Research Article

Exploration of Anti-nociceptive and Anti-inflammatory Activities of Methanolic Extract of *Aralia racemosa* L. Root

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Abstract

Background: Pain and inflammation are usually related to the pathophysiology of several medical conditions. The majority of antinociceptive and anti-inflammatory medications accessible within the merchandise found an outstretched purview of complications. The existing investigation was focused at identify the constituents and investigating the antinociceptive and anti-inflammatory activity of methanol extract of Araliaracemosa L. root.

Methods: The dried root of A. racemosa was extracted with methanol and partitioned between Petroleum ether, chloroform, ethyl acetate, and n-butanol. The organic layer was fractionated by various stationary phases and identified by using spectral analysis. The methanolic extract was assessed for its antinociceptive activity by utilizing hot plate, tail-immersion and acetic acid induced writhing test, while anti-inflammatory activity has been established by carrageen an induced paw edema carried out in vivo. The test group acquired two different doses of the extract (200 mg/kg and 400 mg/kg) orally. The positive control group acquired diclofenac (5 mg/kg), tramadol (2 mg/kg) orally. The negative control group acquired vehicle (2%Tween80, 10ml/kg) orally. Additionally, preliminary phytochemical screening was performed.

Results: Phytochemical investigation of the roots of Aralia racemosa L. (Araliaceae) afforded four known Phytoconstituents identified as Stigmasterol (1), ß-Sitosterol (2), Ursolic acid (3) and Oleanolic acid (4). The structures of those phytoconstituents have been elucidated based on spectral information analysis. Stigmasterol and Ursolic acid were isolated from this plant for the first time. Oral administration of A. racemosa methanol extract (at all doses) considerably ($p < 0.05$) interdict pain sensitivity in the pain models. In the same manner, the extract exhibited anti-inflammatory activity in the inflammation models in the rat. Preliminary phytochemical screening showed the presence of tannins, volatile oils, flavonoids, saponins, triterpenes, and glycosides.

Conclusion: The outcome attained from the current investigation signifies that the extract owned a considerable antinociceptive and anti-inflammatory activity may be attributed to the presence of isolated constituents, indicating the traditional relevance of the plant. There is evidence that crude plant extracts often have greater in pharmacological activity than isolated constituents at a same dose. It is recommended to determine the other active chemical constituents accountable for the antinociceptive and anti-inflammatory activities.

Keywords: Antinociceptive; Anti-inflammatory; Aralia racemose L; Stigmasterol; Ursolic Acid

Introduction

Even though the advancement made in the medical investigation in the elapsed few years, the management of certain severe disorders continuous to be problematic because of their complications and expensive cost related to it. Inflammation is a local response of animals towards injury. Generally, it is a body defense system to hinder the propagate of infection. It is concord with the formation of edema, leucocytes infiltration, and granuloma formation, tissue injury and repair [1]. Inflammation leads to activates inflammatory mediators such as TNF-α, interleukins, and prostaglandins. Anti-inflammatory agents are able of suppressing the cyclooxygenase COX-1 and COX-2 pathway of arachidonic acid metabolism that generates prostaglandins [2]. Even though a large number of treatments are available in the market, such as immune suppressants, NSAIDs, corticosteroids, and histamines, unwanted effects related with them restrict their usage. Osteoporosis, gastric lesions, high blood pressure, and allergy are the typical unwanted effects associated with them. Consequently, the focus has been aimed at the efficiency of plant-based medications with fewer side effects. In Accordance with WHO, 80% of the world populace depend on plant-based remedies for primary healthcareapproximately in 1997, the world marketplace over the counter phytopharmaceuticals had been US$ 10 billion with a yearly growth of 6.5%. WHO incorporates Phytotherapy in its health programs as well as provides specific focus on the validation of drugs from plant origins in developing countries [3].

Pain is an unpleasant and emotional experience related to damaged tissue. Analgesics are the agents accustomed to reducing pain. Traditional analgesics of natural source such as opiates and NSAID drugs but they are associated with unwanted consequences like gastric lesions and tolerance and dependence. Therefore, there is ought to examine naturally available substitute resources to NSAIDs and opiates.

Secondary metabolites of plants like steroids, flavonoids, alkaloids, terpenoids, and glycosides have acquired significance because of their varied pharmacological activities like anti-inflammatory, analgesic and antipyretic, etc. *Araliaracemosa* L. (Family: Araliaceae) is a plant which is indigenous to the exotic and semitropical location of the world. The genus *Aralia* consists of 71 species of plants distributed all over Asia, Mexico, North America, and South America. In 1994 Smith identified the North American species of Araliaceae and recognized the following eight species of *Aralia i.e.,A. racemosa, A. californica, A. nudicaulis, A. spinosa,A. hispida, A. humilis, A. regeliana*and*A. scopulorum.*Standley recognized five species of *Aralia* from Mexico: *A. scopulorum, A. regeliana, A. humilis, A. pubescens*, and*A. racemosa* [4]. Many plants that belong to family Araliaceae were recognized for its

