In- vitro Anti-inflammatory activity in polyherbal gel formulation.

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Abstract: In early times herbal medicines are the only way to cure and prevent various diseases. Which are having a low price, are easily available in nature, are effective on diseases, have fewer side effects, less harmful because they are free from hazardous chemicals. *Ficus racemosa Linn*. is a historical plant used in Ayurveda, Siddha & Unani systems, belongs to the family- Moraceae, also called Gular or cluster fig. All parts of plant leaves, fruits, bark & root are having medicinal use. Like stomachache, skin diseases, inflammation, diabetes, dysentery & as carminative. Hence the present data is, therefore, to give detailed information about the *in vitro* anti-inflammatory property of *ficus racemosa Linn.* fruit extract. Dried ripe fruit extract is obtained from the soxhlet extraction and compared with plain Diclofenac tablet with the help of the protein denaturation method to check the anti-inflammatory property of the given extract of the fruit.

Keywords : Ficus racemosa Linn. Fruit, Anti-inflammatory property, Polyherbal gel, Herbal, Fruit extract.

Introduction –

F. racemosa Linn. is a moderate-sized avenue tree found throughout India either wild or cultivated for its fruits eaten by villagers. It is popular in the Indigenous systems of Medicine like Ayurveda, Siddha, Unani, and Homoeopathy. In the Traditional System of Medicine, all parts of the plant are regarded as medicinally important such as bark, root, leaves, fruits, and latex are used in dysentery, diarrhea, diabetes, bilious affections, stomach-ache, menorrhagia, hemoptysis, piles and as carminative and astringent. The present review is, therefore, an effort to give a detailed survey of the literature on its pharmacognosy, phytochemistry, and traditional and pharmacological uses.[1]

According to the world health organization, 80% of the world's population use botanical medicine for their primary health care needs and primary prevention of diseases.[3]

The demand for herbal medicines is increasing because of their promising pharmacological activity, less side effects, and economical Values that have been proven to be beneficial for all people. Gluanol acetate is the major component of fruits. The other components are glucanol, tiglic acid, taraxesterol, lupeol acetate, friedelin, and hydrocarbons.[4]

The extract of the fruit is used in leukoderma, menorrhagia, and diabetes. It is used locally to relieve inflammation of lymphadenitis, fibrositis, skin wounds and in sprains.[5]

Inflammation is a protective attempt by the organism to remove injurious stimuli and to initiate the healing process. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases. Inflammatory diseases such as rheumatoid arthritis, inflammatory bowel disease, multiple sclerosis, and other connective tissue diseases are cured by the *F. Racemosa linn*.[2]

- *Ficus racemosa* commonly known as figs in English,
- Udumbara in Sanskrit
- Gular in Hindi.
- Atti in Kannada.
- It is a widely cultivated plant all over India.

The phytochemicals present in fruit of *F. racemosa Linn.* exhibit anti-inflammatory activity hypolipidemic, and anti-microbial activity. The polyherbal gel formulation of Udumbara and Aloe vera will be formulated for providing the synergistic effect of anti-inflammatory. The effect of the combined formulation will give increased effect when compared to the individual herb, so combinations are preferred.

1. **Drug and excipient profile:**

- **Name – *Ficus racemosa linn*-**

- **Synonyms** – Cluster fig, Gular, Redwood fig, Jantuphala, phagoora, etc.
- **Biological source**– plant found in Southestasia, India, Australia, etc.

- **Taxonomical hierarchy**–
  - Kingdom – Plantae
  - Subkingdom – Tracheobionta
  - Superdivision – Spermatophyta
  - Division – Magnoliophyta
  - Class - Magnoliopsida
  - Family – Moraceae
  - Genus - Ficus
  - Species – *Ficus racemose linn.*

Chemical Constituents–

Fruits – Glauanol, Gluanol acetate, Glucose, Tiglic acid, Ester of taraxesterol, Higher hydrocarbon, Lupeol acetate, Friedelin and other phytosterol etc.

- **Name – *Aloe Vera***

- **Synonyms -** Aloe, Musabbarkumari.
- **Biological source –**Aloe is the dried juice of aloe barbadense Belonging to the family Liliaceae.
- **Taxonomical hierarchy –**
  - Kingdom – Plantae
  - Sub kingdom – Tracheobionta
  - Division – Magnoliophyta
  - Class – Liliopsida
  - Sub Class – Lilidae
  - Order – Liliales
  - Family – Liliaceae
  - Genus – Aloe
  - Species – *A. vera*

Chemical constituents – It contains active constituents like vitamins, enzymes minerals, lignin, saponin, salicylic acid, and amino acids, etc**.**

Experimental Work

1. **Collection-**

The *F. racemosa* Linn. fruit was collected from the Kolhapur region. Then these are cleaned with water softly. After that, they are dried at room temperature for 5-10 days for completely dry fruit.

