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FREE RADICAL SCAVENGING POWER POTENTIATES ANALGESIC, ANTI-INFLAMMATORY AND CNS DEPRESSANT ACTIVITY OF *ALANGIUM SALVIIFOLIUM* WANG

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ABSTRACT: *Alangium salviifolium* wang. Family Alangiaceae used as a traditional medicinal plant. The objective of this study was to evaluate the comparative phytochemical and *in-vitro* antioxidant activity along with analgesic, anti-inflammatory, and central nervous system (CNS) depressant activities of peel and seed extract of *Alangium salviifolium* fruit. Quantitative phytochemical analysis for total phenolic and flavonoid content measured by Folin-Ciocalteu and Aluminium chloride method was maximum for peel (104.03 ± 0.40 mg of GAE /gm and 34.33 ± 0.24 mg of CAE /gm of dried extract respectively. In the DPPH assay method, total antioxidant capacity and reducing power assay showed significant ($P< 0.05$) antioxidant activity based on IC_{50} value. Peel extract significantly ($P< 0.01$) inhibited writhing (44.44 %) induced by acetic acid in mice compared to seed (37.45 %). The anti-inflammatory activity measured by the carrageenan-induced mice paw edema method was observed to be significant ($P< 0.01$, $P< 0.05$) at 2nd, 3rd and 4th hour. The percent inhibition of peel and seed was 34.97% and 35.50%, which was very close to standard Ibuprofen (37.80%). Our investigation also revealed a significant reduction of locomotor activity of mice in open field and hole cross test compared to standard diazepam. A significant ($P< 0.01$, $P< 0.05$) decline in locomotion at the doses of 75 mg/kg for peel and seed (6 ± 1 and 0) after 120 min which was less than the standard (8.33±0.33). It could be accomplished that *A. salviifolium* may be a promising source of natural antioxidant that potentiate different pharmacological actions.

INTRODUCTION: Free radicals are generated due to imbalance in the body's homeostatic phenomenon between oxidants and antioxidants. The imbalance leads to oxidative stress and the production of reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical and hydrogen peroxide having the root cause of aging and various human diseases like arteriosclerosis, stroke, diabetes, cancer and neurodegenerative diseases such as Alzheimer's and Parkinsonism [1, 2]. Oxidative stress is the potential source of damage to DNA, lipids, sugars and proteins and antioxidant defense mechanisms, resulting in cellular injury [3].

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Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents, and quenches of singlet oxygen formation [4]. However, to keep the integrity of the neuronal cells and for normal functioning, less quantity of reactive oxygen species (ROS) is required as the elevated level of the radicals can lead to neuronal cell death [5].

One principal cellular function of antioxidants is to prevent damage caused by the ROS [6], which has multiple functions in biological systems like maintenance of cell integrity and cell signaling pathways [7]. Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is always a warning signal and primarily protective but often causes many discomforts and many adverse effects [8]. Oxidative stress and inflammation are two linked pathophysiological processes; stimulated by abnormal production of free radicals and proinflammatory mediators causing extreme cellular damage involved in the pathogenesis of chronic human diseases. This oxidative stress-associated inflammation can be neutralized and detoxified by the antioxidant system like superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR), as well as nonenzymatic molecules such as reduced glutathione (GSH) [9, 10].

Fruits are an optimal mixture of antioxidants as they contain phenolic compounds, anthocyanins, carotenoids, and other flavonoids [11]. Scientific evidence indicates intake of fruits has been associated with a reduced risk of cardiovascular disease and cancer at several sites [11]. Currently, available synthetic antioxidants like butylated hydroxyl anisole (BHA), Butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone, and gallic acid esters have been suspected of causing or prompting negative health effects. Hence, there is a trend to substitute them with naturally occurring antioxidants. Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical-induced tissue injury. Many plant extracts and phytochemicals have shown free radical scavenging properties, but generally, there is still a demand to find more information concerning the antioxidant potential of plant species.

Alangium salviifolium wang. is a deciduous, rambling shrub or a tree belonging to the family Alangiaceae. This family consists of one genus with twenty-two species. Different parts of this species are medicinally used for various diseases in India, China, and the Philippines. The root is used in diarrhea, paralysis, piles, and vomiting [12]. The root is also useful for external application in acute rheumatism, leprosy, and inflammation [13]. Fruits are sweet and treat burning sensations, constipation, and hemorrhage. The leaves are used as a poultice in rheumatism; stem barks exert a biphasic action on the blood pressure in cats at lower doses and marked hypotension in higher doses. The plant has been reported for its anti-tubercular, antispasmodic, and anti-cholinesterase activity [14]. It was also reported that the stem bark of *Alangium salvifolium* (Linn. F) Wang had anti-fertility activity in wester female rats [15]. Various phytochemical research revealed the presence of alkaloids, including ipecac alkaloid and benzo pyrido quinolizidine alkaloids. It is also known to produce alangiside, a tetrahydroisoquinoline monoterpene glucoside [16]. Recent phytochemical studies reported that several flavonoids, phenolic compound, irridoid glycosides, and oxyoglucoside of some alcohol were isolated from this plant [17].