analgesic and anti-inflammatory like, Acanthopanax*koreanum* [5] *Dendropanaxmorbifera* [6], *Panaxjaponicus* [7] and *Panaxnotoginseng* [8]. Traditionally, *A. racemosa* roots has a diverse range of reputed medicinal applications as carminative, antiseptic, in cough preparations, pain in the breast, mortifications, rheumatism, Whooping cough, skin diseases, pleurisy, diaphoretic, diuretic, pulmonary diseases, asthma, diarrhea, stimulant, expectorant, syphilis, inflammation and hay fever [9,10] Only a few pharmacological properties have been documented from this plant such as antioxidant, anti-diabetic[11,12]and anti-tubercular[13]. Few phytoconstituents are reported from this plant such as triterpenoid saponins i.e., oleanolic acid, sterols i.e., β-sitosterol and Diterpenoids i.e., *ent*-Kaurenoic acid, continentalic acid[11,14]. As the genus, *Aralia*is enriched with triterpenoid saponins, which may possess antinociceptive and anti-inflammatory activity based on previous studies [15-17].

Therefore, the existing study was intended to identify and isolate the chemical compounds from the methanolic extract of *A. racemosa*L. root for its antinociceptive and anti-inflammatory effects.

Materials and Methods

Plant Material

The plant material was obtained from Tirupati, Chittoor district of Andhra Pradesh, India during the month of March 2016 and authenticated by Dr. K. Madhava chetty, Taxonomist, Sri Venkateswara University Tirupati, India. Voucher specimen No. 1489 was deposited at the herbarium for future reference. The root was shade dried, powdered and sieved through 20 mesh and kept in an airtight container for future use.

Preparation of Extract

The freshly collected roots were shade dried and pulverized. The powder (3 Kg) was treated with petroleum ether for the removal of fatty and waxy material. Then it was air dried and macerated with methanol, filtered and concentrated at 45°C in Buchirot vapor. The weight of methanolic extract obtained was 73g (7.3% w/w yield). The methanolic extract had been suspended in distilled water in a separating funnel and partitioned sequentially with petroleum ether, chloroform, ethyl acetate and n-butanol to acquire fractions in these solvents. Eventually, left residual aqueous fraction at the end was collected. The solvents were removed on a rotary evaporator at low pressure to obtain dried fractions. These extracts were subjected to preliminary phytochemical screening and these extracts were stored in the refrigerator at 4°C for further use [18].

Phytochemical Screening

The methanolic extract of *A. racemosa*L. root was subjected to qualitative chemical analysis by using standard procedures as follows.

The phytochemical screening of carbohydrates was detected by Molisch's test; Proteins were detected by using two tests namely Biuret test and Millon's test and amino acids by Ninhyrdin's test; Steroids was detected by Salkowski, Liebermann-Bur chards and Liebermann's test; Alkaloids was identified with freshly prepared Dragendroff's Mayer's, Hager's and Wagner's reagents and observed for the presence of turbidity or precipitation. The flavonoids were detected using four tests namely Shinoda, sulfuric acid, aluminum chloride, lead acetate, and sodium hydroxides. Tannins were detected with four tests namely gelatin, lead acetate, potassium dichromate and ferric chloride. The froth, emulsion, and lead acetate tests were applied for the detection of saponins. The steroids were detected by (acetic anhydride with sulfuric acid) and (acetic chloride with sulfuric acid) tests. Sample extracted with chloroform was treated with sulfuric acid to test for the presence of terpenoids. Ammonia solution and ferric chloride solutions were used to the presence of anthraquinones [19,22].

Isolation of Constituents

Petroleum Ether Extract (PEE) was subjected to silica-gel (100-200 mesh) column (length 100 cm and diameter 3 cm) chromatography (elution rate of 2 ml min-1 flow with a total elution of 200 ml) and eluted with Petroleum ether and ethyl acetate in different proportions. The consequent fractions (Fr) were collected and spotted over pre-coated silica gel F254 plates (20 × 20 cm, Merck, Germany). The optimum resolution was achieved in the hexane, ethyl acetate and formic acid (7.5: 2: 0.5 v/v) solvent system and the plates were sprayed with anisaldehyde-sulphuric acid reagent to visualize the spots. The fractions showing similar spots were pooled together and concentrated. The fractions which showed prominent spots were taken up for spectral studies which result in the identification of 4 compounds. The compounds PC-1 and PC-2 were identified as phytosterols by Libermann-Burchard's test (Figure 1a). The chloroform fraction was subjected to chromatography on silica gel (60-120 mesh, Merck) eluted with ethyl acetate-hexane (7:3) solvent system. Repeated chromatography to give major two pentacyclic triterpenoids i.e., PC-3 and PC-4 [23,24](Figure 1b).

