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Research Article

Comparative study of two year old Bilwa (*Aegle marmelos*, corr) root with mature Bilwa root and market sample of Bilwa root with special reference to anti-inflammatory activity in albino mice.

Ketki P. Deshmukh[1,*], Amit Diwakar[2]
Assistant Professor[1], Associate Professor[2],
Department of Dravyaguna Vidnyan[1], Department of Swasthvrutta[2]
[1,2]Shree Saptshrungi Ayurved College & Hospital, Nashik, Maharashtra, India-420003

*** *Corresponding Author:*** *Dr. Ketki P. Deshmukh,* **E-mail:** dr.ketkideshmukh@gmail.com
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ABSTRACT:

Inflammation plays an important role in various diseases with high prevalence within populations such as *vatavikaras* like rheumatoid arthritis, atherosclerosis. The aim of the present context to compare anti-inflammatory activity of Two year old cultivated *bilwa(Aegle marmelos,* Corr.*)* root with Mature *bilwa* root and Market sample of *bilwa* root in Albino mice and the objective is to study the authentication and characterization of all samples of *bilwa* root. The anti inflammatory activity was test using anti inflammatory model like Carangeenan induced paw oedema model. Oral administration of root powder of all samples at the doses of 3 gm each as per standard protocol animal dose was calculated (animal dose = 390 mg /kg of body weight in mice. 2 mg Indomethacin in 2ml water of was used as Standard drug for comparison of anti inflammatory activity. But at the end two year old *bilwa* root has shown excellent acute anti inflammatory activity in Carrageenan paw oedema model. The anti inflammatory activity is equivalent to mature *bilwa* root and also to standard drug. So two year old root has potential to be used as substitute of mature *bilwa* plant. The drug *Bilwa* belongs to family Rutaceae and part used is root, root bark, fruit and stem.

KEY WORDS: Inflammation, anti-inflammatory activity, Carrangeenan, Indomethacin, *bilwa* root

INTRODUCTION:

Forest has been main source of plants since time immortal. Concept of medicinal plant forest is also found in some historic texts of India. The natural growth of medicinal plants was allowed to obtain potent plant material and to preserve medicinal plant genes. *Vaidyas* used to collect suitable medicinal plants themselves for *chikitsa* & preparing formulations. Forests were abundant so availability of plants was not an issue until now. Forests are being rapidly destroyed in today's era, due to fast growth of population. Substitutions are very common at least for selective plant material. The current source /practice of collection of medicinal plants includes major role of traders. The forest department permits these traders to collect forest produce.

The entire herbal medicinal system is dependent on the integrity of collectors and traders.

Thus standardization is very important to ensure the properties of drug. Urgent steps are needed to ensure genuineness, purity and to minimize scarcity of medicinal plant material. This can be achieved by plantation of useful medicinal plants and awareness about importance of cultivation and in situ conservation of medicinal plants.

Root collection is very critical among all useful parts as all parts of plant can be easily collected without much harming the plants. Root collection is very difficult in current forest laws where tree cutting is totally banned and is considered as punishable offence.

Cultivation of such plants may prove the best alternative. Govt. also allotted experimental projects to renowned pharmacy like Dabur. They studied the substitution of 1, 2, 3 year old roots of *bilwa.* HPTLC was done for all to find out presence of active principles. Two year old root showed presence of active principle so it was selected for study. It is a important content of *Bhruhatpanchamool* which as anti-inflammatory activity. Thus comparative study of Two year old *bilwa* root with mature *bilwa* root and

Market sample of *bilwa* root with special reference to anti- inflammatory activity in Albino mice was designed.

MATERIALS AND METHODS:

Collection and Authentification of plant material:

Root of two year old *bilwa* saplings were procured from Dapoli Krishi Vidyapeeth and authenticated by Dr. D.N. Mokat (Dapoli krishi vidyapeeth). A plant which yields fruit is considered as mature plant. Roots of mature *bilwa* plants were collected from the own field in Nanded city.

Market sample was procured from Shyamaji Amarji & Sons, Paydhuni, Mumbai. All samples were authenticated at certified lab.

After obtaining the permission from the Institutional Animal Ethics Committee.

Carrageenan-induced Paw Edema model was selected for study and statistical analysis done by **ANOVA test**

Selection criteria:

Criteria for selection of albino mice:

Inclusion criteria:

1. 20-40gms. Albino mice
2. Male and female mice
3. Active and healthy albino mice. Samples were shade dried for 3 days for reducing its moisture content. On next stage, roots were kept in oven at 50˚C and measured its weight after process. Those roots were powdered in the grinder and fine powder was separated by 120 meshes.

