Anti-inflammatory activity of some medicinal plant

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***Abstract*- Medicinal shops and their secondary metabolites are precipitously used in the treatment of conditions as a reciprocal drug. Inflammation is a pathologic condition that includes a wide range of conditions similar as rheumatic and vulnerable- mediated conditions, diabetes, cardiovascular accident, and etcetera. We introduce some sauces which their anti-inflammatory goods have been estimated in clinical and experimental studied of factory material viz., A. obesum (Flower). Performed Determination of Antioxidant exertion, In- vitro Anti-inflammatory exertion, and Heat convinced Haemolysis, Protein denaturation, Proteinase Inhibitory conditioning and Lipoxygenase Inhibition exertion. The results attained may be useful in strengthening the standardization of the named botanicals. Also, the named shops can be considered as a resource for searching new anti-inflammatory agents.**

***Keywords*: Antioxidant Activity, Cyclooxygenase, Antioxidants, Cytotoxicity, Carrageenan, PMA.**

1. INTRODUCTION

Irritation is the immune device's reaction to dangerous stimuli, including pathogens, damaged cells, toxic compounds, or irradiation, and acts with the aid of casting off injurious stimuli and starting up the recuperation procedure [1,2,3]. Inflammation is consequently a defense mechanism this is critical to health. Normally, during acute inflammatory responses, cellular and molecular occasions and interactions effectively decrease approaching damage or contamination. This mitigation technique contributes to healing of tissue homeostasis and backbone of the acute irritation. However, uncontrolled acute infection may also end up continual, contributing to an expansion of persistent inflammatory diseases [4, 5].

At the tissue level, infection is characterized by redness, swelling, warmness, ache, and lack of tissue characteristic, which end result from neighborhood immune, vascular and inflammatory cell responses to contamination or injury [6, 7]. Crucial microcirculatory occasions that arise during the inflammatory technique include vascular permeability adjustments, leukocyte recruitment and accumulation, and inflammatory mediator launch [8, 9]

Various pathogenic elements, together with contamination, tissue harm, or cardiac infarction, can induce infection by way of causing tissue harm. The etiologies of irritation may be infectious or non-infectious. In reaction to tissue damage, the body initiates a chemical signaling cascade that stimulates responses aimed toward restoration affected tissues. Those signals prompt leukocyte chemo taxis from the overall flow to sites of harm [10].

1.1. Inflammatory Response Mechanisms

The inflammatory response is the coordinate activation of signaling pathways that regulate inflammatory mediator levels in resident tissue cells and inflammatory cells recruited from the blood. Irritation is a commonplace pathogenesis of many chronic illnesses, including cardiovascular and bowel sicknesses, diabetes, arthritis, and cancer. Although inflammatory reaction methods depend on the suitable nature of the initial stimulus and its place inside the body, all of them proportion a common mechanism, which may be summarized as follows:

1) Cellular surface pattern receptors recognize unfavorable stimuli;
2) Inflammatory pathways are activated;
3) Inflammatory markers are launched; and
4) Inflammatory cells are recruited.

1.2. Pattern recognition receptor activation

Microbial systems called pathogen-associated molecular patterns (PAMPs) can cause the inflammatory reaction via activation of germ line-encoded sample-recognition receptors (PRRs) expressed in both immune and no immune cells [11, 12]. a few PRRs additionally apprehend numerous endogenous signals activated in the course of tissue or cellular harm and are called chance-associated molecular patterns (DAMPS). DAMPs are host bio molecules that can initiate and perpetuate a non-infectious inflammatory reaction [13, 14]. Disrupted cells also can recruit innate inflammatory cells in the absence of pathogens by using releasing DAMPs [15].

Lessons of PRR families consist of the Toll-like receptors (TLRs), C-type lectin receptors (CLRs), retinoic acid-inducible gene (RIG)-I-like receptors (RLRs), and NOD-like receptors (NLRs) [16]. TLRs are a circle of relatives of notably conserved, mammalian PRRs that participate in the activation of the inflammatory response. greater than ten participants of the TLR circle of relatives had been recognized, and TLRs are the properly-studied of the recognized PRRs. Transmission of PAMPs and DAMPs is mediated via myeloid differentiation aspect-88 (MyD88) at the side of TLRs. Signaling through TLRs turns on an intracellular signaling cascade that results in nuclear translocation of transcription factors, which includes activator protein-1 (AP-1) and NF-κB

or interferon regulatory component three (IRF3) discern, DAMPs and PAMPs percentage receptors, consisting of TLR4, suggesting similarities between infectious and non-infectious inflammatory responses [17, 18].