Experimental Animals

The study was carried out on wistar albino rats and swiss albino mice of male sex (Mahaveer Enterprises, Hyderabad.) and was kept at an animal house in V. V. Institute of Pharmaceutical Sciences, Gudlavalleru bearing CPCSEA registration number 1847/PO/Re/S/16/CPCSEA. They were allowed to take standard pellet food and water *adlibitum*. Before the experiment, the rats and mice were kept in standard environmental conditions at room temperature 25-27°C relative humidity (55 ±5)% and 12 h light/12 h dark cycle for 7 days. All rats and mice received humane care in accordance with the "Guide for the Care and Use of Laboratory Animals"[25].
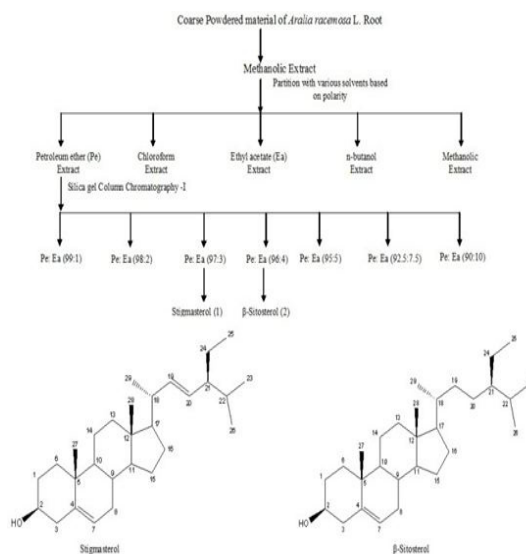

Figure 1 (a): Separation scheme of active compounds from the Petroleum ether fraction of Araliaracemosa L. root and structures of isolated compounds.
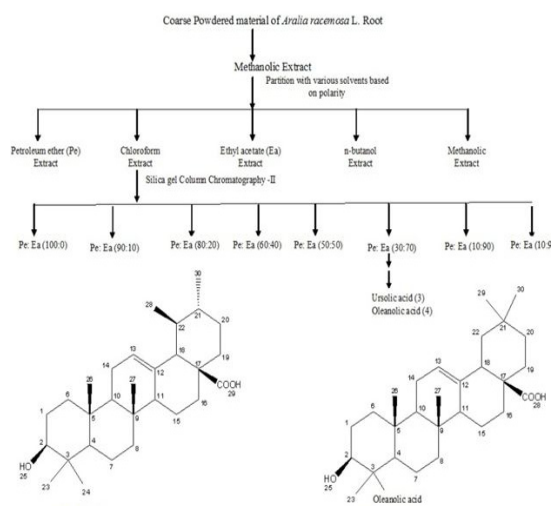

Figure 1 (b): Separation scheme of active compounds from the chloroform fraction of Araliaracemosa L. root and structures of isolated compounds.

Acute Toxicity Study

To evaluate the toxicity of *A. racemosa* extract the acute toxicity study was performed based on OECD (Organization for Economic Cooperation and Development) 423 guidelines up to the dose of 2000 mg/Kg.The rats and mice were observed for 1 h continuously and then hourly for 4 h and finally after every 24 h up to 14 days for any physical signs of toxicity, such as writhing, gasping, palpitation and decreased respiratory rate or mortality. No animals died. Therefore, the LD_{50} is greater than 2000 mg/kg. Pre-

screening investigation with 200 and 400mg per body weight was done [25].

Antinociceptive Activity

The antinociceptive activity of methanolic extract of root was evaluated by using three models: (1) Hot plate method based on pain sensation at 55°C (2) Tail Immersion and (3) Acetic Acid Induced Writhing method

Hot Plate Method

Central analgesic activity was evaluated using the hot plate method. The analgesic activity of methanolic extracts of the root and isolated constituents were evaluated in the rat by the method given by Eddy and Leimback[26]. This method was specifically used to evaluate the central action of the methanolic extract of *A. racemosa*. The rats were divided into four groups comprising five animals each. Group, I received the only vehicle and served as control. Group II received tramadol (2 mg/kg b.wt, i.p.) and served as standard. Groups III and IV were administered with methanolic extract of root of *A. racemosa* in different doses of 200 and 400 mg/kg b.wt, i.p. respectively. Each rat was placed on the hot plate maintained at $(55 \pm 2)°C$, and the response time was recorded as the time at which animals reacted to pain stimulus by either paw licking response or jumping response, whichever appeared first at 0, 30, 60, 90 and 120 min after treatment. The following calculation was

% analgesic activity = $(T_a - T_b / T_b)$ X 100

T_a = Average of reaction time after the administration of extract

T_b = Average of Initial reaction time

Tail Immersion Method

Tail immersion test was performed by immersing extreme 3 cm of the albino-wistar rat tail in a hot water at a temperature of $(55 \pm 0.5)°C$. Within few second each rat was reacted by withdrawing the tail, and the reaction time was recorded with a stopwatch. The drugs were given orally to the respective groups. The experiment was repeated at 0, 30, 60, 90 and 120 min following the administration of extracts and the standard drug [27].