2. **Extraction-**

The dried fruits are then grounded into a coarse powder. The main extraction is carried out by 2 processes. One is direct coarse powder is extracted and the second one is carried out like the cold maceration was carried out the coarse powder of fruit is macerated in the 95% ethanol for 24 hours and then extraction was done.

The *Ficus racemosa linn.*ripe fruits were collected from Kolhapur.

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At room temperature, the collected fruits were allowed to dry for 8-10 days.

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The dried fruits were grounded into the coarse form of powder.

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With 95% of ethanol, cold maceration was carried out for the grounded fruits.

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After 24 hours of cold maceration, the extract was filtered.

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Soxhlet apparatus with 95% ethanol is used for extraction of the grounded fruits.

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Filtrates are obtained from cold maceration.

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A water bath thermostatic is used for evaporating the solvent remains in the extract which is being concentrated

3. Pre- formulation studies-

A. Phyto-chemical investigation- The general Phyto-chemical investigations are carried out for the detection of general chemical classes like alkaloids, carbohydrates, flavonoids, tannins and starch.

- Test for Alkaloids - i) Dragendroffs Reagent.
  ii) Mayer's Reagent.
  iii) Hager's Reagent.
- Test for Carbohydrates – i) Fehling's test.
  ii) Benedict's test.
- Test for Tannins - i) Lead acetate test.
  ii) Ferric chloride test.

B. T. S. of *Ficus racemosa:*

The fruit is first washed with water to get clean from any type of dust particles. the transverse section of fruit is taken by the blade. The section obtained is now treated with phloroglucinol and dilute HCL on the glass slide. Then to this a drop of glycerol is added so that section doesn't dry and cover slip is placed on it. then it was observed under 45X and 10X both.

4. Formulation development –

The herbal gel is formulated using the different concentration of each ingredient. Listed below, batch like B1, B2, B3

Table No. 1: Formulation of Batches

	B1	B2	B3
Drug	0.5 mg	0.5 mg	0.5 mg
Aleo vera	25 ml	30 ml	35 ml
Agar	1.5 gm	1.5 gm	1.0 gm
Vitamin C.	0.5 mg	0.5 mg	0.5 mg
Water	q/s	q/s	q/s
Total weight	50 gm	50 gm	50 gm

5. Evaluation of herbal gel-

- Physical appearance – i) Cosistency.
  ii) Colour.
  iii) Appearance.
  iv) Homogeneity
- Washability.
- pH.
- Spredability.
- Viscosity.

6. *In-vitro* anti- inflammatory study-

1) **Protein denaturation test** –The plant extract (sample) 0.2ml of 1% bovine albumin and 4.78 ml of PBS (phosphate buffer saline) with pH 6,now this mixture is mixed properly and then incubated for 15 min.(37°C) and then heated on water bath for 5 mins. At 70°C and cooled. Once the mixture gets cooled the absorbance is checked of the solution by using UV-Spectrophotometer at the wavelength of 660 nm. Phosphate Buffer solution is used as the control in this process. The

% Of inhibition of the protein denaturation is calculated accordance with the formula,

% Of inhibition of denaturation -100(1-absorbance of sample/
Absorbance of the control.