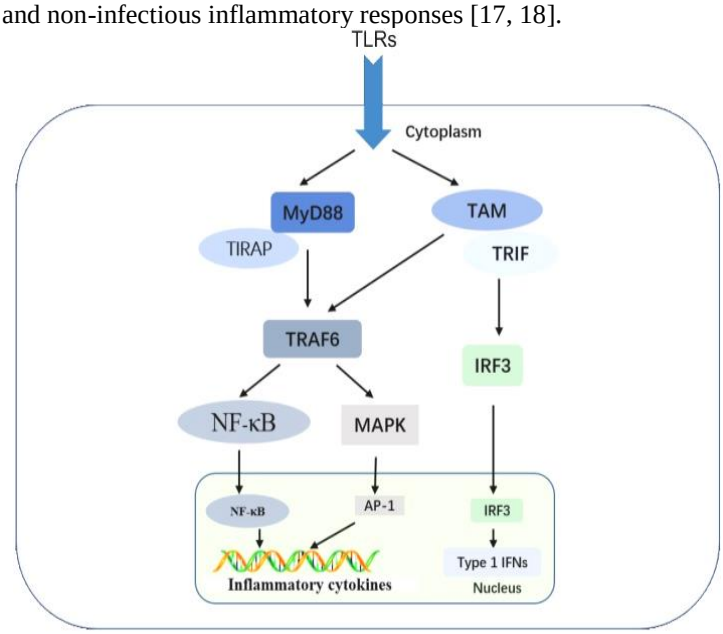

Figure 1.2: TLR Signaling, representing inflammatory cytokines attack by NF kB and AP-1 pathway.

1.3. Activation of inflammatory pathways

Inflammatory pathways affect the pathogenesis of a number of chronic illnesses, and involve not unusual inflammatory mediators and regulatory pathways. Inflammatory stimuli set off intracellular signaling pathways that then spark off production of inflammatory mediators. Primary inflammatory stimuli, which includes microbial merchandise and cytokines consisting of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumors necrosis aspect-α (TNF-α), mediate inflammation via interplay with the TLRs, IL-1 receptor (IL-1R), IL-6 receptor (IL-6R), and the TNF receptor (TNFR) [19, 20].

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Collection and authentication of Plant Materials

The isolation of active chemical constituent of *A. obesum* flowers were done by collecting them forms the local botanical garden of Ajmer, Rajasthan, India. The plant was authenticated by Herbal garden and vouchers specimen number DL/20/03.

2.1.2. Chemicals

Methanol, ethyl acetate, hexane, chloroform and butanol were purchased from Merck (Dramstadt, Germany). Sodium sulfate and charcoal were purchased from Sigma-Aldrich. Vacuum liquid chromatography was performed on silica gel $60GF_{254}$ and flash column chromatography on silica gel 60 (E. Merck 1.09385, Model Aldrich), while size exclusion chromatography was achieved by using Sephadex LH-20 (Ameshem Bioscience), pre-swollen in the specified solvent before loading on to the column.

2.2.Methods

2.2.1. Extraction, Fractionation and Purification

Powder of flower of *A. obesum* was extracted in methanol and the crude extract was portioned between aqueous, ethyl acetate, butanol, methanol and 70: 30 (methanol: water). The EA-phase was dried with sodium sulfate (anhydrous), charcoaled and evaporated invacuo to give a thick residue which was subjected to normal pressure column chromatography on gradient system by using hexane, chloroform and methanol and afforded to five pure compound Honghelin, Obeside B and C, Betulin, Rosmerinic acid and flavones.

2.2.2. Preliminary Phytochemical Screening of Extract

The methanolic extracts of flowers of *A. obesum* were subjected to preliminary phytochemical tests to detect the presence of alkaloids, steroids, saponins, glycosides, anthraquinones, tannins, terpenoids, coumarins, carbohydrates and flavonoids using standard techniques.

a. Alkaloids

Small portion of alcoholic extract stirred separately with a few drops of dilute hydrochloric acid and then filtered. The filtrate is then tested carefully with various alkaloid reagents such as:

- **Mayer's Reagents**
  Alkaloids give precipitate with Mayer's reagents. One ml of Mayer's reagent (Potassium mercuric iodide solution) was added to 1 ml extract, whitish yellow or cream-colored precipitate indicated the presence of alkaloids.
- **Dragendroff's Reagents**
  Alkaloids give orange brown precipitate with Dragendorff's reagents. One ml of Dragendorff's reagent (Potassium bismuth iodide solution) was added to 1 ml extract, an orange-red precipitate indicated the presence of alkaloids.
- **Hager's Reagents**

Alkaloids give yellow colored precipitate with Hager's reagents. In to the 1 ml extract, 3 ml of Hager's reagent (saturated aqueous solution of picric acid) was added, a yellow colored precipitate indicated the presence of alkaloids.

- **Wagner's Reagents**
  Alkaloids give reddish brown precipitate with Wagner's reagents. In to 1 ml extract, 2 ml of Wagner's reagent (iodine in potassium iodide) was added and the formation of reddish-brown precipitate indicated the presence of alkaloids.

b. Carbohydrates and Glycosides

A small quantity of each extract dissolved separately in distilled water and was filtered. The filtrate is subjected to the following test for Carbohydrates.