Acetic Acid induced Writhing Method

The antinociceptive action of the extracts was assessesemploying acetic acid induced writhing method in mice. In this approach, acetic acid was given intraperitoneally to the mice to produce pain sensation. As a positive control, any standard NSAID drug may be utilized. In the current investigation, diclofenac sodium utilized to assist the purpose. About 200 and 400 mg/kg bodyweight of the plant extract had beengiven orally to the Swiss albino mice after an overnight fast. Test samples as well as vehicle have been given orally 30 min just before to intraperitoneal administration of 0.7% v/v acetic acid solution (0.1 ml/10g) how-

ever diclofenac sodium has beengiven 15 min ahead to acetic acid injection. Then the animals were subjected an observation table. Each mouse of all groups was noticed separately for counting the number of writhing they produced in 15 min beginning merely 5 min as soon as the intraperitoneal administration of acetic acid solution. Full writhing was not usuallyachieved by the animal due tothe fact occasionally the animals began to give writhing however they failed to complete it. This rudimentary writhing was regarded as half-writhing. Consequently, two half-writhing had beenconsidered as one full writhing. The number of writhes in every treated group was in comparison with control group while diclofenac sodium (5 mg/kg) utilized as a reference substance (positive control). The percentage inhibition was calculated using the formula described below

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

Anti-inflammatory Activity

Carrageenan-Induced Paw Edema in Rats

All the animals were fasted overnight before the commencement of the experiment and only water was permitted*ad libitum*. A mark has been done on the tibiotarsal junction in the right hind foot of the rat so that every time the foot was dipped to a fixed volume. Acute inflammation was created in all the groups through administering 1% w/v, 0.1 ml carrageenan in normal saline into the sub-plantar tissue of the right hind foot, and the linear paw circumference was observed at 1h, 2h and 3h by volume displaced by foot using Plethysmometer (IITC, Life Science, USA).Extracts and standard had been given intraperitoneally, 1 h prior to the administration of carrageenan. The average increase in paw volume of each group was calculated and compared with the control group and the standard group[28].Percentage inhibition of paw edema was calculated using the following formula

$$\% \text{ Inhibition} = \{1 - [(V_d - V_p) / (V_c - V_p)]\} \times 100$$

$V_d - V_p$ = Difference in paw volume after Carrageenan injection (V_d) and before Carrageenan injection (Vp) in Drug-treated group

$V_c - V_p$ = Difference in paw volume after Carrageenan injection (V_c) and before Carrageenan injection (V_p) in Vehicle-treated group

Statistical Analysis

Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA). All results were expressed as Mean ± SD. The data were analyzed by one-way ANOVA followed by Tukey multiple comparison tests.

Results

Acute Toxicity Studies

The methanolic extract of *A. racemosa* was proved to be safe at 2000 mg/kg since even with this high dose no noticeable harm-

ful or deleterious effects and fatality were noticed instantly or up to 14 days of the observation period. The outcome indicates that the extract possesses an LD_{50} of greater than 2000 mg/kg.

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).

Phytoconstituents	Method	Pet. Ether Extract	Chloroform Extract	Ethyl Acetate Extract	Methanolic Extract	n-butanol Extract	Aqueous Extract
Flavonoids	Shinoda Test	-	-	+	+	-	+
	Zn+HCltest	-	-	+	+	-	+
	Lead acetate Test	-	-	+	+	-	+
Volatile oil Alka-loids	Stain test	+	-	-	+	-	+
	Wagner Test	-	+	-	+	-	+
	Hager's Test	-	+	-	+	-	+
Tannins & Phenols	Fecl3 Test	-	-	-	+	+	+
	Potassium dichromate test	-	+	-	+	+	+
Saponins	Foam Test	-	-	-	+	+	+
Phytosterols	Liebermann's test	+	+	-	+	-	-
Carbohydrates	Molish test	-	-	-	+	-	-
Acid compounds	Litmus test	-	-	-	-	-	-
Glycoside	Bontrager's test	-	-	-	+	-	+
Amino acids	Ninhydrin test	-	-	-	+	-	+
Proteins	Biuret test	-	-	-	+	-	+
Fixed oils & fats	Spot test	+	-	-	-	-	-

Table 1: Preliminary phytochemical screening of various extracts of Aralia racemosa L. root.

Characterization of isolated Phytoconstituents

Stigmasterol

White powder, $C_{29}H_{48}O$, MW 412.69. UV λ_{max} (CHCl$_3$) nm: 257; IR (KBr) ν_{max} 3418 (-OH), 2934, 2866, 2339, 1602, 1566, 1461, 1409, 1383, 1251, 1191, 1154, 1109, 1089, 1053, 1020, 791 cm^{-1}; ESMS m/z (%):409.2, 395.3, 335, 161, 144, 121.1, 105.1, 97.1, 85.1, 69, 67.2, 65, 50.2; ^{1}H NMR (400 MHz, CDCl$_3$) δppm: 7.25 (1H, s, OH-2), 5.34-5.35 (1H, d), 5.12-5.18 (1H, m), 4.99-5.05 (1H, m), 3.48-3.56 (1H, m), 2.18-2.31 (2H, m), 1.93-2.09 (3H, m), 1.82-1.87 (2H, m), 1.66-1.75 (1H, m), 1.37-1.54 (13H, m), 1.05-1.31 (m, 7H), 0.99-1.01 (m, 8H), 0.90-0.98 (m, 2H), 0.78-0.85 (m, 9H), 0.66-0.70 (3H, t); ^{13}C NMR (400 MHz, CDCl$_3$) δ ppm: 140.85 (C-4), 138.31 (C-19), 129.40 (C-20), 121.72 (C-7), 77.34 (C-2), 71.86 (C-11), 56.95 (C-17), 56.09 (C-21), 51.29 (C-10), 50.29 (C-12), 42.41 (C-3), 42.30 (C-18), 40.46 (C-13), 39.77 (C-5), 37.35 (C-6), 36.59 (C-8), 32 (C-9), 31.96 (C-1), 31.91 (C-22), 31.77 (C-16), 28.91 (C-15), 25.41 (C-24), 24.41 (C23-),21.24 (C-26), 21.14 (C-14), 21.06 (C-29), 19.42 (C-27), 19.03 (C-25), 12.23 (C-28). PC-01 was identified as Stigmasterol.

