anti-inflammatory activity of *A. blanchetii* was evaluated using the carrageenan induced paw edema. The Analgesic activity of *A. blanchetii* was evaluated in tail flick and hot plate method. **Result:** The phytochemical screening *A. blanchetii* leaves has successive extraction investigated to be flavonoids, terpenoids, sugars, phenol, alkaloids, cardiac glycoside, saponin are present. The anti-inflammatory activity in carrageenan induced paw edema effect in *A. blanchetti* showed moderate significance was absorbed at a dose of 500 mg/kg body weight as compared to control. The standard drug showed significant effect compared to control. The combination group has shown a highly significant effect, when compared to the standard group. The Analgesic activity in Tail flick and Hot plate method effect in *A. blanchetti* shown moderate significance was absorbed at a dose of 500 mg/kg body weight as compared to control. The standard drug showed significant effect compared to control. The combination group has shown a highly significant effect, when compared to the standard group. **Conclusion:** The *A. blanchetii* leaves extract has peripheral analgesic properties as well as anti- inflammatory activities. Our results support the therapeutic activities of *A. blanchetii* proclaimed by indigenous and justify its use in traditional medicine.

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INTRODUCTION:

Currently used anti-inflammatory drugs are associated with some severe side effects like NSAIDs stimulate the gastrointestinal tract and damage the kidneys, selective cyclooxygenase inhibitors increase the risk of cardiovascular diseases and glucocorticoid drugs may activate or aggravate infections [1,2]. While pain has been found to be more expensive than heart disease, cancer or diabetes in terms of health care and economic costs. The impact of pain on patients' and their families' psychological, social and economic well-being provides

an indirect reason to consider pain as a public health issue. As a result, pain treatment and prevention should be safe, effective and accessible. Therefore, the development of potent anti-inflammatory and analgesic drugs with fewer side effects is necessary. Medicinal plants have great value of phytochemicals because of their medicinal properties.

The secondary metabolites such as some types of alkaloids, glycoside, and phenolic compounds give remarkable anti-inflammatory and analgesic properties. Drugs from plant origin are considered to be comparatively safe and cost-effective options for treatment of any diseases. In recent years, scientific attention has been paid to saponin due to their structural diversity and significant biological activities [3].

Allamanda blanchetii commonly known as purple Allamanda, is an ornamental plant of the Allamanda genus and it belongs to Apocynaceae family. All parts of the plant are poisonous if ingested. *A. blanchetii* is commonly used as an ornamental plant. Only a few reported that *A. blanchetii* shown to have antioxidant, cytotoxic, thrombolytic, membrane stabilizing and antimicrobial activities. *A. blanchetii* is a shrubby climbing species with showy purple to pink flowers that has been cultivated as an ornamental in tropical and subtropical climates. It is a rapidly spreading species that spreads by layering. *Allamanda blanchetii* is a native of Brazil that is considered invasive in Anguilla and a weed in Australia. In Hawaii, *A. blanchetii* is also classified as a potentially invasive cultivated species. It's listed in the world's weeds compendium.

When climbing over other vegetation, it forms a clump about 2 m tall and 2-3 m wide, but it can grow much bigger (up to 5m height). Leaves sessile or sub sessile, 8 to 12 cm long, in whorls of usually 4, oblong or obviate-oblong, abruptly acuminate; petioles up to 2 mm long leaf blades broadly elliptic to orbicular with trichomes on both surfaces; stems and leaves with milky sap leaves sessile or sub sessile, 8 to 12 cm long, in whorls of usually 4.

Corolla rose-purple, darker in throat, tube not basally swollen, calyx pubescent, corolla 6 to 9 cm long 5 to 6 cm across at limb, tube not basally swollen. The fruits are spiny follicles with many flattened seeds with a membranous wing [4]. With the objective to study the effect of *A. blanchetti* the study was performed to evaluate the analgesic and anti-inflammatory activity of the plant.

MATERIALS AND METHODS:

Chemicals and reagents:

Diclofenac and pentazocine were procured from RS Sales Corporation. Nagpur, Maharashtra. Carrageenan was procured from Ozon International, Mumbai. All other chemicals and reagents of analytical grade were procured from authorized dealers.

Animals:

Animals were Approved by Institutional Animal Ethics Committee (reg.no:1103/PO/ReBi/S/07/CPCSEA) for protocol no: SDPC/IAEC/02/10/2021 on dated 23-10-2021 for the conduction of experiments. They were maintained under controlled conditions of temperature (23 ± 2 °C), relative humidity (55 ± 10 %), and 12 h/12 h light/dark cycles. They were randomized into experimental and control groups and housed each in sanitized polypropylene cages containing sterile paddy husk and with free access to standard pellets as basal diet and *water ad libitum*.