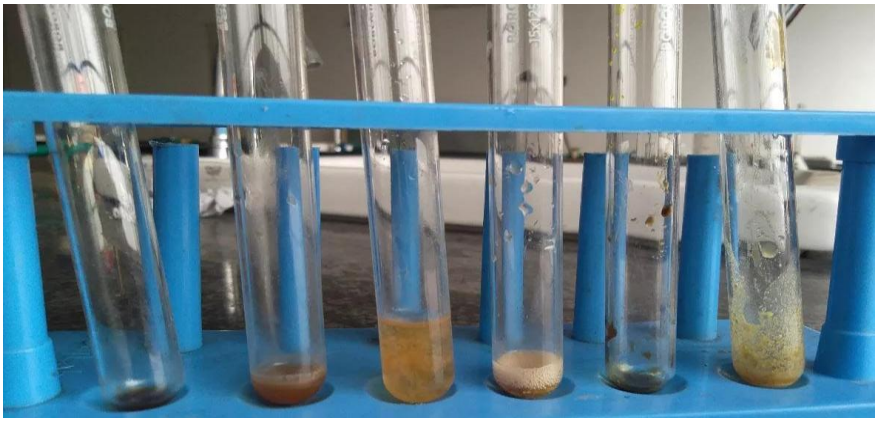
RESULTS AND DISCUSSION -

1. **Preformulation Result-**

Phyto-chemical investigation-

Table No.2: Phytochemical investigation result

Phytochemical test	Ethanol extract of *Ficus racemosa linn.*
For Alkaloids	
Dragendroffs reagent	+
Mayer's reagent	+
Hager's reagent	+
For carbohydrates	
Fehling's test	+
Benedict's test	+
For Tannins	
Lead acetate test	+
Ferric chloride test	+



2. Evaluation results of gel-

Physical appearance-

1. Consistency-

Table No. 3: Consistency of gel

Formulation	Consistency
B1	Very Good
B2	Good
B3	Good

2. Colour-

Table No. 4: formulation of colour

Formulation	Colour
B1	Olive green
B2	Olive green
B3	Olive green

3. Appearance-

Table No.5: Appearance of gel

Formulation	Appearance
B1	Semi-solid
B2	Semi-solid
B3	Semi-solid

4. Homogeneity-

Table No.6: Homogeneity of gel

Formulation	Homogeneity
B1	Good
B2	Good
B3	Good

5. Washability-

Table No. 7: Washability of gel

Formulation	Washability
B1	Washable
B2	Washable
B3	Washable

6. pH-

Table No.8: pH of gel

Formulation	pH
B1	5.9
B2	5.7
B3	5.6

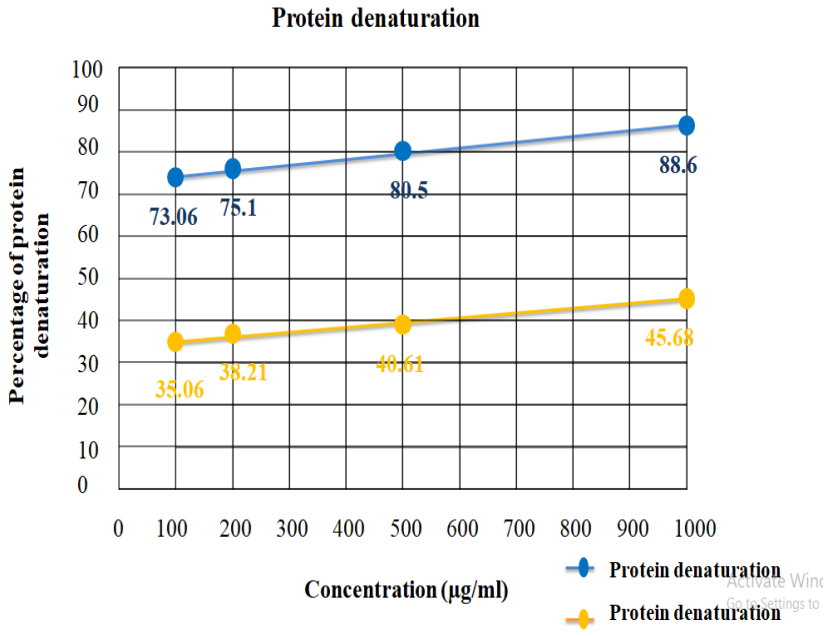
7. Spredability-

Table No.7.8: Spredability of gel

Formulation	Spredability
B1	8.49
B2	8.40
B3	8.00

Table No. 7.9: Inhibition of protein denaturation

Concentration (µg/ml)	Protein denaturation	
	Diclofenac	*F.racemosa linn.* extract
100	73.20	35.06
200	75.12	38.21
500	80.52	42.61
1000	88.64	45.68



Conclusion:

In conclusion the extraction procedure is carried out for the plant material that is further evaluated. The plant material and other excipients are used and herbal gel is formulated by use of aloe vera Gel base. The in-vitro activity for anti-inflammation, study is carried out and lastly the accelerated stability study. The in-vitro study has shown promising results which can be used as support for studying further as only in-vitro study. So, the future scope is the pre-clinical and clinical studies can be conducted and if promising results are obtained can be further utilized.

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Author's Contribution :

All the authors have contributed equally for the outcome of the present work.

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