- **Molisch's Test**
  One ml of α-naphthol solution and concentrated sulphuric acid was added in 2 ml of the extract, through the side of the test tube. The formation of purple or reddish violet color at the junction of the two liquids reveals the presence of carbohydrates.
- **Fehling's Solution**
  Equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (potassium tartrate and sodium hydroxide in distilled water) reagent was mixed along with few drops of extract solution and boiled, a brick red precipitate of cuprous oxide forms.
- **Benedict's test**
  Extract solution was treated with few drops of Benedict reagent (alkaline Solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate forms, if reducing sugar is present.
- **Tollen's test**
  To 100 mg of compound add 2ml of Tollen's reagent and heat gently; a silver mirror is obtained inside the wall of the test tube. It indicates the presence of aldose sugar.
- **Keddes reagent test**
  Cardenolides give blue or violet color with this reagent which fads after 1-2 hour. This reagent is prepared by mixing equal volume of 0.2% solution of 3, 5 di-nitro benzoic acid in 100ml of 0.5 N KOH solutions in 50% methanol.
- **Legal's test**
  Treat the extract with pyridine and add alkaline sodium nitroprusside solution, blood red color appears.
- **Keller Killiani Test**
  1gm of powdered drug extracted with 10ml of 70% alcohol for few minutes and filtered. To 5 ml of this filtrate 10 ml of hydrogen peroxide solution and 0.5 ml of strong solution of lead acetate is added. Precipitate thus obtained is filtered. Filtrate is shaken with 5 ml of chloroform layer is separated and to this 1ml of mixture of 1 volume of 5% ferric sulfated and 99 volume of glacial acetic acid is added. To this mixture 1 or 2 drops of concentrated sulfuric acid is added. Appearance of blue color confirms the presence of de-oxy sugars.
- **Antimony trichloride Test**
  Solution of glycoside is heated with antimony tri chloride and trichloroacetic acid to obtain blue or violet color. Both cardenolides and bufadinolides give this test.
- **Bontrager's Test**
  Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonical layer acquires rose pink color shows the presence of glycosides.
- **Bromine water test**
  Test solution when treated with bromine water gives yellow precipitate.

c. Test for Saponins

- **Foam test**
  Dilute 1ml of extract with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. A one-centimeter layer of foam indicates the presence of Saponins.

d. Test for Triterpenes

- **Hirschorn Test**
  1 ml of all the extracts was warned with trichloroacetic acid. A yellow color, which change to red was formed indicated the presence of triterpenes.
- **Libermannstorch mora sky Test**
  1ml of all the extracts heated with 0.5 ml of acetic acid and added a drop of sulphuric acid. A red color that changed to blue color indicates the presence of Triterpenes.

e. Test for Steroids

- **Liebermann Burchard Test**
  2 ml of the extracts were dissolved separately in a 0.5 ml chloroform and 1 ml of acetic anhydride. After these 1 or 2 drops of concentrated sulfuric acid were added to the above solution. A strong blue or violet color changed gradually to green, indicated the presence of steroids.
- **Salkovaski Test**
  1 ml of all the extracts was dissolved separately in 0.5 ml of chloroform and to this added a drop of concentrated sulfuric acid. Formation of red color was observed in all the extracts indicated the presence of steroids.

f. Test for flavonoids

- **Shinoda test (Magnesium hydrochloride reduction test):**

To the test solution add few fragments of magnesium ribbon and add conc. hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue color appears after few minutes.

- **Zinc hydrochloric reduction test:**
  To the test solution add a mixture of zinc dust and conc. hydrochloric acids. It gives red color after few minutes.
- **Alkaline test:**
  To the test solution add few drops of sodium hydroxide solution. Formation of an intense yellow color, which turns to colorless on addition of few drops of dilute acid indicate presence of Flavonoids.

g. Test for tannins and phenolic compounds

- **Gelatin test:**
  Test solution with 1% gelatin solution containing 10% sodium chloride gives white precipitate.
- **Ferric chloride test:**
  Test solution gives blue green color with ferric chloride.
- **Vanillin hydrochloride test:**
  Test solution when treated with few drops of vanillin hydrochloride reagent gives purplish red color.
- **Proteins and Free Amino Acids**
  Small quantity of alcoholic extract was dissolved in to the few ml of water and subjected to Millon's, Biuret and Ninhydrin tests.
- **Gums and Mucilage**
  About 10 ml of extract were slowly added to the 25 ml of absolute alcohol with constant stirring, filtered, dried in air and examine for its swelling properties.

h. Tests for detection of inorganic chemical constituents

The Ash prepared from drug material was added to 50% v/v HNO_3 solution. It was kept for one hour and was filtered. The filtrate was used to perform the following:

- **Tests for Calcium**
  To 10ml. filtrate one drop of dilute *ammonium hydroxide and ammonium oxalate* solution were added. White precipitate of calcium oxalate was formed. Precipitate formed was soluble in HCl but insoluble in acetic acid.
  To 2ml of filtrate ammonium carbonate was added. The formation of white precipitate that was insoluble in ammonium chloride solution indicated the presence of Calcium.
- **Tests for Magnesium**
  The calcium oxalate precipitate formed in the test for calcium was filtered and separated. Then the filtrate was heated and cooled. To the filtrate solution, the solution of sodium phosphate in dilute ammonia was added which gave white crystalline precipitate to confirm the presence of magnesium.
  White precipitate was formed with *ammonium carbonate solution* but not with ammonium chloride solution, when magnesium was present.
- **Test for Sodium**
  A thick paste of ash of drug was prepared with concentrated hydrochloric acid. The paste was taken on a platinum wire loop that was introduced into flame of a burner. *Golden yellow flame* was observed which indicated the presence of Sodium in the drug.
- **Tests for Potassium**
  To 2-3 ml test solution, few drops of *cobalt nitrite* solution was added. Yellow precipitate of potassium cobalt nitrite was observed.
  Flame test: Gave violet color to the flame
- **Tests for Sulphate**
  To 5 ml filtrate, few drops of 5% *barium chloride* solution was added. The formation of white crystalline *barium sulphate* precipitate which was insoluble in HCl indicated the presence of Sulphate. With *lead acetate solution* white precipitate was formed which was insoluble in NaOH.
- **Test for Phosphate**
  To 5ml solution, few drops of *ammonium molybdate solution* were added. It was then heated for 10 min and cooled. Yellow crystalline precipitate of ammonium phosphomolybdate was observed to indicate the presence of Phosphate.
- **Tests for Iron**
  To 5ml of test solution, few drops of 2% *potassium ferrocyanide* were added. Dark blue coloration indicated the presence of Iron in the drug. To 5ml of test solution few drops of 5% *ammonium thiocyanate* were added. Development of blood red color indicated the presence of Iron.
- **Tests for Chloride**
  To 3ml test solution few drops of 10% $AgNO_3$ were added. White precipitate of $AgCl_2$, which was soluble in dilute ammonia, indicated the presence of Chloride.
  To about 5 to 7 ml filtrate, 3 to 5ml of *lead acetate solution* was added. Formation of white precipitate indicated the presence of Chloride.
- **Test for Carbonate**
  Solution of *magnesium sulphate* was added to the test solution. Formation of white precipitate indicated the presence of Carbonate.
- **Test for Nitrates**

Red fumes were liberated when test solution was warmed with sulphuric acid and copper. Solution of ferrous sulphate was added and it yielded no brown color whereas when sulphuric acid was added a brown color was produced at the junction of two liquids which indicated the presence of Nitrates.

2.2.3. Determination of antioxidant Activity

The evaluation of the antioxidant activity was carried out by free radical scavenging method (DPPH and ABTS) and ferric reducing antioxidant power (FRAP)

- **DPPH scavenging activity (DPPH)**
  The antiradical power of substances was measured by the decrease of absorption of DPPH (1,1-Diphenyl-2- picrylhydrazyl). To 950 µl of a methanol solution of DPPH (0.1 mM) were added to 50 µl of the plant extract. After 30 min, the absorbance of the mixture was measured at 517 nm. The ability to scavenge DPPH radical was calculated using the following formula:

$$\%\ \boldsymbol{Inhibition\ of\ DPPH} = \frac{\boldsymbol{A_c - A_s}}{\boldsymbol{A_c}} \boldsymbol{X\ 100}$$

  A_c = Absorbance of control
  A_s = Absorbance of sample

- **ABTS scavenging activity (ABTS)**
  The technique is based on the scavenging of $ABTS^{\bullet+}$ [(3-ethyl benzothiazoline 6-sulfonic acid) diammonium salt] radical cation which was generated by mixing solutions of ABTS (7 mmol/L) and potassium persulfate (2.45 mmol/L). The mixture was then incubated in the dark at room temperature for 16h. The product was diluted for optimal absorbance of 0.7 at 734 nm. The decolorization of the $ABTS^{\bullet+}$ solution by 100 µg/mL of the test sample or reference compound (Trolox) was monitored by a decrease in absorption at 734 nm during 30 min. The antioxidant activity expressed in µM trolox equivalent antioxidant capacity (TEAC)/ mg dry weight (DW).
- **Ferric reducing antioxidant power (FRAP)**
  The method is based on reduction of ferric tripyridyltriazine (Fe^3 – TPTZ) to ferrous complex tripyridyltriazine (Fe^2 – TPTZ) by an antioxidant in acidic pH. The ferrous Fe (II) complex -TPTZ develops a blue color with maximal absorbance at 593 nm. The methodology of Benzie and Strain20 was used. FRAP mixture consists of 10 parts of acetate buffer solution (300 mM) at pH 3.6, 1 volume of 10 mmol/l 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mmol/ l HCl and 1 volumes of a solution of $FeCl_3.6H_2O$ (20 mM). To 2 ml of the FRAP mixture were added 10 µl of the plant extract. After incubation of 15 min at room temperature, the absorbance was measured at 593 nm. The calibration range was prepared with Trolox. Results are expressed as µmol Trolox equivalent antioxidant capacities (TEAC)/mg DW.