β-Sitosterol

White powder, $C_{29}H_{50}O$, MW 414.70; UV λ_{max} (CHCl$_3$) nm: 251; IR (KBr) ν_{max} 3424, 2959, 2936, 2867, 1602, 1565, 1465, 1382, 1332, 1242, 1191, 1154, 1051, 779, 450, 432, 416cm^{-1}; ESMS m/z (%): 411.2, 397.3, 383.3, 311.2, 161.1, 81.2; ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm: 7.30 (1H, s), 5.34-5.35 (1H, d), 4.98-5.19 (1H, m), 3.47-3.55 (1H, m), 2.19-2.31 (2H, m), 1.03-1.30 (9H, m), 1.00 (4H, s), 0.90-0.98 (4H, m), 0.76-0.86 (9H, m), 0.68-0.69 (3H, d), 1.94-2.07 (2H, m), 1.79-1.88 (4H, m); ^{13}C NMR (400 MHz, CDCl$_3$) δppm: 140.84 (C-4), 121.70 (C-7), 71.82 (C-2), 56.94 (C-11), 56.85 (C-17), 50.25 (C-10), 45.95 (C-21), 42.39 (C-7), 42.36 (C-3), 39.87 (C-13), 37.34 (C-5), 36.57 (C-6), 36.19 (C-18), 33.78(C-19), 32.15 (C-8), 31.99 (C-9), 31.97 (C-7), 30.39 (C-22), 26.28 (C-20), 25.90 (C-15), 25.40 (C-16), 24.40 (C-24), 23.2 (C-23), 21.17 (C-26), 21.06 (C-14) 21.06 (C-29), 19.32 (C-27), 19.34 (C-25), 12.11 (C-28). PC-02 was identified as β-Sitosterol.

Ursolic acid

White powder, $C_{30}H_{48}O_3$, MW 456.7; UV λ_{max} (EtOH) nm: 203; IR (KBr) ν_{max} 3450, 2925, 2869, 2339, 1556, 1456, 1387, 1247,

1157, 822, 444, 433, 422, 415cm^{-1}; ESMS m/z (%): 455.2 (M-1)$^+$, 456.2, 457.3; ^{1}H NMR (400 MHz, DMSO) δ ppm: 11.91 (1H, s), 5.14 (1H, s), 4.27 (1H, s),3.01 (1H, s), 2.51 (1H, s), 2.10-2.13 (1H, d) 1.85-1.93 (4H, t),1.26-1.32 (4H, t), 1.05 (1H, s), 0.91-0.92 (8H, d), 0.88 (1H, s) 0.82-0.83 (4H, d), 0.76 (3H, s), 0.69 (4H,s); ^{13}C NMR (400 MHz, DMSO) δ ppm: 178.16 (C-29), 138.17 (C-12), 124.58 (C-13), 76.86 (C-2),56.01 (C-4), 54.82 (C-18), 52.40 (C-11), 47.05 (C-10), 46.82 (C-17), 41.64 (C-9), 40.41 (C-3), 40.21 (C-22), 40 (C-6), 39.79 (C-5), 39.58 (C-19), 39.37 (C-8), 39.16 (C-20), 38.96 (C-1), 38.49 (C-15), 38.46 (C-16), 38.36 (C-23), 38.28 (C-24), 36.53 (C-14), 36.31 (C-30), 32.73 (C-7), 30.2 (C-28), 28.24 (C-26), 27.55 (C-27), 26.99 (C-15). PC-03 was identified as Ursolic acid.

