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In Vitro Evaluation of Anti-Inflammatory Activity of *Woodfordi Fruticosa Leaves*

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ABSTRACT:

Medicinal plants contain numerous biologically active compounds which are helpful in improving the life and treatment of diseases and these are the primary source of synthetic and traditional herbal medicine.Inflammation is a body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological function. It is triggered by the release of chemical mediators from injured tissue migrating cells.The present study was carried out by egg protein denaturation method for in-vitro assessment of anti-inflammatory activity of leaves of woodfordia fruticosa. Denaturation of tissue proteins is one of the main reasons for inflammatory diseases. Hence the In vitro study of different extract of woodfordia fruticosa leaves showed the presence of significant anti-inflammatory activity.

KEYWORDS: Anti-inflammatory activity, Albumin,Woodfordia fruticosa.

INTRODUCTION

Since early time man has depended on nature for both health and illness. Primitive man used plants, animal parts and minerals for treating diseases. Herbal medicines are now in great demand in the developing world for primary health care not because they are inexpensive but also for better cultural acceptability, better compatibility, better use with the human body and minimum side effects. So valuable contribution to be done to develop and identify such plants which cure and prevention thedisease **(Jarald and Jarald, 2007).**

A World Health Organization (WHO) Expert Group defined traditional Medicines as the sum total of all knowledge and practices, whether explicable or not, used in diagnosis prevention and elimination of physical, mental, or social imbalances and relying exclusively on practical experience and observation handed down from generation, whether verbally or in writing **(WHO, 1976)**

Worldwide, there is several typestraditional system of medicines can be used for the treatment of diseases. They have natural products as the main ingredient of their therapeutic preparation .AYURVEDA, SIDDHA, UNANI, HOMEOPATHY SYSTEM of medicine and even the allopathy has many drugs derived from natural sources. Ayurveda which is the oldest holistic medicines system has been derived from a Sanskrit w ord ‘AYUS’(life) and ‘VED’(knowledge) which means the ancient “ science of life” is believed to be prevalent for last 5000 year in India. It is one of the most

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noted systemsof medicine in the world. The origin of Ayurveda has been taken from the gods. The "Tridoshic" concept is the fundamental principle in Ayurveda. Authentic information on Ayurveda has been compiled by ancient Indian medicine practitioner in forms called *SAMHITA* and other similar books. It is believed that five characters of medicinal herbs that are *rasa, guna, virya, vipak* and *prabhava* can be applied to treat various pathological conditions**. (kokate et al,2014)**

Woodfordia fruticosa belongs to a class of medicinally important plant which, when mixed with probiotics generate enhanced activity of the medicinal value in question. A method to demonstrate such function, an issue of commercial importance, has been patented. **(Das et al, 2007)**

1.1 INFLAMMATION

Inflammation is a body response to injury, infection or destruction characterized by heat, redness, pain, swelling and disturbed physiological function. It is triggered by the release of chemical mediators from injured tissue migrating cells. **(Gram et al, 1997)**

Inflammation is a defense mechanism in the body. The immune system recognizes damaged cells, irritants and pathogens, and it begins the healing process. When something harmful or irritating affects a part of our body, there is a biological response to try to remove it. The signs and symptoms of inflammation can be uncomfortable but are a show that the body is trying to heal itself. Inflammation is part of the body's immune response (**Waugh, 2010**).

According to Medical Dictionary of Pathology "Inflammation is a localized protective response elicited by injury or destruction of tissues which serves to destroy, dilute or wall off both the injurious agent and injured tissue"

1.2 SYMPTOMSOFINFLAMMATION

Joint pain

Joint stiffness

Loss of function

Redness (Rubor)

Swelling (Tumor)

Dolar (pain)

Calor (heat)

1.3 THEAGENT CAUSING INFLAMMATION MAY BE AS

1. **Physicalagent** like heat,cold, radiation. Mechanical trauma.
2. **Chemicalagent** like organic and inorganic
3. **Infectiveagent** like bacteria,viruses and their toxins.
4. **Immunologicalagent** like cell-mediated and antigen –antibody reaction.

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(Waugh, 2010)

1.4 TYPES OF INFLAMMATION

Acute inflammation

Acute inflammation is short term process occurring in response to tissue injury, usually appearing within minutes or hours.

It is associated with increased vascular permeability, capillary infiltration and emigration of leukocytes. It is from the increased movement of leukocytes and plasma from bloody to the site of tissue injury. This leads to inflammatory response through various biochemical events.