A new alkaloid, ankorine was isolated from leaves [18]. Plant is also rich in tetrahydroisoquinoline monoterpene glycoside. *e.g.,* alangiside-1 oripecoside- 2 whose structures are closely related to the ipecac alkaloid [19]. To the best of the author's knowledge, there are no previous scientific reports on the antioxidant, analgesic, anti-inflammatory, and CNS depressant activity of peel and seed of *A. salviifolium.* Therefore, the present study was undertaken to investigate the analgesic, anti-inflammatory, and CNS-depressant activity due to a phytochemical and antioxidant compound of ethanol extract of fruits of *A. salviifolium* in Swiss albino mice.

MATERIALS AND METHODS:

Collection and Authentication of Sample: The fresh fruits of *A. salviifolium* were collected from the roadside of Kazla, Rajshahi, Bangladesh, on May 2019. An expert taxonomist identified the plant at the Department of Botany, University of Rajshahi, where a voucher specimen (Voucher No. 173) was deposited. Then the fruits were washed, the peel was separated from the seed, and shade-dried for several days with occasional sun drying. The plant material was also oven dried at 40^0 C for 24 hours for better grinding. Then the dried plant material is pulverized into a coarse powder with a grinding machine. The powder material was poured

into an airtight container and placed in a cool, dark, and dry place for extraction.

Preparation of the Extract: About 230 gm of peel and 295 gm of seed powdered materials were placed separately in fresh, amber-colored extraction bottles and soaked with 1.5 L of ethanol. The sealed bottles were preserved for 15 days with frequent shaking and stirring.

The mixture was filtered separately through a fresh cotton plug and finally with a Whatman No. 1 filter paper. Then the filtrate was concentrated using a rotary evaporator (BibbySterilin Ltd., UK) under reduced pressure. The mixture looked like a gummy greenish and yellowish color concentrate, referred to as crude ethanol extract of *A. salviifolium* peel (ASP) and seed (ASS). The extract was stored in an airtight amber glass vial for further use.

Drugs and Chemicals: All the chemicals used in this study were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Diazepam (Square Pharmaceuticals Ltd.), Ibuprofen (ACI Pharmaceuticals Ltd.), and Diclofenac Sodium (Beximco Pharmaceuticals Ltd.) was used for conducting the tests.

Experimental Animals: Healthy Swiss albino mice, six weeks of age and weighing about 25-30 g of both sexes, were obtained from the central animal house of the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh. Under ambient temperature, all animals were kept with 12-hour light followed by a 12-hour dark cycle.

Before research, the animals were acclimatized for 7 days and fed formulated rodent food and water. The study with an animal was conducted in the department of Pharmacy, Varendra University, Rajshahi, Bangladesh, after getting approval from the Institutional Ethics Committee, Varendra University, Rajshahi (Ref. VU/ERC/2021-2022/004).

Chemical Analysis: Chemical Analysis was done in the drugs and toxins research division, Bangladesh Council of Scientific and Industrial Research (BCSIR), Rajshahi, Bangladesh. The ethanolic extract was subjected to qualitative chemical analysis to identify various phytoconstituents using standard procedure [20].

Quantitative Phytochemical Evaluation:

Total Phenolic Content: The total phenol content was determined by Folin- Ciocalteu's assay method using gallic acid as standard [21]. In this procedure, 0.5 ml of plant extract was mixed with 2 ml Folin-Ciocalteu's reagent (FCR) (Previously diluted with water 1:10 v/v) and 2 ml of 7.5% sodium carbonate solution. The tubes were vortexes for 1 min and allowed to stand for 20 min at 25° C for color development. Then, the absorbance of the sample was measured against a blank at 760 nm using UV-Spectrophotometer (Shimadzu, USA). The experiment was repeated three times at each concentration for precision. Total phenol content was expressed in terms of gallic acid equivalent to GAE per gm of dry extract.