Oleanolic acid

White powder, $C_{30}H_{48}O_3$, MW 456.71; UV λ$_{max}$ (EtOH) nm: 210; IR (KBr) ν$_{max}$3443, 2941, 2862, 1694, 1602, 1566, 1462, 1388, 1364, 1304, 1273, 1208, 1185, 1161, 1093, 1028, 960, 791 cm^{-1}; ESMS m/z (%): 455.3, 456.2; 1H NMR (400 MHz, DMSO) δ ppm: 12 (1H, s), 5.16 (1H, s), 4.27 (1H, s), 3 (1H, s), 2.73-2.77 (1H, m), 1.88-1.95 (1H, s), 1.80-1.83 (2H, m), 1.58-1.70 (3H, m), 1.42-1.50 (8H, m), 1.23-1.38 (5H, m), 1.07-1.10 (4H, t), 0.98-1.01 (1H, m), 0.86-0.93 (14H, m), 0.72 (3H, s), 0.68 (5H, s); ^{13}C NMR (400 MHz) 178.52 (C-28), 143.83 (C-12), 121.49 (C-13), 76.83 (C-2), 54.81 (C-4), 47.09 (C-11), 45.70 (C-10), 45.44 (C-22), 41.32 (C-17), 40.82 (C-22), 40.20 (C-18), 39.99 (C-12), 39.58 (C-9), 39.37 (C-6), 39.16 (C-3), 38.95 (C-5), 38.89 (C-8), 38.36 (C-19), 38.07 (C-21), 36.60 (C-1), 33.34 (C-29), 32.80 (C-30), 32.43 (C-16), 32.09 (C-14), 30.35 (C-23), 28.21 (C-24), 27.20 (C-7), 26.94 (C-26), 14.82 (C-27). PC-04 was identified as Oleanolic acid.

Antinociceptive Activity

Hot plate method

The results (mean±SEM) of hot plate showed that the crude methanolic extract (200 and 400 mg/Kg) exhibited an increase in basal reaction time from 8.7±0.435 and 8.66±0.596 at 0 min to 11.06±0.896 and 12.96±0.353 at 90 min respectively (Table 2).

Treatment	Reaction Time (s)				
	Time after Treatment (min)				
	0	**30**	**60**	**90**	**120**
Control	9.4±0.122	9.53±0.085 (1.27)	9.50±0.083 (0.95)	9.6±0.058 (1.91)	9.4±0.040 (-0.21)
Tramadol (2mg/kg)	9.2±0.15	20.3±0.674* (121.08)	17.1±0.2* (46.19)	13.7±0.680* (49.23)	11.7±0.526* (26.8)
MEAR (200mg/Kg)	8.7±0.435	8.1±0.520^a (-7.01)	9.4±0.241^a (8.16)	11.1±0.896^a (27.12)	10.5±1.237^a (20.68)
MEAR (400mg/Kg)	8.7±0.596	9.4±0.788 (8.54)	10.6±0.626 (22.4)	13±0.353 (49.65)	11.7±0.853 (34.64)

Table 2: Effect of Methanolic extracts of *Araliaracemosa*L. root on Hot-Plate Method. All the values are expressed as mean ± SEM; n = 5 rat in each group, by one way ANOVA followed by Tukey's Multiple Comparison Test. *, p<0.05 significant compared to control and a, p<0.05 significant compared to Standard.

Tail Immersion Method

The tail immersion method revealed a well-marked increase in basal reaction time of 6.48±0.353 in methanolic extract (200 mg/Kg) and 7.10±0.555 in methanolic extract (400 mg/Kg) at 90 min. The basal reaction time of control groups were 3.27±0.112 and 3.38b ±0.125 in tail immersion methods respectively (Table 3). The inhibition was the highest at 90 min at 400 mg/kg dose which was slightly lower than standard.

Treatment	Reaction Time (s)				
	Time after Treatment (min)				
	0	30	60	90	120
Control	3.4±0.162	3.5±0.210	3.7±0.127	3.4±0.163	3.5±0.220
Tramadol (2mg/kg)	3.3±0.224*	18±0.36*	14.9±1.34*	7.6±1.738*	5.8±0.510*
MEAR (200mg/Kg)	3.3±0.112	3.4±0.087	4.1±0.327	6.5±0.353	3.5±0.332
MEAR (400mg/Kg)	3.4±0.125*	4±0.222*	5±0.347*	7.1±0.555*	3.9±0.340*

Table 3: Protective effect of Araliaracemosa methanolic extract on tail withdrawal reflexes induced by tail immersion method in rat. All the values are expressed as mean ± SEM; n = 5 mice 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.

Acetic Acid Induced Wrighting

Control group showed maximum writhing (24.4±1.140), while Methanolic extract of *A. racemosa* at a dose of 200 and 400 mg/Kg demonstrated a significant antinociceptive effect against acetic acid induced writhing, inhibiting pain by 39.34% and 63.93% as compared to the control respectively (Table 4, Figure2). Diclofenac at 5 mg/kg had 69.67% ($p<0.001$) inhibition of wrighting response.