ChronicInflammation

Itrefers to a prolonged inflammatory response that involves a progressive change in the type of cells present at the site of inflammation. It is associated with infiltration of mononuclear immune cells, macrophages, monocytes, neutrophils, fibroblast activation, proliferation and fibrosis**. (Tripathi, 2015)**

This leads to a destruction of the tissue and proceeds to heal the damaged site.

1.5 TREATMENT

Professional rehabilitation therapists treat pain and inflammation. Such treatment makes movement easier and enables people to participate more fully rehabilitation.

There are many drugs available to decrease joint pain, swelling, and inflammation and prevent or minimize the progression of the inflammatory disease.

1.6 CLASSIFICATION

Selective COX-2 inhibitors

Celecoxib, Etoricoxib , Parecoxib

Nonselective-COX inhibitors which are used for the treatment.

CLASS	DRUG
Salicylic	Aspirin
Propionic acid	Ibuprofen, ketoprofen
Anthranilic acid derivative	Mefenamic acid
Aryl acetic acid derivative	Dicofenac, Aceclofenac
Oxicam derivative	Piroxicam, Tenoxicam
Pyrrolo-pyrrole derivative	Ketorolac

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Indole derivative	Indomethacin
Pyrazolone derivatives	Phenylbutazone
Preferential COX-2 inhibitor	Meloxicam, Nabumetone
Selective COX-2 inhibitor	Celecoxib, Etoricoxib

Preferential COX-2 inhibitor

Nimesulid, Diclofenac, Aceclofenac , Meloxicam etc.

Analgesic-Antipyretic with Poor Anti-inflammatory Action

Para-aminophenol derivative	paracetamol
Pyrazolone derivative	Metamizo
Benzoxazocine derivative	Nefopam

SOMEHERBS HAVE ANTI-INFLAMMATORY PROPERTIES-

Turmeric- (curcuma longa) – treating arthritis, Alzheimer,s disease, and some other inflammatory condition.

Cannabis – Contain a cannabinoid called cannabichromene, which has been shown to have anti-inflammatory properties.

Ginger – Used as carminative dyspepsia, constipation

Neem – (Azadirachta indica) used as leprosy, cardiovascular disease.

1.7COLLECTION AND AUTHENTICATION OF PLANT

The leaves of the plant Woodfordia Fruticosa was collected from Bhauwala, Dehradun (Uttarakhand) and was authenticated at Botanical Survey of India (BSI, Dehradun) with **Acc.No, 109.**

The fresh leaves were washed under running tap water to remove adhere dirt, followed by rinsing with distilled water, shade dried and use grinder to obtain coarse powder.

1.8PREPARATION OF EXTRACT

Total gram of powered drug is weigh accurately and then 10 gm of powder drug was weighed and soaked with 100ml of distilled water , then again 10 gm of drug is weighed and soaked with 100ml of ethanol, again 10 gm of drug is weighed and soaked it with 100 ml of chloroform in a sealed container. Shake it and after shaking the extraction mixture was kept for 3 days (maceration).

After 3 days the extracts are filtered through whattman filter paper. Crude extract were obtained by evaporating the solvents in a water bath at a low temp. (45-50 ^{0}c).After that the extract is kept in shade until a solid extract was obtained.

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Paste from the extract obtained was used to phytochemical screening.

1.9DETERMINATIONOFEXTRACTIVEVALUE **(Trease and Evans, 2009)**

Extractive values are useful for the evaluation of a crude drug. The extractive value is defined as the amount of extract present in the crude drug. The extract is basically chemical constituent which are present in the drug. The extractive value of the crude drug determines the quality as well as purity of the drug. The chemicals are of two types. They are either water soluble or Water insoluble. Some of the constituents are solvent specific; it means they dissolve in some specific solvent. Hence these also provoke a type of method known by solvent specific extraction. In this extractive value is calculated by a common method which is same for every type of extractive value depends on specific solvent. In this about 4-5 gm of powdered drug was weighed and was kept for maceration for 24 hrs with solvent. After 24 hrs the solution was filtered and the residue was discarded. To the filtrate 5 ml was discarded and to previously weighed china dish 10 ml of filtrate was taken and was evaporated under water bath. When the filtrate was distilled off by leaving a residue in dry form the china dish was cooled and then weighed to calculate the amount of extract present in filtrate.

At last percentage yield was calculated and result is given in Table No. 4.