   Experimental material: Swiss Albino mice bred in animal house of National Toxicology Centre (N.T.C) Pune, weighing between 20-40 gm and of either sex were selected and housed in polypropylene cages of dimensions 30 ×20×13 cm in standard conditions (18 to 21˚C) and relative humidity (50-60 %) for 2 days before performing the experiment. They had free access to water and a normal diet. After which they were examined for their normal health and subjected to the experimental study. The experiment was conducted

Exclusion criteria:

1. Below or above 20-40gms.
2. Diseased albino mice
3. Animals already used for other experiments.

Preparation for animals:

Selected animals were weighed on digital balance. Cages were marked with label indicating Research project number, Weight, Sex of animal & Group **Analysis of Variance (ANOVA)**

Instruments and equipments:

Feeding tube, Watch, Weighing machine, Vernier caliper, Gloves, syringe, Needle, Carrangeenan solution, Experimental drug, Albino mice.

Drugs: Root powders of Two year old *Bilwa*, Mature *Bilwa*and Market sample of *Bilwa*, Distilled water, Carrangeenan and Indomethacin

Dose design :

For root powder : Dose in ml = Weight of mouse × 0.01 ml of stock solution

For Careengenan: 5 mg of Carboxy methyl cellulose (CMC) mixed with 1 ml of water and 10 mg of Carrageenan.

For Stock solutions: Weight of mouse × 0.01 ml of regarding solution of each group.

Administration of Drug: Orally with infant feeding tube.

Duration of study: Two days.

36 albino mice weighing 20-40 gm taken and divided into following groups (in each group 3 male, 3 female)

1. Control Group (Group A (control group) and Group B (Disease control group)
2. Standard Group (Indomethacin standard control group)
3. Experimental Group (Group D) root powder of two year old *bilwa*), group E (root powder of mature *bilwa*) and Group F (root powder of Market sample)

   After dividing the animals the solutions were administered in respective dose as per weight of the animal and injection Carrangeenan was given half n hour later for induction of artificial inflammation. By maintaining the animal in vertical, upright position, paw volume (oedema) was measured with digital Vernier caliper. The highest point of inflammation was noted and arms were placed at that point, one at planter surface and one at the dorsal surface of paw. Paw volume was measured at specific time interval i.e. after 30 min's, 60 min's, 120 min's, 240 min's and after 1440 min's. Statastical Analysis was done by Anova test.

OBSERVATIONS AND RESULTS:

Macroscopic evaluation of root of two year old plant: Yellowish brown, occasionally ridged, rootlets rarely presents.

Microscopic evaluation:

Transverse sections of two year old root showed Xylem vessels, only few medullary rays. Central core consisted of thin walled parenchyamatous cells possessing starch grains more than mature *bilwa* root. Central pith was also observed. All three samples showed presence of Xylem tissue. Market sample showed presence of prismatic crystals and very few starch grains.

Table No. 1: Organoleptic evaluation

Sr. No.	Tests	Two year old *bilwa* root	Marure *bilwa* root	Market sample of *bilwa* root
1	Colour	Yellowish brown	Creamish yellow	brown
2	Odour	Characteristic	Characteristic	No smell
3	Taste	Astringent	Astringent and bitter	Slightly bitter
4	Touch	coarse	coarse	fine

Table No. 2: Physico Chemical Analysis

Parameters	Two year old *bilwa* root	Mature *bilwa* root	Market sample of *bilwa* root	API Values
Moisture content	4.23%	3.53%	5.44%	Not given
Total ash	9.9%	5%	12.28%	Not more than 6%
Acid soluble ash	8.68%	4.46%	10.90%	Not more than 1%
Alcohol soluble extractives	3.13%	14.36%	3.21%	Not less than 7%
Water soluble extractives	6.87%	12.24%	7.96%	Not less than 7%

Phytochemical study:

Sample: Methanolic extracts of *bilwa*root powders

Mobile Phase: n Butenol: Acetic Acid: Water (4:1:5)

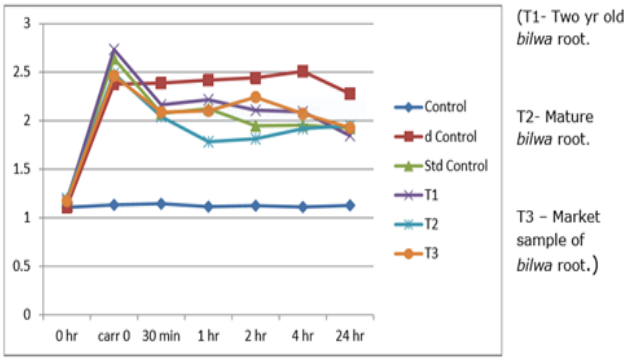
Stationary phase: Precoated silica gel 60 F254 TLC Plate (E. Merk