2.2.4. Determination of *In-Vitro* Anti-inflammatory Activity

2.2.4.1. Preparation of the Extracts

One gram of freeze-dried sample was mixed with 20 mL of methanol (70% *v*/*v*) and vortexes at high speed for about five minutes, and then centrifuged (Hettich, EBA 20) for 10 min at 4500 rpm, and the supernatants were collected. Then, the extracts were filtered through a filter paper (WhatmanNo.42), and then the residue that remained was re-extracted with 70% methanol with the same procedure, and the supernatants obtained were combined with those from the first extraction. The solvent in the combined mixture was evaporated in a rotary evaporator (HAHNVAPOR, Model HS-2005 V, HAHNSHIN Scientific, Seoul, South Korea) at 40 °C. The prepared concentrated extracts of flowers of *A. obesum* dried at 40 °C for 12 h in an oven, and then dried extracts were stored at −18 °C in air-tight screw-capped glass vials, until used for the anti-inflammatory bioassays, within one week. The extracts collected were dissolved in methanol to obtain a concentration of 3 mg/mL for each assay.

2.2.4.2. Membrane Lyses Assay

a. **Preparation of Erythrocyte suspension:** Whole human blood was collected from a healthy human subject. The blood was centrifuged at 3000 rpm for 5 min in heparinized centrifuge tubes, and washed three times with equal volume of normal saline (0.9% NaCl). After the centrifugation, the blood volume was measured and reconstituted as a 10% (*v*/*v*) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). Composition of the buffer solution (g/L) used was NaH_2PO_4 (0.2), Na_2HPO_4 (1.15), and NaCl (9.0).

b. **Heat-Induced Hemolysis:** Briefly, 0.05 mL of blood cell suspension and 0.05 mL of hydromethanolic extracts of flowers were mixed with 2.95 mL phosphate buffer (pH 7.4). The mixture was incubated at 54 °C for 20 min in a shaking water bath. After the incubation, the mixture was centrifuged (2500 rpm for 3 min), and the absorbance of the supernatant was measured at 540 nm using a UV/VIS spectrometer (Shimadzo 1800, Mumbai, India). Phosphate buffer solution was used as a control for the experiment.
   The level of haemolysis was calculated using the following equation:
   % Inhibition of Haemolysis = 100 X (1-A2/A1)
   Where A1 = absorption of the control, and A2 = absorption of test sample

c. **Effect of Protein Denaturation:** The reaction mixture (5 mL) consisted of 0.2 mL of 1% bovine albumin, 4.78 mL of phosphate buffered saline (PBS, pH 6.4), and 0.02 mL of extract, and the mixture was mixed, and was incubated in a water bath (37 °C) for 15 min, and then the reaction mixture was heated at 70 °C for 5 min. After cooling, the turbidity was measured at 660 nm using a UV/VIS spectrometer (Shimadzo 1800, Mumbai, India). Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula:
   % Inhibition of Haemolysis = 100 X (1-A2/A1)
   Where A1 = absorption of the control, and A2 = absorption of test sample

d. **Proteinase Inhibitory Activity:** Briefly, the reaction solution (2 mL) consisted of 0.06 mg trypsin, 1 mL of 20 mMTris-HCl buffer (pH 7.4), and 1 mL test sample (0.02 mL extract 0.980 mL methanol). The solution was incubated (37 °C for 5 min), and then 1 mL of 0.8% (*w*/*v*) casein was added, and the mixture was further incubated for an additional 20 min. At the end of incubation, 2 mL of 70% perchloric acid was added to terminate the reaction. The mixture was centrifuged, and the absorbance of the supernatant was measured at 210 nm against buffer as the blank. Phosphate buffer solution was used as the control. The percentage inhibition of protein denaturation was calculated by using the following formula:
% Inhibition of Haemolysis = 100 X (1-A2/A1)
Where A1 = absorption of the control, and A2 = absorption of test sample

2.2.4.3. Lipoxygenase Inhibition Assay

Briefly, a mixture of a solution of sodium borate buffer (1 mL, 0.1 M, pH 8.8) and lipoxygenase (10 µL, final concentration 8000 U/mL) was incubated with 10 mL flower extract in a 1 mL cuvette at room temperature (30 ± 2 °C) for 5 min. The reaction was initiated by the addition of 10 µL linoleic acid substrate (10 mmol). The absorbance of the reaction solution was measured at 234 nm using a UV/VIS spectrometer (Shimadzo 1800, Mumbai, India). Phosphate buffer solution was used as the control, and the percentage inhibition of lipoxygenase was calculated using the following equation:

% Inhibition = 100 X (absorbance of the control- absorbance of the sample)/absorbance of the control

3. RESULT AND DISCUSSION

3.1. Preliminary Phytochemical Screening of Extract

The extract obtained after extraction of the plant material viz., *A. obesum* (Flower) was subject to phytochemical screening which revealed the present of various active phytocontituents. The result was presented in Table 3.1.