Formula used for calculating the extractive value:-

Extractive value (%) = weight of dried extract / weight of plant material x 100

5.4 DETERMINATION OF ASH VALUE

The ash of any organic material is composed of their non-volatile inorganic components. The object of ashing crude to remove the traces of organic matter which may be interferes in an analytical determination. On incineration, the crude drugs normally produce ash which is usually consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium. In certain drugs, the percentage variation of the weight of ash from sample to sample is very small and any marked difference indicates change in quality. The ash value can be determined by 3 different methods: total ash, the acid insoluble ash, the water soluble ash .

Total Ash

A high ash value indicates contamination, substitution, adulteration or carelessness in preparing crude drug for marketing. Total ash is designed to measure the total amount of material produced after complete incineration of the ground drug at high temperature as possible to remove all the carbons. To determine total ash, weigh accurately 2-3 gm of the powdered drug in silica crucible. Incinerate the powdered drug by increasing the heat (450^0 C) until the sample was free from carbon. Then cool it by keeping it indesiccators. Weigh the ash and calculate the percentage of total ash with reference to the air dried drug. Results are showed in Table No. 5.

AcidInsolubleAsh

Usedfor the determination of earthy matter present on roots, rhizomes, and also on the leaves. To

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determine the acid insoluble ash value, boil the total ash obtained as above for 5 minutes with 25ml of dilute HCL.

Filter and collect the insoluble matter on the ash less filter paper, wash the filter paper with hot water , ignite in crucible , cool and kept in desiccators. Weigh the residue and calculate the acid insoluble ash of the drug. The percentage ash was calculated of acid insoluble ash with reference to the air dried drug.

WaterSolubleAsh

Water soluble ash is that part of the total ash content which is soluble in water. To the ash obtained as total ash 25 ml water was added and boiled for 5 minutes. The insoluble matter was collected on an ash less filter paper, then wash with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450^0C. The weight of the residue was subtracted from the weight of total ash. The content of water soluble ash with reference to dried drug was calculated **(Trease and Evans, 2009).**

SulphatedAsh

The drug powder was taken into a crucible and was incinerated till fumes arise. Than 10 ml of conc. Sulphuric acid was taken and incinerated matter was moistened. It was than incinerated for 15 minutes at 800^0C. It was than cooled in desiccators and weighed. The procedure was repeated two times and it should be noted down that there should be noted down that there should not be deviation of 0.5 mg in two observation

5.5 DeterminationofMoistureContent

Moisture content determination is important, not only to know excess water, but also in conjunction with suitable temperature moisture will lead to the activation of enzymes and gives suitable conditions to the proliferation of living organism.
There are various methods to determine moisture content like loss on drying, separation and measurement of moisture, chemical methods, electrometric methods and spectroscopic methods. Moisture is an inevitable component of crude drugs, which must be eliminated as far as practicable. The preparation of crude drug from the harvested drug plant involves cleaning to remove soil or other extraneous material followed by drying which place a very important role in the quality as well as purity of the material. Removal of dryness of the drug is important and also equally the rate at which the moisture is removed. The duration of drying process varies from a few hours to several weeks, depending upon the water content and other features of the drug. A weighed sample of crude drug is dried at 100^0C and weighed periodically until more than 0.25% is lost in 1 hours drying. The total weight loss is expressed as the percentage of the initial weight of sample. The test for loss on drying determines both water and volatile matter in crude drug. It can be carried out either by heating at 100^0C -105^0C.

Loss on drying is the loss of mass expressed as per percent w/w. To estimate the loss on drying 5 gm of drug is accurately weighed in a dried and tared petridish. The substance is to be dried to constant

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mass or for the prescribed time as specified and result is mentioned in TABLE NO. 6.

PHYTOCHEMICAL SCREENING (Khandelwal 2008,)

Test for Alkaloids

Dragendroff"s Test: - 2 ml of test solution was mixed with 2 ml of Dragendroff"s reagent (Potassium bismuth iodide solution). And orange-red precipitate indicates the presence of alkaloid.

Mayer'stest: - 2 ml of test solution was mixed with 2 ml of Mayer's reagent (potassium mercuric iodide solution). Whitish or cream colored precipitation indicates the presence of alkaloid.

Hager's Test:-2 ml of test solution was mixed with Hager;s reagent (saturated aqueous solution of picric acid). Yellow color precipitate indicated the presence of alkaloid.

TestforCarbohydrate

Molisch's test (General test) :- To 2-3 ml extract was taken and few drops of alpha-napthol solution in 95% alcohol were added. Then it was shaken and conc. H_2SO_4 was added from sides of the test tube. Violet ring formed at the junction of two liquids.