Collection and Authentication of Plant material:

The leaves were collected from the local region of Surat district and authenticated by Dr. Farzine Parabia, Associate Professor, Department of Biosciences, Bapalal Vaidya Botanical Research Centre (BVBRC) V NSGU, Udhna Magdalla Road, Surat. A voucher specim en was deposited at VNSGU herbarium the voucher number assignedto “VNSGU/BVBRC/2022/05/TC-07”.

Preparation of Extract:

A. blanchetii leaves were collected, washed and dried using a shade drying method. The dried leaves were reduced to fine powder by using a mechanical mixer. Then stored in an air tight container until the time of use. The dried powder of leaves was subjected to an extraction procedure by Soxhlet apparatus using Ethanol as solvent (in 1:3 ratio) at 40 ºC for 6 to 8 h. Extract was filtered using muslin cloth and then Whatman filter p aper Residual extract was evaporated to dryness at 35ºC, then stored at room temperature in an airtight container till use (Fig 1).

Preliminary Phytochemical Investigation [5]:

Phytochemical screening of ethanolic extracts of *A. blanchetii* leaf had been performed using different phytochemical tests for confirming the presence of different phytochemical components. Alkaloids, Saponin, Carbohydrates, Steroids, Terpenoids, Amino acid, Phenol, Tannins, Protein.

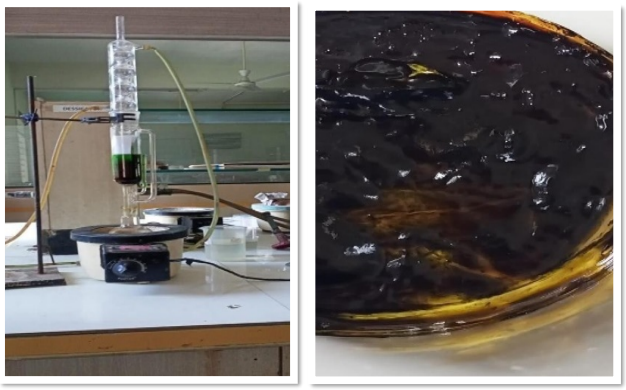

Fig 1. Extraction using Soxhlet Extractor and Extract.

Test for Carbohydrate (Molisch Test):

In 2 ml extracts, some droplets of α- naphthol solution were placed and shaked them. After ward 2 ml conc. Sulphuric acid was put in a glass tube through the wall. Formation of violet ring on the junction of two liquids signifies carbohydrates presence.

***Test for reducing sugar (Fehling Test)*:**

About 1ml Fehling's A and 1 ml Fehling's B solutions were mixed, boiled for one minute. Equal volume of test solutions was added. It was heated in a boiling water bath for 5 to 10 min. First yellow, then brick red ppt is observed.

Test for reducing sugar (Benedict Test):

Equal volume of Benedict's reagent and test solution in the test tube were mixed. The solution was heated in a boiling water bath for 5 min. Solution was appeared green, yellow or red depending on the amount of reducing sugar present in the test solution.

Test for proteins (Biuret's Test):

In 3 to 4 ml of extracts, 4 % NaOH solution as well as 1% CuSO4 solution were added. Appearance of violet or pink colour confirmed proteins.

Test for protein containing sulphur:

About 5 ml test solution was mixed with 2 ml 40 % NaOH and 2 drops 10 % lead acetate solution. The mixture was boiled. Solution turns black or brownish due to PBS formation.

Test for alkaloids (Dragendorff's Test):

In 2 to 3 ml filtrates, a small amount of Dragendorff's reagent was added. Occurrence of orange brown precipitates confirmed alkaloids presence.

Test for alkaloids (Wagner's test):

In 2 to 3 ml filtrates, a small amount of Wagner's reagent was added, giving reddish brown precipitate.

Test for alkaloids (Mayer's test):

In 2 to 3 ml filtrates, a small amount of Meyer's reagent was. A cream colour precipitation was observed.

Test for alkaloids (Tannic acid test):

Test solution was treated with a tannic acid solution that gives buff coloured precipitate.

Test for tannins and phenol:

To 2 to 3 ml of aqueous or alcoholic extract, few drops of following reagent were added.

Lead acetate solution: White precipitate, Dilute iodine solution: transient red colour, Acetic acid solution: red colour solution, and Potassium dichromate: red precipitate.