Total Flavonoid Content: Total flavonoid determination of *A. salviifolium* peel and the seed was done by Aluminum Chloride colorimetric method [22] using catechin as standard.0.5 ml of samples/ standard was taken in a test tube, and added 1.5 ml of methanol and 2.8 ml of distilled water. After 5 min, 100 µl of 10% $AlCl_3$ and 1M potassium acetate solution were added thoroughly. The test tubes were incubated at room temperature for 30 minutes to complete the reaction. The absorbance of the solution was measured at 420 nm against blank using UVspectrophotometer. The experiment was repeated three times at each concentration for precision. The total flavonoid content of the extractives was expressed as mg of CAE/gm of dried extract.

Antioxidant Assay: The antioxidant activity of *A. salviifolium* peel and seed extract was determined by the following procedure.

Total Antioxidant Capacity: Total antioxidant capacity of *A. salviifolium* peel and seed extract was determined by the method described by Prieto *et al.,* [23] using catechin as standard. The assay was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. In a test tube, 0.5 ml of sample/standard at different concentrations (20-100 µg/ml) was

mixed with 3 ml reaction mixture containing 0.6 M sulphuric acid, 28 Mm sodium phosphate, and 1% ammonium molybdate. All the test tubes were incubated at 95°C for 10 min to complete the reaction. Sample absorbance was measured at 695nm by UV spectrophotometer using a reaction mixture as a blank solution. The experiment was repeated three times for each concentration.

Reducing Power Capacity Assay: The reducing power of the test samples was determined according to the Oyaizu [24] method. The reductive ability was measured by the reduction of $FeCl_3$ in the presence of antioxidants. Ascorbic acid is used as a standard substance at various concentrations (5 – 40 µg/ml). In a test tube, 0.25 ml sample/standard solution was taken at different concentrations. To this 0.625 ml of 0.2M phosphate buffer & 0.625 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$] was added and incubated at 50^0C for 20 min. Then 0.625 ml solution of 10 % of Trichloroacetic acid (TCA) was added into test tubes. The total mixture was then centrifuged at 3000rpm for 5 min, after which 1.8 ml of supernatant was collected & mixed with 1.8 ml of distilled water and 0.36 ml of 0.1% ferric chloride solution. The absorbance of the solution was measured at 700 nm against blank using UV spectrophotometer.

DPPH Assay: The DPPH (1,1-diphenyl-2-picryl hydrazyl) was used to detect the free radical scavenging potency of experimental parts as described by Blois [25] and Desmarchelier *et al.,* [26]. Plant extract's hydrogen atom donating ability was determined by the decolorization of methanol solution in DPPH. In the presence of antioxidant, DPPH changes the violet / purple color in methanol solution to shades of yellow. A solution of 0.1 mM DPPH in methanol was prepared. 2.4 ml of this solution was mixed with 1 ml methanol solution of extract at various concentrations (12.5 – 150 µg/ml). To complete the reaction, the solution was thoroughly vortexed and left in a dark place at room temperature for 30 minutes. The absorbance was measured against blank at 517 nm using a UV spectrophotometer. Butylated hydroxyl toluene (BHT) was standard for this test. The following equation calculated the percentage of DPPH radical scavenging activity:

$$\% \text{ DPPH radical scavenging activity} = \{(A_o - A_1) / A_o\} \times 100$$

Where, A_o is the absorbance of the control and A_1 is the absorbance of the extract/standard. Then % of inhibition was plotted against concentration and IC_{50} was calculated from the graph. The experiment was repeated three times at each concentration.

Determination of Analgesic Activity:

Acetic Acid-Induced Writhing Method: Evaluation of the analgesic activity of the extract by the acetic acid-induced writhing model in mice was conducted according to the procedure described by Whittle *et al.,* [27]. The acetic acid-induced writhing method is an analgesic behavioral observation assessment method demonstrating noxious stimulation in mice. The test samples (ethanol extract of *A. salviifolium* peel and seed at 100 and 200 mg/kg body weight), standard (Diclofenac Sodium 10 mg/kg body weight), and control (1% Tween 80 in water at the dose of 10 ml/kg body weight) were administered orally.

After 30 min, 0.7% acetic acid was injected intra-peritoneally. Approximately 5 minutes after the injection of acetic acid, a wave of contraction and elongation of the abdominal musculature, referred to as writhing, was started. The number of writhing for the next 10 minutes was counted for each mouse. The percent inhibition (% analgesic activity) was calculated by:

$$\% \text{ Inhibition} = [(A-B)/A] \times 100$$

Where, A=Average number of writhing of the control group, and B=Average number of writhing of the test or standard groups.