TestforReducingSugars

Fehling's Solution test: - 1 ml of extract was boiled on water bath with 1 ml of each of Fehling Solutions A and B. Boil for 5-10 min. First yellow, then brick red ppt. was indicates the presence of sugar.

Benedict's test:-To 2ml of extract, 2 ml of Benedict's reagent was added. The mixture is heated on a boiling water bath for 5 minutes. A orange red precipitate indicates the presence of sugar.

TestforGlycosides

Foam test (saponins):- Small amount of extract was shaken with 2ml of extract. Persistence of foam produced for 10 minutes indicated the presence of saponins.

Legal test: - 3 ml of chloroform and ammonia solution (10%) was added to 2ml of extract. Formation of pink color indicated the presence of glycoside.

Keller –killani test (cardiac glycoside) :- Treat the extract with 2 ml of glacial acetic acid , 1 drop 5% of ferric chloride solution. Add 1 ml conc. Sulphuric acid, appearance of reddish brown ring at the interface indicates the deoxysugar characteristic of Cardenolides. Appearance of violet ring below the brown ring & a greenish ring in the acetic acid layer confirmed the result.

Test for proteins

Biurettest: - Treat the test solution with few drops of 2% of copper sulphate solution , add 1ml of ethanol followed by excess of potassium hydroxide pellets. Formation of pink color in the extract layer indicates the presence of protein.

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Test for proteins containing Sulphur :- 5ml of test solution was mixed with 2ml of 40% NaOH and 2 drops of 10% Lead acetate solution. Boil the mixture and solution turned black due to the formation of lead sulphide.

Test for amino acid

NinhydrinTest: - 3 ml test solution was heated with 3 drops of 5% of ninhydrin reagent on boiling water bath for 5-10 minutes. Formation of purple colour indicates the presence of amino acid.

Test for steroids

Salkowskitest:-To2 ml of extract, add 2ml of chloroform and add carefully 2ml of conc. Sulphuric acid. A red color of chloroform layer and greenish yellow color layer of acid indicates the presence of steroids.

Testforgumsandmucilage

Dilute small quantity of ethanolic extract with water, add ruthenium red solution. A pink color production shows the presence of gums and mucilage.

TestforFlavonoids

Shinodatest: - 2-3 ml of test solution was mixed with 5ml 95% ethanol then few drops of conc. HCL and 0.5 g magnesium turning were added. Pink color indicates the presence of flavonoids.

Lead acetate Test: - Treat the extract with few drops of lead acetate solution. Formation of yellow precipitate indicates the presence of flavonoids.

Test for Tannins and Phenolic compounds

1ml of test solution was mixed with 5ml of 5% $FeCL_3$ solution. Deep blue-black color was indicates the presence of phenol.

Lead acetate Test: - Treat the extract with 3ml of 10%nlead acetate solution. A bulky white ppt indicates the presence of phenolic compound.

Take 0.5 gm the dried powdered of the drug, boil it with 20 ml of water in test tube, filter the mixture, and add few drops of 0.1% ferric chloride. Development of a brownish green or a blue – black coloration indicated the presence of tannins.

1ml of test solution was mixed with 5ml of dilute iodine solution. Transient red color gave positive result.

1ml test solution was mixed with 5ml of gelatin solution. White ppt indicates the presence of phenols and tannins.

5.6 DETERMINATIONOF*IN- VITRO*ANTI-INFLAMMATORY ACTIVITY

In vitro protein denaturation by using Egg albumin

The5ml of reaction mixture was comprised of 0.2ml of eggs albumin (from fresh hen's egg), 2.8 ml

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of phosphate buffered saline (pH 6.4) and 2ml of varying concentration of woodfordia fruticosa so that the final concentration becomes 100, 200, 300 mcg/ml. similar volume of double distilled water served as control. Then the mixture was incubated at 37^0C in incubator for about 15 minutes and then heated at 70^0C for 5 minutes. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance. The percentage inhibition of protein denaturation was calculated by the formula mentioned below:

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

RESULT

TABLE NO. 6.1 : Extractive value of various extracts

S. No.	Typeofextract	Extractive Value (%)
1.	Distilled Water	12%
2.	Ethanol	16%
3.	Chloroform	5.2%
4.	Pet. Ether	2.52%

TABLENO.6.2 : **Different ash value of woodfordia fruticosa Leaves**

S. No.	Type of ash value	Observation (%)w/w
1.	Total ash	5.05%
2.	Acid insoluble ash	0.05%
3.	Water soluble ash	0.09%
4.	Sulfated ash	1.2%