Protein test by Ninhydrin Test:

In test tubes 2 to 3 ml extracts, 4 droplets of 5 % Ninhydrin solution were placed. Samples were then heated using a boiling water bath till 10 min. Bluish or Purple colour appears to show amino acid presence.

Test for steroids/ terpenoids (Salkowski reaction):

About 2 ml of extract was added with 2 ml chloroform and 2 ml conc. H_2SO_4 were shaken well; the chloroform layer appears red and the acid layer shows greenish yellow fluorescence.

Test for steroids/ terpenoids (Liebermann-Burchard reaction):

About 2 ml extract wax mixed with chloroform added 1 top 2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of the test tube. First red, then blue and finally green appeared.

Test for saponin *(Foam Test):*

The drug extract or dry powder was vigorously shaken with water persistent stable foam observed.

Table 1. The Acute Toxicity study.

Groups	Treatment (mg/kg)	Number of Animals
Group 1	5	5
Group 2	50	5
Group 3	300	5
Group 4	2000	5
Group 5	5000	5

Acute toxicity studies:

According to OECD Guideline- 423 we used 5, 50, 300, 2000, 5000 mg/kg doses of extract given by oral route of administration in female mice to determine LD50 of the *Allamanda blanchetii* leaves. (Table 1).

Anti-inflammatory activity:

Carrageenan induced rat paw edema [6]*:*

Male Wistar rats (150 to 250 g each) were divided into 5 groups each having 8 animals. Inflammation was induced by subcutaneous injection of 0.1 ml of 1 % freshly prepared carrageenan in the right hind pawof rats. Left leg of animals were serves as control noni nflamed paw for comparison. Percentage increased volu me was calculated. Theexperimental group were receive d three graded concentrations (125, 250, and 500 mg/kg) of *A. blanchetii* leaves extract (ABLE). Diclofenac sodium (15 mg/kg) was standard to compare the response of drug and readings were recorded at 0, 30, 60, 120, and 180 min after dosing shown in Table 2.

Table 2. Anti-inflammatory Activity.

Groups	Treatment	Dose	Route
Group 1	Carrageenan induced rat paw edema	0.1 ml	Sub planter
Group 2	Lower dose of Allamanda Blanchetii in Carrageenan induced rat paw edema	125 mg/kg	Oral
Group 3	Moderate dose of Allamanda Blanchetii in carrageenan induced rat paw edema	250 mg/kg	Oral
Group 4	Higher dose of Allamanda Blanchetii in carrageenan induced rat paw edema	500 mg/kg	Oral
Group 5	Standard drug in Diclofenac	15 mg/kg	Oral

Analgesic activity:

Tail-Flick method*:*

Male Wistar 40 rats were divided into 5 groups and each group 8 animals were used. In this method proximal one third of the tail was placed 1.5 cm in nichrome wire. The experimental groups received three graded doses (125, 250, and 500 mg) concentrations of *A. blanchetii* leave extract pentazocine (15 mg/kg) was used as standard drug. The reaction time was noted at 0, 15, 30, 60, and 180 min after drug administration shown in Table 3.

Hot-plate method:

Male Wistar 40 rats were divided into 5 groups and each group used 8 animals. The hotplate is maintained at a temperature of 56±0.5 °C. The latency to lick the hind paws or jump from the hot plate is taken as the reaction time.

Table 3. Analgesic Activity (Tail Flick Method and Hot Plate Method).

Groups	Treatment	Dose	Route
Group 1	Vehicle treated control animals	0.2 ml	Oral
Group 2	Lower dose of test drug for analgesic activity	125 mg/kg	Oral
Group 3	Moderate dose of test drug for analgesic activity	250 mg/kg	Oral
Group 4	Higher dose of test drug for analgesic activity	500 mg/kg	Oral
Group 5	Standard drug in Pentazocine	15 mg/kg	Oral

The experimental groups were receiving three graded (125, 250, and 500 mg) concentrations of *A. Blanchetii* leaf extract. Pentazocine (15 mg/kg) as a standard drug. The reaction time was noted at 0, 15, 30, 60, and 180 min after drug administration shown in Table 3.

Tail Immersion Method:

Analgesic activity was assessed by the Tail immersion method. The Wistar rats weighing 150 to 250 g were fasted overnight with ad libitum access to water. The animals were divided into five groups. The animals were allowed to adapt to the cages for 30 min before testing. The distal part of the tail of each animal was marked (5 cm). This marked part of the tail was immersed in a beaker of freshly filled water of exactly 55 °C. Within a few seconds the rat reacted by withdrawing the tail. The time taken to withdraw the tail was noted as reaction time. A cut-off time of 10 s was maintained at 55 °C to prevent tissue damage. After respective drug treatment, the tail of each animal was immersed in a beaker of freshly filled water of exactly 55 °C and reaction time was measured at 0, 15, 30, 45, and 60 min, respectively shown in Table 4.