Table 3.1: Preliminary Phytochemical Screening of *A. Obesum*

S. No.	Chemical Constituents	Methanolic	Aqueous	Pet. Ether	Chloroform
1	Alkaloids	+	+	+	+
2	Carbohydrates	+	+	+	+
3	Glycosides	+	+	+	+
4	Steroids	+	+	+	+
5	Flavonoids	+	+	+	+
6	Saponins	+	+	+	+
7	Fixed oils and fats	-	-	-	-
8	Tannins	+	+	+	+
9	Proteins and amino acids	+	-	+	-
10	Terpenoids	+	-	-	-

Abbreviations: (+) is positive; (-) is Negative

3.2. Determination of Antioxidant Activity

Table 7.3 shows the antioxidant activity evaluated by three techniques. The IC_{50} of the DPPH radical scavenging was 135.6 ± 16.07 µg/ml respectively for the plants flowers. For the ABTS test, flowers exhibited values of 1448.06 ± 25.41 µM TEAC/mg DW respectively. Whether for the DPPH test or the ABTS test, differences between the male and female trees were not significant. With respect to FRAP, the MEOAs of flowers recorded significantly the values of 66.55 ± 4.52 µM TEAC/mg DW. The literature reports that the antioxidant activity of plant extracts depends on both the nature of the test and the phenolic compounds. For example, quercetin showed very low antioxidant activity in the FRAP test but very high activity in the DPPH and ABTS assays. Indeed, polyphenols are compounds which have more than one hydroxyl group attached to one or more benzene rings. They are usually encountered as esters or glycosides rather than as free compounds. Then, the antioxidant activity of the Polyphenols depends on the arrangement and the number of hydroxyl groups in the phenolic rings and their connections with the saccharine. Polyphenols could act as reducing initiators, chelating agents or by the prevention of oxidative reactions caused by active singlet oxygen. For our study, the antioxidant activity could be explained by the presence of cardenolide, triterpenoids, pregnanes, carbohydrate, phenolic acids, flavonoids, and amino acids. The previous chemical studies deduced that *A. obesum* is a very rich plant with different compounds, including Flavonoids triterpenes, pregnanes, cardenolides, cardiac glycosides, and carbohydrate. Finally, due to the presence of a single hydroxyl in the B ring. These data make it possible to conclude that the antioxidant activity recorded in our work results from the synergy of the various phenolic compounds, even with other non-phenolic antioxidants.

Table 3.2: Antioxidant activity of *A. obesum* flower extract assessed by DPPH, ABTS and FRAPS methods

Activity	DPPH (%)	IC_{50} (µg/ml)	ABTS (µM TEAC/ mg DW)	FRAP (µM TEAC/mg DW)
A. Obesum Flowers	82.53 ± 0.68	135.6 ± 16.07	1448.06 ± 25.41a	66.55 ± 4.52

Values are expressed in mean ± SEM. Means in each column followed by different letters are significantly different (P<0.05).

3.3.*In-vitro* Anti-inflammatory Activity

3.3.1. Heat induced Haemolysis

The percent inhibition of heat-induced haemolysis of red blood cells at different concentrations of flower extract of *A. obesum* (DW), in the range of 25–100 µg/mL, is shown in Figure 3.2. Methanolic extracts of extract of flowers were able to inhibit haemolysis in a concentration-dependent manner. Inhibition % of haemolysis from these leaf extracts were within the range from 9.23% to 24.2%, at the concentrations of 25–100 µg/mL.

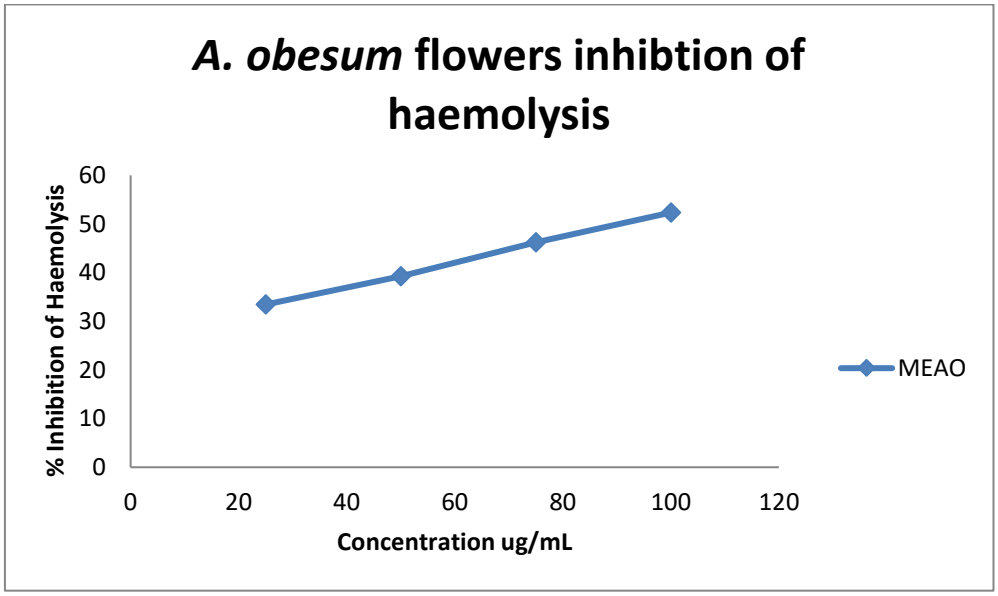

Figure: 3.3.1. *A. obesum* flowers inhibition of haemolysis (Data are represented as the means± standard deviations of three replicate determinations)

3.3.2. Protein denaturation

Methanolic extracts of leafy vegetables were able to inhibit protein denaturation in a concentration-dependent manner, and the inhibitory effect of extract off lowers at different concentrations (25–100 µg/mL) on protein denaturation is shown in Figure 7.2.