TABLE NO. 6.3 : Moisturecontentof *woodfordiafruticosa* leaves

S. No.	Weight of drug (gm)	Weight of drug with petridish	Weight of drug with petridish after drying	% yield
1.	5gm	59.31	58.91	0.67%
2.	5gm	59.31	58.84	0.79%
3.	5gm	59.31	58.80	0.86%
4.	5gm	59.31	58.75	0.95%

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TABLENO. 6.4.PhytochemicalScreening

S. No.	Test	Method	Ethanol	Dist. Water	Chloroform
1.	**Alkaloids**	Dragondroff's test	+	+	+
		Mayer's test	+	+	+
		Hager's test	+	+	+
2.	**Carbohydrate**	Molisch's test	+	+	+
3.	**Reducing sugar**	Fehling'ssolution	+	+	-
		Benedict's test	+	+	-
4.	**Glycosides**	Foamtest	+	+	+
		Legaltest	+	+	+
		Killer-killanitest	+	+	=
5.	**Proteins**	Biurettest	-	-	-
		Protein containing Sulphur	+	+	+
6.	**Amino acid**	Ninhydrin test	-	-	-
1.	**Steroids**	Salkowski test	+	+	+
2.	**Gums & mucilage**	Rutheniumred	-	-	-
3.	**Flavonoids**	Shinodatest	+	+	+
		Lead acetate test	+	+	+
4.	**Tannins & phenolic compounds**	5% $FeCl_3$ Solution	+	+	+
		Lead aceatate test	-	-	-
		0.1% ferric chloride solution	+	+	+
		Dilute Iodine solution	+	+	+
		Gelatin Solution	+	+	+

+Sign indicates the presence of phytochemical constituent and – sign indicates the absence of phytochemical constituents.

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TABLE NO. 6.5 : In-Vitro anti-inflammatory activity of woodfordia fruticosa by Egg albumin protein denaturation method

S.No.	Type of extract	Concentration mcg/ml	Absorbance	% Inhibition
1.	Distilledwater	100	0.375	12.99%
2.	Distilledwater	200	0.281	34.80%
3.	Distilledwater	300	0.176	59.16%
4.	Ethanol	100	0.134	15.18%
5.	Ethanol	200	0.123	22.15%
6.	Ethanol	300	0.090	43.30%
7.	Chloroform	100	0.115	15.55%
8.	Chloroform	200	0.110	19.11%
9.	Chloroform	300	0.100	26.4%
10.	Standard	50	0.227	33.43%
11.	Standard	100	0.135	60.99%
12.	Control/Blank	-	-	-

Fig No. 6.1

DISCUSSION

The leaves of woodfordia fruticosawere subjected to In-Vitro anti-inflammatoryactivityinvariousconcentrationsbyegg albumin protein denaturation. The aqueous and ethanolic extract show the more anti-inflammatory activity as compare to chloroform extract. Aqueous and alcoholic extract were found to give potent anti-inflammatory activity. The activity

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might be due to the presence of high content of secondarymetaboliteswhichmay be soluble in high polarity solvents. The preliminary phytochemical investigation of *woodfordiafruticosa* revealed the presence of various secondary metabolites such as, carbohydrates, tannins, phenolic compounds, saponins and flavonoids, glycosides and proteins. The In-vitro anti-inflammatory activity of *woodfordiafruticosa* extracts was assessed by egg protein denaturation method the aqueous extract at the concentration of 300mcg/ml and the alcoholic extract at the concentration of 200mcg/ml exhibited maximum degree of anti-inflammatory activity.

SUMMARY AND CONCLUSION

TheHerbal medicines have its origin in ancient culture. Herbs have potent ingredients and should be taken with the same level of caution as pharmaceutical medication. The present study was carried out by egg protein denaturation method for in-vitro assessment of anti-inflammatory activity of leaves of woodfordia fruticosa. Denaturation of tissue proteins is one of the main reasons for inflammatory diseases. Hence the In vitro study of different extract of woodfordia fruticosa leaves showed the presence of significant anti-inflammatory activity. The aqueous extract shows more anti-inflammatory activity. The activity may be due to the presence of alkaloids, glycosides flavonoids, tannins and phenolic compounds. Protein denaturation method is important in vitro method to check anti-inflammatory activity of plant constituent. In this method Woodfordia Fruticosa revealed most significant anti-inflammatory activity.

In future aspect this plant could be beneficial in the management of inflammatory disease.

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