RESULTS AND DISCUSSION:

Preliminary phytochemical test:

Phytochemical screening of ethanolic extracts of *A. blanchetii* leaves was performed using different

phytochemical tests for confirming the presence and absence of constituents shown in Table 5.

Table 4. Analgesic Activity (Tail Immersion Method).

Groups	Treatment	Dose	Route
Group 1	Vehicle treated control animals	0.4 ml	I.V
Group 2	Lower dose of test drug for analgesic activity	125 mg	Oral
Group 3	Moderate dose of test drug for analgesic activity	250 mg	Oral
Group 4	Higher dose of test drug for analgesic activity	500 mg	Oral
Group 5	Standard drug of Pentazocine	30 mg/kg	Oral

Table 5. Preliminary Phytochemical test (+)-Shows presence and (-) – show absence of the phytochemicals.

Sl. No.	Phyto-chemicals	*Allamanda blanchetii*
1	Alkaloids	+
2	Saponin	+
3	Tannin	+
4	Phenol	+
5	Terpenoids	+
6	Carbohydrates	+
7	Steroids	+
8	Amino acid	-

Anti-inflammatory activity:

Anti-inflammatory effect of *A. blanchetii* extract of carrageenan induced rat paw edema described in table below, ABLE show significant value of against inflammation induced by carrageenan. Higher dose (500 mg/kg) of ABLE is given similar action of diclofenac (standard drug) shown in Table 6.

Analgesic Activity:

Analgesic effect of *A. blanchetii* extract of Hot plate method (Eddy's Hot plate) described in table below, *A. blanchetii* shows significant value against Eddy's hot plate. Higher dose (500 mg/kg) of *Allamanda Blanchetti* is given similar action of pentazocine shown in Table 7.

Tail Flick Method:

Analgesic activity of *A. blanchetii* extract of Tail flick method described in table b elow, ABLE show significantvalue of against Tail-flick method. Higher dose (500 mg/kg) of ABLE is given similar action of pentazocine shown in Table 8.

Tail Immersion Method:

Analgesic activity of *A. blanchetii* extract of Tail immersion method described in table below, ABLE show significant value of against Tail-immersion method. Higher dose (500 mg/kg) of ABLE is given similar action of pentazocine shown in Table 9.

DISCUSSION:

Inflammation is a complex process initiated by several factors ranging from bacterial infection and chemical injury to environmental pollution that result in cell injury or death. Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs in the world today. Pain and fever are the most common complaints associated with inflammation. The NSAIDs used in the inflammatory conditions do not cure and remove the inflammatory response to the diseases. There is a market need for orally active molecules that can treat inflammatory disease processes, rather than just the symptoms, more effectively than currently available drugs. Therefore, the research for new Anti-inflammatory agents is in process ABLE shows good data against pain source and inflammation may be the presence of alkaloids and tannins present in leaves extract. Diclofenac used as a standard drug to compare the inflammatory activity and higher dose of ABLE show good and comparable results. Lower and medium dose shows significant inhibition against the inflammatory stimulus but not potent as diclofenac but higher dose is more potent ABLE shows good data against pain source and Eddy's hot plate, may be the presence of alkaloids and tannins present in leaves extract. Pentazocine used as a standard drug to compare the Analgesic activity and higher dose of ABLE show good and comparable results. Lower and medium dose shows significant inhibition against the Eddy's hot plate stimulus but not potent as pentazocine but higher dose is more potent ABLE shows satisfactory data against pain source and Tail-flick method may be the presence of alkaloids and tannins present in leaves extract. Pentazocine used as a standard drug to compare the Analgesic activity and higher dose of ABLE show good and comparable results. Lower and medium dose shows significant inhibition against the Tail-flick method stimulus but not potent as pentazocine but higher dose is more potent ABLE shows very good data against pain source, Tail-immersion method may be the presence of alkaloids, and tannins present in leaves extract. Pentazocine used as a standard drug to compare the

Table 6. Anti-inflammatory activity using carrageenan induced paw edema.