Anti-inflammatory activity

Carrageenan-Induced Paw Edema in Rats

The results of the inhibitory effect of methanolic extracts on carrageenan-induced rat paw edema are shown in Figure3 and Table 5. The crude methanolic extract (200 and 400 mg/Kg) inhibited the edema volume of 11.85% and 17.28% with a mean edema volume of 2.08±0.184 and 2.06±0.056 at 2 h respectively. The carrageenan control induced inflammation with a mean edema volume from 2.48±0.298 at 0 h to 3.5±0.2 at 3 h. The standard drug (reference), diclofenac sodium showed inhibition of edema volume of 29.94%.
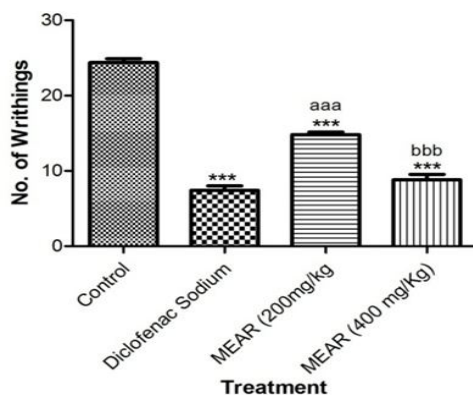

Figure 2: Effect of Methanolic extract of *Araliaracemosa* L. on acetic acid induced Writhing in Mice. Results are presented as mean±SEM, (n=5), ***, $p<0.001$ versus Control, aaa, $p<0.001$ versus Diclofenac sodium and bbb,$p<0.001$ versus MEAR (200 mg/Kg).

Group	Difference in Paw Volume at hrs(Mean±SD)			% Inhibition at hrs		
	1 hr	2 hr	3 hr	1 hr	2 hr	3 hr
Carrageenan (1%0.1ml)	2.48±0.298	2.95±0.3	3.5±0.2	-	-	-
Carrageenan+Diclofenac (200 mg/Kg)	2.08±0.095*	1.95±0.211*	1.74±0.106*	19.75	24.42	29.94
Carrageenan + MEAR (200 mg/Kg)	2.15±0.175	2.08±0.184	2.06±0.348	8.5	11.85	8.54
Carrageenan + MEAR (400 mg/Kg)	2.16±0.065	2.06±0.056	2.02±0.27	9.12	17.28	13.98

Here, MEAR: Methanolic Crude Extract of *Aralia racemosa* L. M-1= Mice 1, M-2 = Mice 2,M-3 = Mice 3, M-4 = Mice 4, M-5 = Mice 5.

Table 5: Effect of Methanolic extract of *Araliaracemosa* L. root on Carrageenan-Induced Paw edema method. Results are presented as Mean±SEM, (n=5), ***, $p<0.001$ versus Control.
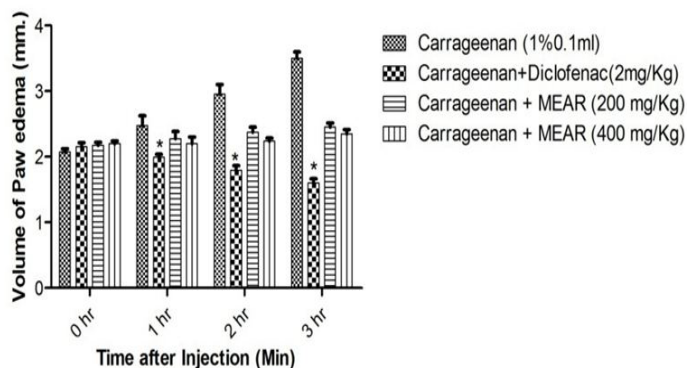
Figure 3: Effect of Methanolic extract of Araliaracemosa L. root on Carrageenan-Induced Paw edema method. Results are presented as Mean±SEM, (n=5), ***, p<0.001 versus Control.

Discussion and Conclusion

The safety of the herb had been affirmed by the acute toxicity study, carried out based on OECD test guidelines-423, which revealed that the root extract at a higher dose of 2000 mg/kg developed no indication of toxicity or fatality in rat and mice.

It is hypothetic that alcoholic solvents effectively permeate cell membranes, enabling the extraction of formidable amounts of endocellular elements compared with lower polarity solvents including chloroform, which are constrained to extracting mainly extra-cellular material. In this way, alcohols dissolve primarily polar constituents along with moderate and lower polarity constituents. Therefore, methanol utilized as the solvent of preference in the current research for extracting the root of *A. racemosa* [29].

In our Investigation, for those antinociceptive and anti-inflammatory models, male rat and mice had been utilized. The primary reason for utilizing male sex regarding inflammation models is a result of the fact that estrogen exerts anti-inflammatory activity. In terms of pain, studies have demonstrated that variance in experimental pain reactivity is present in the various stages of the menstrual cycle in health females of reproductive age group. A meta-analysis about reports of experimental pain reactivity indicated females in the follicular stage possessed greater pain thresholds compared to later phases. Therefore, hormonal changing within the various stages of the menstrual cycle involving the female rat and mice might have an effect on pain reactivity. Consequently, to prevent this kind of distinction, male rat and mice were utilized [30,31].

In the current investigation, we evaluated the effects of the crude methanolic extract of *A. racemosa* against the animal models to ascertain the anti-nociceptive and anti-inflammatory effects. The anti-nociceptive activity was assessed through acetic acid writhing in mice, hot plate, and tail immersion assays in the rat, whereas anti-inflammatory activity had been carried out by carrageenan-induced paw edema in rats.