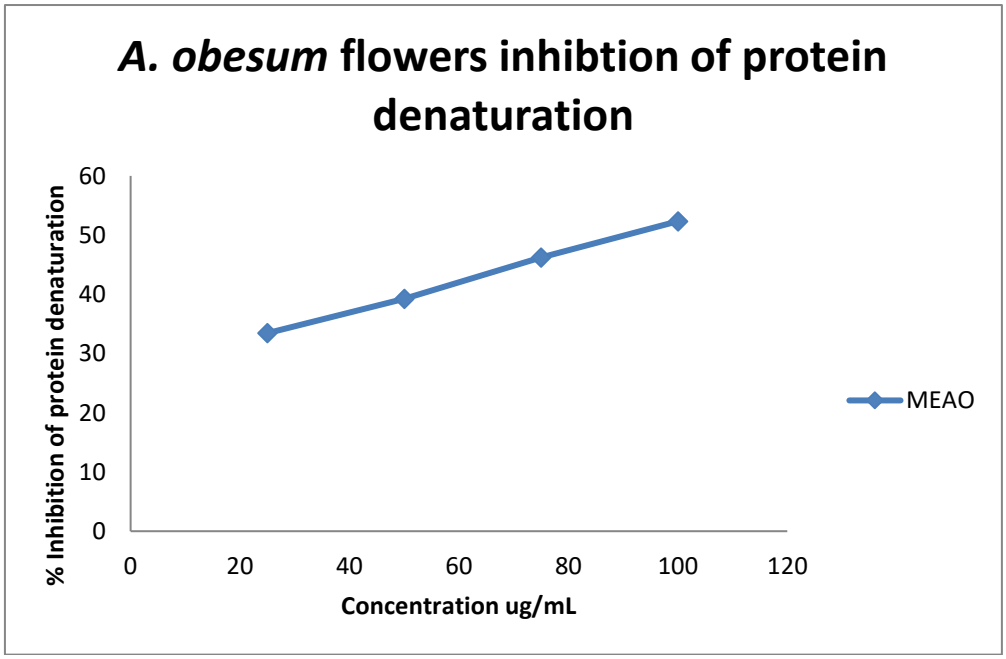

Figure no. 3.3.2: *A. obesum* flowers inhibition of protein denaturation (Data are represented as the means± standard deviations of three replicate determinations)

Inhibition % of protein denaturation of these leafy vegetables was within the range from 43.12% to 72.12% at the concentration range of 25–100 µg/mL.

3.3.3. Proteinase Inhibitory activities

Proteinase inhibitory activity of extract of flowers is shown in Figure 7.3, and the inhibition levels were within the range of 22.23–36.56%.

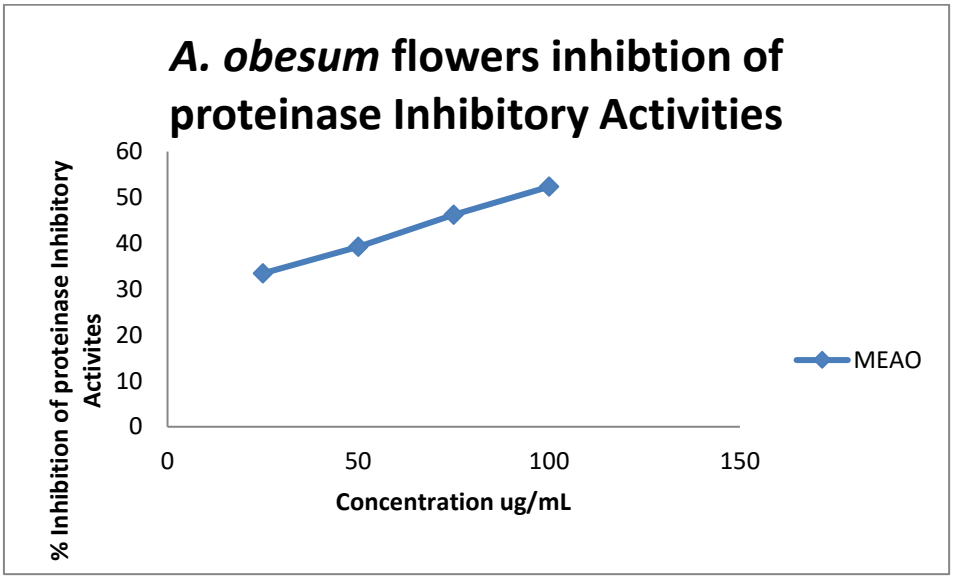

Figure 3.3.3: *A. obesum* flowers inhibition of proteinase activity (Data are represented as the means ± standard deviations of three replicate determinations)

3.3.4. Lipoxygenase Inhibition Activity

Results for lipoxygenase inhibitory activity of different leafy vegetables are shown graphically in Figure 7.4.