Groups	0 min/% of Inhibition	30 min/% of inhibition	60 min/% of inhibition	120 min/% of inhibition	180 min/% of inhibition
Control	0.58±0.01	0.66±0.04	0.74±0.03	0.68±0.02	0.61±0.04
ABLE Dose 125mg	0.57±0.03 (0%)	0.52±0.02* (14.75%)	0.45±0.01 (26.23%)	0.36±0.02 (40.98%)	0.30±0.02* (50.82%)
ABLE Dose 250mg	0.55±0.04 (0%)	0.53±0.02 (13.11%)	0.47±0.02* (22.95%)	0.36±0.03* (42.62%)	0.26±0.02* (57.38%)
ABLE Dose 500mg	0.53±0.03 (0%)	0.49±0.02* (19.67%)	0.39±0.03* (36.07%)	0.29±0.02*** (52.46%)	0.20±0.15*** (67.21%)
Diclofenac 15mg/kg	0.56±0.04 (0%)	0.46±0.02 (24.59%)	0.37±0.03 (39.34%)	0.23±0.03 (62.3%)	0.11±0.03 (81.98%)

p values: *p<0.05*p<0.001 when compared to 0h paw edema volume of respective groups % inhibition.**
All values are expressed in second as Mean ± Standard deviation (n-8).

Table 7. Analgesic activity (Eddy's Hot plate).

GROUP	0 MIN	30 MIN	60 MIN	120 MIN	180 MIN
CONTROL	12.45±0.50	12.25±0.92	11.85±1.15	11.35±1.10	10.15±0.89
ABLE Dose 125mg	9.45±0.87	8.85±1.24	8.78±0.87	8.50±0.95	7.95±0.58
ABLE Dose 250mg	8.83±0.94	8.25±0.76	8.18±0.91	7.85±0.96	7.45±0.80
ABLE Dose 500 mg	8.75±1.2	8.12±0.88	8.09±0.95	7.54±1.02	7.12±0.68
Pentazocine 15mg/kg	8.72±0.34	8.09±1.07	8.05±0.34	7.25±0.58	7.04±0.78

All values are expressed in second as Mean ± Standard deviation (n-8).

Table 8. Analgesic activity (Tail flick method).

GROUPS	0 MIN	15 MIN	30 MIN	60 MIN	180MIN
CONTROL	12.45±0.5	12.36±0.88	12.21±0.44	11.95±0.37	11.78±0.20
ABLE Dose 125mg	11.85±1.02	11.68±0.99	11.49±0.18	11.25±0.94	10.85±0.40
ABLE Dose 250 mg	11.79±1.2	11.53±0.95	11.35±0.95	11.14±0.24	10.72±0.06
ABLE Dose 500 mg	10.96±0.94	10.82±0.5	10.76±0.29	10.51±0.35	10.38±0.20
Pentazocine 15mg/kg	10.85±0.34	10.71±1.07	10.65±0.34	10.42±0.58	10.26±0.78

All values are expressed in second as Mean ± Standard deviation (n-8).

Table 9. Analgesic activity (Tail immersion method).

Groups	0 min	15 min	30 min	45 min	60 min
Control	4.83±0.46	4.79±0.41	4.45±0.54	4.25±0.41	3.85±0.41
ABLE Dose 125 mg	4.75±0.35	4.72±0.50	4.38±0.45	4.19±0.48	3.78±0.39
ABLE Dose 250 mg	4.68±0.30	4.62±0.43	4.31±0.41	4.12±0.19	3.68±0.33
ABLE Dose 500 mg	4.52±0.34	4.45±0.42	4.28±0.38	4.09±0.25	3.56±0.27
Pentazocine 15mg/kg	4.48±0.31	4.36±0.43	4.18±0.50	4.09±0.39	3.45±0.5

All values are expressed in second as Mean ± Standard deviation (n-8).

Analgesic activity and higher dose of ABLE show good and comparable results. Lower and medium dose shows significant inhibition against the Tail-immersion method stimulus but not potent as pentazocine but higher dose is more potent.

CONCLUSION:

Synthetic molecules are generated tolerance and dependence over the time in that condition plant shows good replacement as a new drug therapy. Medicine or drugs, which belong to plants, produce fewer side effects and high potency. In this study, we compared ABLE

(125,250,500 mg/kg) with diclofenac, which is a reported NSAID'S drug and ABLE was found to have satisfactory data regarding anti-inflammatory and analgesic activity in comparison to standard drug. As per literature survey we can say, when we compare the main constituent of plants with standard drugs, maybe we get very good results, because in leaves extract we can't say how many main constituents are present. Plant shows presence of alkaloids, glycosides, tannin, phenols, carbohydrates and saponin may be for anti-inflammatory and analgesic activity shows due to presence of alkaloids and tannins containing extract.

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