The antinociceptive activity of the root extract of *A. racemosa* was evaluated by using the hot plate, tail-immersion and acetic acid induced writhing methods. Hot Plate and Tail immersion tests are a central model. Tail immersion test provides discernment for opioid derived analgesics. The potency of agents in this particular model demonstrates antinociceptive activity through central mechanism acting via the opioid receptor and is extremely related with the alleviation of human pain perception. Acetic acid-evoked writhing method, alternatively, is employed for the evaluation of the peripheral nociceptive activity. The constriction reaction of the abdomen entails the release of arachidonic acid from cellular phospholipids through cyclooxygenase and has usually been related to prostanoids, for instance, elevated levels of Prostaglandins (PGE2 and PGF2α) in peritoneal fluids along with Lipooxygenase(LOX) product. PGs stimulates abdominal constriction through triggering and sensitizing the peripheral chemo-sensitive nociceptors, that are mainly accountable for resulting in inflamed pain [32].

This investigation suggested that *A. racemosa*methanolic extract possesses both peripheral and central antinociceptive attributes. Its peripheral antinociceptive activity has been deduced through its inhibitory effects on chemical (acetic acid) evoked nociceptive stimuli. At 400 mg/kg (63.93%), the peripheral antinociceptive action of the extract on acetic acid-evoked pain has been observed to be similar to 5 mg/kg (69.67%) of diclofenac. The centrally acting protective effects of the extract have been confirmed by the hotplate as well as tail immersion tests results. The tail immersion test illustrated that the biological activities were mediated through mu (μ) opioid receptors instead of kappa (κ) and delta (δ) receptors [33].

In all analgesic models, the dose (400 mg/kg) was found to be more effective than the (200 mg/kg) dose. In the case of hot plate model, 400 mg/kg dose of the extract produced a rapid onset of action (Table 2) and the highest percentage protection than 200 mg/Kg dose of the extract. The dose was also shown more effective than 200 mg/kg in reducing the writhing response (Figure 2). This possibly shows that 400 mg/kg dose of the extract is the optimum dose in producing both central and peripheral analgesic effect.

In the current research, carrageen a utilized as a chemical agent to induce edema compared to formalin for the acute inflammation study due to the fact carrageen an is found to be more trenchant in producing edema compared to formalin, showing a more trustworthy model for inflammation[34]. In the acute inflammation model, the root extract of *A. racemosa* demonstrated the inhibitory effect on edema formation (Figure 5). This impact began from the 1 h and was retained until 3 h. Its effect throughout the first phase implies that it activity entails inhibition associated with mediators like 5-HT and histamine. Diclofenac produced a more obvious edema inhibition in the late stage, which is in commitment with the undeniable fact that NSAIDs restricts inflammation considerably more in the second phase [35]. Production of arachidonic

metabolites through the COX-2 enzyme is the significant factor liable for the late phase of carrageen an evoked inflammation. The extract considerably inhibited paw edema in the second phase furthermore. These specify together revealed that the root extract of *A. racemosa*might exert its activity furthermore through curbing COX and subsequent PG synthesis.

The preliminary phytochemical screening in our investigation revealed that methanol root extract of *A. racemosa*possibly consists of secondary metabolites such as tannins, volatile oils, flavonoids, saponins, triterpenes and glycosides.

The anti-nociceptive effect of several plants has been gained via their flavonoid [36], terpenoids [37], tannin [38], phenol [35], steroid [35], alkaloid [35] and saponin constituents [36]. Therefore, it may be stated that the antinociceptive activity of the root extract of *A. racemosa*notices in Hot plate, tail immersion and acetic acid induced writhing models in the current research might be as a result of existence of saponins, flavonoids, steroids i.e., β-Sitosterol and Stigmasterol [39], triterpenes i.e., ursolic acid and Oleanolic acid [40].

The anti-inflammatory action of several plants relates to the existence of saponins [38], flavonoids [41], terpenoids [42], triterpenes [15], glycosides [43] and tannins [35]. Thus, it may be stated that the anti-inflammatory activity of the extract noticed in carrageen an and formalin-induced paw edema models might be due to the occurrence of glycosides, triterpenes i.e., ursolic acid and Oleanolic acid, flavonoids, steroids i.e., β-Sitosterol and Stigmasterol and saponins.

The co-existence of both anti-nociceptive and anti-inflammatory effects that was noticed with this extract is well described for many NSAIDs. Therefore, it is fascinating that this extract behaved like NSAIDs in this research that correlates perfectly with the conventional application of the plant.

Conclusion

The antinociceptive and anti-inflammatory action of *A. racemosa*root extract might be because to its secondary metabolites, that potentially include flavonoids, saponins, triterpenes i.e., Ursolic acid and Oleanolic acid, steroids i.e., β-Sitosterol and Stigmasterol and glycosides as well as rationalize the conventional utilization of this plant in folk medicine, being an antinociceptive and anti-inflammatory agent. There is evidence that crude plant extracts often have greater in their pharmacological activity than isolated constituents at asame dose. But, further phytochemical, as well as biological tests, are recommended to determine the other active chemical constituents accountable for the antinociceptive and anti-inflammatory activities.

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