Determination of Anti-Inflammatory Activity:

Carrageenan-Induced Paw Edema Method: Evaluation of anti-inflammatory activity by the Carrageenan-induced paw edema method in mice was conducted according to the procedure described by Winter *et al.,* [28]. The six groups of mice (each containing 3 mice) were taken for the test. To create edema, 0.1 ml of 1% carrageenan was injected intraperitoneally into the mice's left hind paw plantar surface. After 30 minutes of carrageenan injection, the test samples, control (1% tween 80 in water), and standard (Ibuprofen) were administered orally with the help of a feeding needle. A thirty minutes interval was given to ensure proper absorption of the administered substances. The volume of paw edema was

measured at 1h, 2h, 3h and 4h using a vernier caliper to determine the diameter of edema.

Determination of CNS Depressant Activity:

Open Field Test: This experiment was carried out in accordance with a modified method of Gupta *et al.*, [29]. The mice in the control group received the vehicle 1% Tween 80 in water (at the dose of 10 ml/kg body weight), and the standard group received diazepam orally at the dose of 5 mg/kg. The test group received the crude ethanol extract at 25 and 75 mg/kg body weight, respectively. The animals were then placed on the floor of an open field (100 cm x 100 cm x 4 cm) and divided into a series of squares with alternative color black and white. After administering test drugs, the number of squares visited by each animal was counted for 3 min duration starting at 0, 30, 60, 90, and 120 min.

Hole Cross Test: The method described by Takagi *et al.*, [30] was followed to conduct this test using a cage (30 cm x 20cm x 14cm) with a steel partition fixed in the middle. A hole of 3 cm diameter was made at the height of 7.5 cm in the middle of the cage. The animals were divided into control, standard, and test group. The test groups received crude ethanol extract at the dose of 25 mg/kg and 75 mg/kg body weight orally. In contrast, the control group received vehicle (1% Tween 80 in water) at 10ml/kg body weight orally. The standard group received diazepam at the dose of 5mg/kg body weight orally with the help of a feeding needle. The number of mouse passages from one chamber to another through the hole was recorded for 3 min at 0 min, 30 min, 60 min, 90 min, and 120 min after oral administration of test drugs.

Statistical Analysis: Results of all chemical analyses were expressed as mean ± standard deviation and for biological analysis were mean ± S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's post Hoc test and independent-sample t-test was carried out with SPSS 20.0 for windows software and the results obtained were compared with the control group. *P*-value (***P*< 0.01, **P*< 0.05) was considered to be statistically significant.

RESULTS: Qualitative chemical analysis for the identification of various phytochemicals confirms the presence of alkaloid, phenol, flavonoid, steroid, and saponin in ethanol extract of *A. salviifolium*.

Total Phenolic (TPC) and Flavonoid Content (TFC): The total phenolic and flavonoid content of ASP and ASS was calculated using a standard curve for gallic acid and catechin, respectively shown in **Table 1**. The result revealed that the amount of total phenol and flavonoid content were higher in peel extract. The phenol content for peel was104.03 ± 0.40 mg of GAE /gm of dried extract in compared to seed extract 85.74 ± 1.82 mg of GAE /gm at 100 µg/ml with reference to standard curve($Y = 0.0657x + 0.0481$, $R^2 = 0.9984$). Flavonoids content in seed extract was 16.80 ± 0.18 mg of CAE /gm at 100 µg/ml which was lower than peel 34.33 ± 0.24 mg of CAE /gm of dried extract with reference to standard curve ($Y = 0.0033x - 1E\text{-}04$, $R^2 = 0.9829$). These phytochemical compounds were known to support bioactive chemicals in medicinal plants and are responsible for this plant extract's antioxidant activities.

TABLE 1: PHENOLIC AND FLAVONOID CONTENT OF *A. SALVIIFOLIUM*

Polyphenols	Peel extract (ASP)	Seed extract (ASS)
TPC (mg of GAE /gm of dried extract)	104.03 ± 0.40	85.74 ± 1.82
TFC (mg of CA /gm of dried extract)	34.33 ± 0.24	16.80 ± 0.18

TPC = Total phenolic content; TFC = Total flavonoid content. n=3, each value is the mean of three analysis ± standard deviation.

Total Antioxidant Capacity (TAC) and Ferrous Reducing Power Capacity (FRPC): The TAC and FRPC of different parts of *A. salviifolium* showed considerable antioxidant activity compared to standard catechin (CA).

At the concentration of 100 µg/ml, the absorbance of CA, ASP and ASS extract was 0.619 ± 0.002, 0.465± 0.002 and 0.367 ± 0.002 respectively.