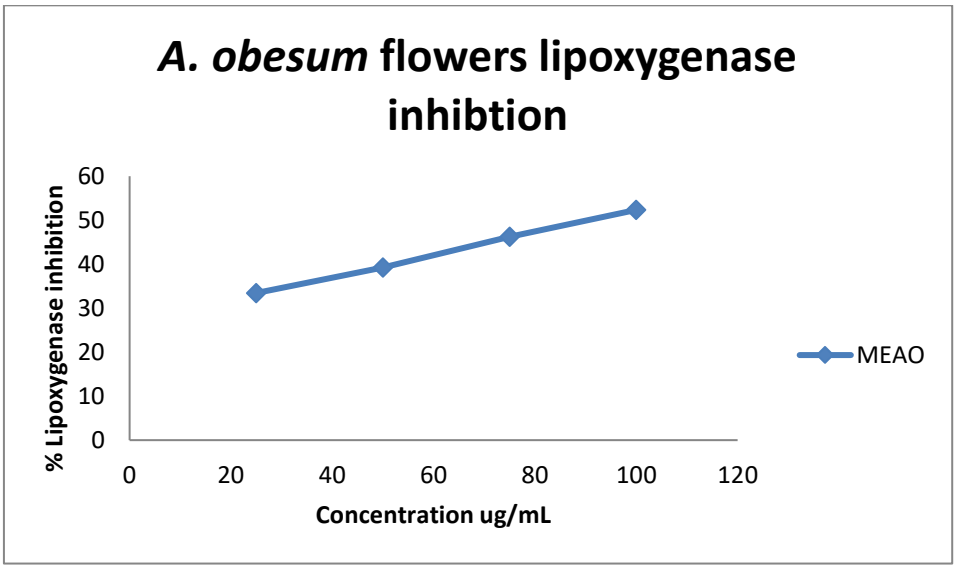

Figure 3.3.4: *A. obesum* flowers Lipoxygenase inhibition (Data are represented as the means± standard deviations of three replicate determination)

Inhibition levels were within the range of 33.45–52.34% within the concentrations of 25–100 µg/mL. The flowers of *A. obesum* showed an improved ability to inhibit lipoxygenase activity (about 50.0%) at 100 µg/mL concentration.

CONCLUSION

On the basis of above results it is concluded that selected medicinal plant extracts, fractions and most of the sub-fractions showed moderate to high antioxidant activities while the pure isolated compounds from these fractions were found to be very weak antioxidants, except 5-O-caffeoyl quinic acid which was isolated from the active fraction of *I. coccinea* flowers and exhibited the potent antioxidant activity towards DPPH free radical. The antioxidant capacity of these plants revealed due to the emergence of bioactive composition, which are promising source of natural antioxidants and can be exploited for multiple industrial and domestic applications. Our study identified presence of cardenolide, triterpenoids, pregnanes, carbohydrate, phenolic acids, flavonoids, and amino acids. The previous chemical studies deduced that A. obesum is a very rich plant with different compounds, including Flavonoids triterpenes, pregnanes, cardenolides, cardiac glycosides, and carbohydrate. The same was true for the antioxidant activity except for the FRAP test which showed a greater activity in the flowers.

During inflammation, as part of their defensive roles, leukocytes release their lysosomal enzymes, including proteases, causing further tissue damage and subsequent inflammation. Damage to cell membranes will further make the cell more susceptible to secondary damage by means of free radical-induced lipid per oxidation. Regulation of the volume and water content of cells may occur, through membrane proteins, by controlling the movement of sodium and potassium ions and damage to the membrane will affect this function. As the red blood cell membrane is similar to that of lysosomal membrane, inhibition of red blood cell hemolysis may provide insights into the inflammatory process. Stabilization of these cell membranes may retard or inhibit the lysis and subsequent release of the cytoplasm contents which, in turn, minimize the tissue damage and, hence, the inflammatory response. Therefore, substances that contribute significant protection of cell membrane against injurious substances are important in the event of inhibiting the progression of inflammation. In conclusion, the result indicate that the methanolic extract of flowers of *A. obesum* possess anti-inflammatory activity.

Denaturation of protein molecule is due to the inflammation process in conditions like arthritis. Inhibition of protein denaturation may play an important role in the antirheumatic activity of NSAIDs. Various plant extracts have shown their protein denaturation ability, as mentioned earlier in different literature reviews. The flowers of A. obesum possess higher protein denaturation inhibition properties whereas showed higher lipoxygenase inhibition ability also. Lipoxygenases are the key enzymes in the biosynthesis of Leukotrienes. Leukotrienes play an important role in several inflammatory diseases, such as arthritis, asthma, cancer, and allergic diseases. The mechanism of anti-inflammation may involve a series of events in which the metabolism of arachidonic acid plays an important role.

Results indicate that these anti-inflammatory activities may be due to the occurrence of bioactive compounds, such as Polyphenols, Flavonoids, and carotenoids in these leafy types.

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