With increasing concentration, peel and seed showed moderate to high FRPC. At 30 µg/ml concentration, the absorbance of standard ascorbic acid (AA), ASP and ASS was 2.440 ± 0.001, 1.889 ± 0.005and 1.033 ± 0.002 whereas at 40 µg/ml, the absorbance was 3.434 ± 0.003, 2.453 ± 0.001 and 1.682 ± 0.003 respectively. A higher absorbance value indicated higher reducing power.

These results demonstrated that the ethanol extract of ASP possesses higher TAC and FRPC than ASS, almost resembling the standard CA and AA **Table 2.**

TABLE 2: RESULTS OF TOTAL ANTIOXIDANT CAPACITY AND FERROUS REDUCING POWER CAPACITY (FRPC)

Extractives	TAC		FRPC	
	At 80 µg/ml	At 100 µg/ml	At 30 µg/ml	At 40 µg/ml
ASP	0.325 ± 0.002	0.465 ± 0.002	1.889 ± 0.005	2.453 ± 0.001
ASS	0.203 ± 0.002	0.367 ± 0.002	1.033 ± 0.002	1.682 ± 0.003
CA	0.493 ± 0.003	0.619 ± 0.002	-	-
AA	-	-	2.440± 0.001	3.434 ± 0.003

ASP and ASS represented *A. salviifolium* peel and seed, CA and AA representing standard Catechin and Ascorbic acid respectively. Each value is the average of three analysis (n= 3, X ± SD).

DPPH Radical Scavenging Activity: Free radical scavenging activity of peel and seed of *A. salviifolium* was shown **Fig. 1** in the form of percent of scavenging using BHT as a standard. Among the extracts, ASP possessed the higher activity. At a concentration of 100µg/ml, the free radical scavenging activity of BHT, ASP and ASS was 82.04 ± 0.23 %, 64.87 ± 0.94% and 59.39 ± 0.49 %. On the other hand, at 150 µg/ml concentrations, the scavenging activity of ASP and ASS was 72.20 ± 0.22 % and 66.75 ± 0.26 %, whereas at the same concentration, the standard BHT was 88.51 ± 0.57%. The IC_{50} value of BHT, ASP, and ASS ethanol extract was 11.5, 38, and 60 µg/ml, respectively. Therefore, BHT's free radical scavenging activity and different extracts were in the order of BHT >ASP >ASS.

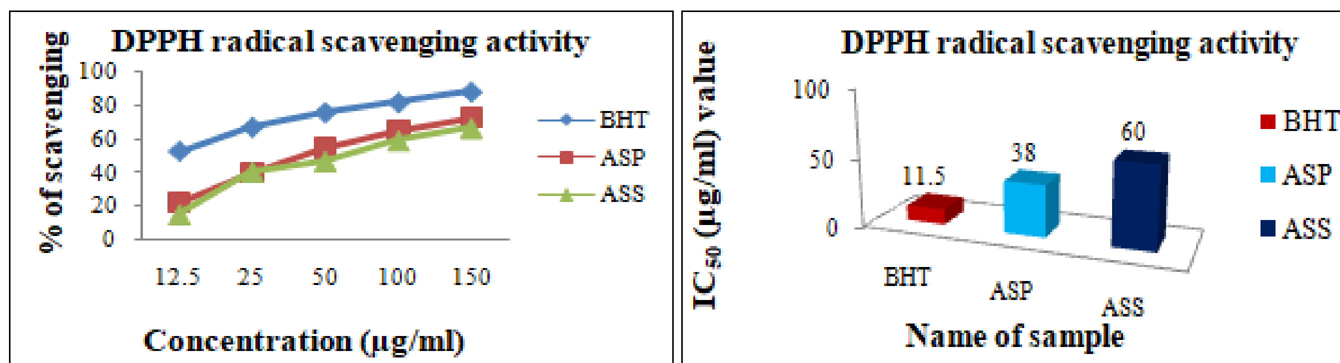

FIG. 1: DETERMINATION OF A) DPPH RADICAL SCAVENGING ACTIVITY AND B) IC_{50}VALUE OF *A. SALVIIFOLIUM* PEEL AND SEED. EXPERIMENT WAS PERFORMED AS TRIPLICATE (n=3, p< 0.05) FOR ALL TESTED DOSES

Analgesic Activity: In mice, the acetic acid-induced writhing method was applied to determine the analgesic activity of ethanolic extract of different parts of *A. salviifolium*. All doses of the extract showed significant (***P*< 0.01) reduction of writhing provoked by the intraperitoneal administration of acetic acid in a dose-dependent manner shown in **Table 3**. All tested doses showed a significant reduction of writhing when the dose was increased from 100 mg/kg to 200 mg/kg doses. At 100 mg/kg doses, ASP and ASS exhibited 35.05 % and 26.34 % of inhibition, whereas, at 200 mg/kg doses, this extract exhibited 44.44 % and 37.45 % of inhibition, respectively, compared to standard Diclofenac Na (47.74%).

TABLE 3: ANALGESIC ACTIVITY DETERMINATION AT DIFFERENT DOSES OF *A. SALVIIFOLIUM* PEEL AND SEED EXTRACT

Animal group	Dose (mg/kg body weight)	No of writhing ± SEM	Percent inhibition (%)
Control	1ml/10gm	81 ± 0.58	
Diclofenac Na	10 mg/kg	42.33** ± 0.67	47.74
ASP	100 mg/kg	52.61**± 1.53	35.05
ASP	200 mg/kg	45**± 1	44.44
ASS	100mg/kg	59.66**± 2.08	26.34
ASS	200 mg/kg	50.67**± 2.52	37.45

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (***P*< 0.01).

Anti-inflammatory Activity: Anti-inflammatory activity of ethanolic extract of *A. salviifolium* fruits were evaluated using carrageen an induced paw edema method in mice. There was a dose and time dependent significant reduction (***P*< 0.01, **P*< 0.05) of paw edema at 100 and 200 mg/kg of extract compared to Ibuprofen (10mg/kg) over a period of 4 hours, as shown in **Table 4**. The percent inhibition activity of ASP and ASS at 100 mg/ kg was 32.67 % and 33.44 %, whereas at 200 mg/ kg doses was 34.97% and 35.50% compared to standard Ibuprofen (37.80%) after 4 hours.

TABLE 4: RESULT OF ANTI-INFLAMMATORY ACTIVITY ANALYSIS OF *A. SALVIIFOLIUM* AT DIFFERENT DOSES

Animal group	Dose(mg/kg)	Paw edema diameter in mm					% of inhibition of paw edema			
		Before treatment	1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
Control	1ml/10gm	8.17± 0.60	15.83± 0.20	15.37± 0.26	14.00± 0.53	13.07± 0.15	-	-	-	-
Ibuprofen	10mg	10.27± 0.50	12.20± 0.17**	10.23± 0.24**	9.10± 0.17*	8.13± 0.23**	22.93	33.4 4	35	37.8 0
ASP	100 mg	10.23± 0.15	13.4± 0.15**	12.7± 0.26**	10.73± 0.15*	8.8± 0.17**	15.35	17.3 7	23.3 6	32.6 7
ASP	200 mg	9.33± 0.44	12.43± 1.09*	11.07± 0.34**	10± 0**	8.5± 0.29**	21.48	27.9 8	28.5 7	34.9 7
ASS	100 mg	11.07± 0.58	13.67± 0.23**	10.63± 0.86*	9.5± 0.58*	8.7± 0.42**	13.64	30.8 4	32.1 4	33.4 4
ASS	200 mg	8.4± 0.47	12.67± 0.33**	10.33± 0.17**	9.17± 0.44**	8.43± 0.13**	19.96	32.7 9	34.5	35.5 0

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (***P*< 0.01, **P*< 0.05).

CNS Depressant Activity: After statistical analysis of the experimental data, it was observed that in open field test, the number of squares travelled by the mice was significantly decreased in the test group throughout the study period **Table 5**.

A noticeable decline in locomotion of test animals at 30 min was observed, which continued up to 120 min in a dose-dependent manner. Test animals showed significant (***P*< 0.01, **P*< 0.05) decrease in number of movements at the doses of 75 mg/kg for ASP and ASS (6 ± 1 and 0*) after 120 min compared to control (4.33±0.67), which was less than the standard (8.33±0.33). The hole cross-test also observed the reduction of locomotor activity for the plant extract. A reduction in a number of movements was observed at the 2nd observation (30 min) period and was sustained up to the 5th observation period (120 min).

TABLE 5: RESULT OF CNS DEPRESSANT ACTIVITYOF *A. SALVIIFOLIUM PEEL AND SEED*

Animal group	Dose (mg/kg of body weight)	Number of movements							
		After several times in open field test				After several times in hole cross test			
		After 30 min	After 60 min	After 90 min	After 120 min	After 30 min	After 60 min	After 90 min	After 120 min
Control	10ml/kg	78± 1.52	40.67± 1.20	26.66± 0.88	4.33± 0.67	2.33± 1.20	2.33± 0.88	3.67± 0.67	3.33± 0.88
Diazepa m	5 mg/kg	68.67± 0.88*	50.33± 1.20*	18.67± 0.89*	8.33± 0.33*	3.67± 0.88	1.33± 0.67	0*	0*
ASP	25 mg/kg	60 ± 1.52**	39.33 ±1.20	14.65 ± 0.88**	10 ± 1**	9± 0.58**	6.67± 0.33**	4.33 ± 0.67	1.66 ± 0.33
ASP	75 mg/kg	41.66±1.20 **	32 ± 1.52*	9 ± 0.58**	6 ± 1	5.67 ± 1.20	4.33 ± 0.33	0.67± 0.67*	1 ± 0*
ASS	25 mg/kg	63.33 ± 1.20*	41.33 ± 0.88	20.67 ± 0.88*	15.33± 0.58**	2.33± 0.88	1± 0.58	0**	0**
ASS	75 mg/kg	17.67± 1.20**	9.67 ±0.33**	1 ± 1**	0*	2.67± 1.20	1± 0.58	0.33± 0.33*	0*

All values are expressed as mean ± standard error mean (SEM). Differences with respect to the control group were calculated using the independent sample T –test (***P*< 0.01, **P*< 0.05).

The extract diminished the movement of the tested animals in a dose-dependent manner **Table 5**. After 120 min of administration, ASP and ASS showed significant (*$P< 0.05$) CNS depressant activity for all doses compared to control (3.33± 0.88). These results were also similar to the standard drug diazepam at the dose of 5 mg/kg at the same time.

DISCUSSION: In this research work, we wanted to reveal various pharmacological activities of ethanolic extract of *A. salviifolium* fruits in mice along with phytochemical and antioxidant potency. Estimating bioactive compounds may be useful for treating chronic as well as infectious disease [31] as these secondary metabolites (phytochemical) present in plant extract were responsible for various pharmacological actions. The antioxidant properties of different extracts of *A. salviifolium* were significantly corroborated by the phytochemical constituents of the extracts.

Our study revealed the presence of alkaloids, flavonoids, phenol, saponin, and steroid of *A. salviifolium* peel and seed. Phenolic compounds are powerful chain-breaking antioxidants and due to the presence of the hydroxyl group, they have a high scavenging ability [32]. Flavonoids are a group of polyphenolic compounds exhibiting several biological effects such as anti-inflammatory, anti-hepatotoxic, antiulcer, anti-allergic, antiviral, and anticancer activities [33]. They are capable of effectively scavenging the reactive O_2 species because of their phenolic hydroxyl groups and are also potent antioxidants [34].

Qualitative and quantitative analysis showed the presence of high phenolic and flavonoid content in peel and seed extracts of *A. salviifolium,* which contributed directly to the antioxidant activity by neutralizing the free radicals. The plant extract also revealed the presence of saponins which were known to produce an inhibitory effect on inflammation [35]. Various studies reported that alkaloid and their synthetic compound possessed analgesic, antispasmodic and antibacterial activity [36]. The significant antioxidant activity shown by the extract can be attributed due to the presence of phenolic in the extract. Free radical is an unpaired electron molecule involved in bacterial and parasitic infection, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, aging, and neoplastic diseases [37]. Our results demonstrated that the different extracts of peel and seed of *A. salviifolium* possess free radical scavenging activity with different *in-vitro* models like DPPH scavenging activity, total antioxidant capacity and ferrous reducing power capacity assays.

The result of DPPH scavenging activity assay in this study indicated that the plant was potentially active. This result suggested that the plant extract contained compounds capable of donating hydrogen to a free radical to remove the odd electron responsible for radical's reactivity[38]. In our study, the IC_{50} value of BHT, ASP and ASS ethanol extract was 11.5 µg/ml, 38 and 60 µg/ml, respectively. Among these, peel extract exhibited more radical scavenging activity in minimum concentration.

Nasrullah *et al.,* reported in a study that ascorbic acid (AA), dichloromethane (DASR), and chloroform (CASR) extract of *A. salviifolium* root have shown potent antioxidant effect with the IC_{50} value of (12.58±1.45) µg/ml, (11.26 ± 1.29) µg/ml and (16.48 ± 1.12) µg/ml respectively [39]. The result of the total antioxidant capacity and ferrous reducing power capacity of *A. salvifolium* peel and seed suggest a significant indicator of potential antioxidant activity. The electron donation capacity of the extract stabilizes the free radical and breaks the free radical chain reaction, thus producing a reducing power of extract [40].

Pain sensation in the acetic acid-induced writhing method is elicited by triggering localized inflammatory response resulting release of free arachidonic acid from tissue phospholipid [41] *via* cyclooxygenase (COX) and prostaglandin biosynthesis [42]. Increasing prostaglandin levels within the peritoneal cavity enhance inflammatory pain by increasing capillary permeability [43]. The acetic acid-induced writhing method was found efficient for assessing peripherally active analgesics effect. The agent reducing the number of writhing will render an analgesic effect by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition [44]. *A. salviifolium* peel and seed exhibited 44.44% and 37.45 % of inhibition, respectively, compared to standard Diclofenac Na (47.74%) at the doses of 200 mg/kg.

Therefore, it is likely that our extract might exert peripheral antinociceptive action by interfering with the irritant or by inhibiting the synthesis, release or antagonizing the action of pain mediators at the target sites. A study by Tanwer *et al.,* [45] showed that the analgesic activity of *Alangium salviifolium* Linn. root ethanolic extract was dose-dependent. The writhe movements were reduced 63.0 ± 0.70 in control to 47.5 ± 0.48 at 100mg/gdw of crude ethanolic extract and 18.5±1.7 at 300 mg/gdw concentration. The analgesic activity of 300mg/gdw is comparable to diclofenac sodium. carrageenan induced paw edema method is a well-known experimental animal model for acute inflammation and is believed to be biphasic. The early phase is mainly mediated by histamine, serotonin, and increased synthesis of prostaglandins. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandin produced by tissue macrophages [46].

There was a dose and significant time-dependent reduction (**$P< 0.01$, *$P< 0.05$) of paw edema at 100 and 200 mg/kg of extract compared to Ibuprofen (10mg/kg). The percent inhibition activity of ASP and ASS at 200 mg/ kg doses was 34.97% and 35.50% compared to standard Ibuprofen (37.80%) after 4 hours.

This finding suggested that the possible mechanism of the observed anti-inflammatory activity might be its ability to reduce the release of histamine, serotonin, or kinin-like substances or the biosynthesis of prostaglandins. In a study, Ahad *et al.,* [47] reported that the root extract of *A. salviifolium* showed anti-inflammatory activity in rats' carrageenan-induced paw edema model. Significant percent inhibition of paw edema was observed within 6 hours, supporting its traditional use for the treatment of inflammation.Two neuropharmacological models, open field and hole cross test, were used to study the CNS depressant activity of *A. salviifolium* fruit extract. In evaluating CNS drug action, observing its effect on the animal's locomotor activity. The activity measures the level of the CNS's excitability and decreased activity resulting from CNS depression [48]. In this study, open field and hole cross-test results demonstrated the movement's lowering after 30 minutes and continued up to 120 minutes.

All doses tested animals possessed a maximum no of lowering movement from 90 minutes than the standard drug. The result also observed that seed extract caused more CNS depressant and anxiolytic activity than peel and standard diazepam. Several plants have been reported to have CNS depressant and anxiolytic activity due to triterpenoids [49], saponins [50] and flavonoids [49, 50].

As mentioned in results, ASP and ASS possessed various phytochemical substances such as triterpenoid saponins alkaloids, steroid and flavonoids. CNS depressant and anxiolytic activity of *A. salviifolium* was supposed to be attributed to these phytochemicals found in the extract. Triterpenoid saponins are reported to have agonistic / facilitatory activities at $GABA_A$ receptor complex [51, 52] which led to the hypothesis that they act as benzodiazepine-like molecules. This is supported by their behavioral effects in animal models of CNS depression and anxiety [49, 50].

From the results, we can conclude that ASP and ASS possess considerable analgesic and anti-inflammatory activity along with CNS depressant and anxiolytic activity due to the presence of various phytochemicals and antioxidant compounds and thus may be used in a variety of painful and excitatory conditions.

CONCLUSION: On the basis of the findings, it can be stated that the ethanolic extract of *A. salviifolium* fruits contain various phytochemical compounds and may be the promising source of antioxidant activity which was comparable to that of the standard compound. The results also suggested that the fruits have potent analgesic, anti-inflammatory and CNS depressant activity.

Therefore, *A. salviifolium* fruit is a natural source of antioxidant substances of high significance, and consumption of the fruit can offer health benefits in the prevention of diseases caused by oxidative stress, diabetes and cancer. However, further research is needed to determine the responsible bioactive components for the above-mentioned pharmacological activities.

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