Table No. 3: Common Rf Values of Two year old Bilwa root/Mature Bilwa root and Market sample of Bilwa root

Detection	Common spots
254 nm	0.13,0.26, 0.41, 0.53, 0.64,0.80, 0.84, 0.88
UV 365 nm	0.84, 0.88
Iodine vapours	0.13, 0.26, 0.31, 0.53, 0.64, 0.84,.0.88
5% methanolicsulphuric acid	0.13, 0.84, 0.88

Experimental Study: Following graphs shows the anti inflammatory activity of all groups against Time interval.

Graph No. 1: Time interval against Mean response of inflammation

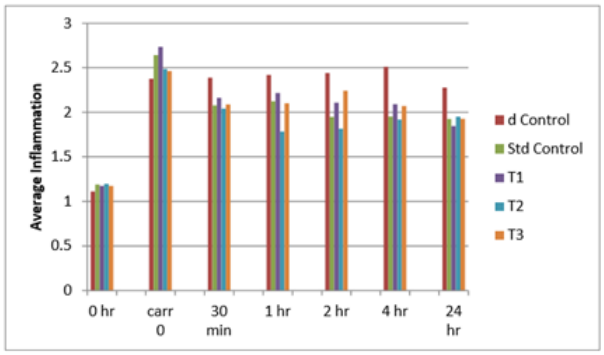


(T1- Two yr old *bilwa* root.

T2- Mature *bilwa* root.

T3 – Market sample of *bilwa* root.)

Graph No. 2 : Time Interval against mean inflammation response of each group



Time interval on X-axis & Average Mean response of inflammation of groups on Y axis.

DISCUSSION:

The drug Two year old *Bilwa* root is Yellowish brown, occasionally ridged, rootlets rarely presents. Microscopic evaluation of two tear old root shows Xylem vessels, only few medullary rays., starch grains

more than mature root. All three samples showed presence of Xylem tissue. Market sample showed presence of prismatic crystals and very few starch grains. So there is resembles of microscopic characters between two year old root and mature bilwa root. Organoleptic characters also resembles such as colour, odour, taste etc.

Rf values of two year *bilwa* root & mature *bilwa* root showed nearly equal differentiating spots and also comparable with standard spots. It indicated that active principles of these two samples were nearly similar.

As per experimental study Animals in **Group A** did not receive inj. carrageenan and study drug either. Inflammation was not produced. This indicated that the oedema produced in other groups was due to inj. carrageenan.

Animals in **Group B**– received inj. Carrageenan but no intervention (test and standard drug) was done so inflammatory response continued further till 4th time interval. Oedema was constant. Animals did not move their leg. Movements were sluggish.

When all groups compared for anti inflammatory activity, following results were seen.

In first time interval(0-30 mins)animals in Group C, Group D, Group E and Group F showed almost same Anti inflammatory response at first time interval.

In second time interval(31 -60 mins)animals in Group C showed increase in inflammation; Group D indicated decrease in inflammation further whereas Group E showed sustained anti inflammatory activity. Group F indicated decrease inflammation during this time period. Group F had shown superior anti inflammatory activity during this interval.

In Third Time Interval (61-120 mins): Animals in Group C indicates decreased in inflammation further.

Group D and Group E showed no change in inflammation. Group F showed slight increase in inflammation. So, Group C was superior in anti inflammatory activity than all others group.

In Fourth Time Interval (121- 240 mins): Group C and Group D showed no change in inflammation. Group E indicated slight increase in inflammation. Group F showed better response. Group F was superior in anti inflammatory response than standard drug, two year old *bilwa* root and mature *bilwa* root.

In Fifth Time Interval – (241-1440 mins): Group C , Group E and Group F showed no change in inflammation; Group D indicated decrease in inflammation. Two year old *bilwa* root showed better response at this interval as compared to all other groups.

The data was analyzed by ANOVA test. It indicated that difference observed in Group C, D, E and F was significant. Two year old *Bilwa* root showed significant anti inflammatory response than other groups. So two year old root has potential to be used as substitute of mature *bilwa* plant.

CONCLUSION:

1. Two year old *bilwa* root has shown excellent acute anti inflammatory activity in Carrageenan paw oedema model. The anti inflammatory activity is equivalent to mature *bilwa* root and also to standard drug. So two year old root has potential to be used as substitute of mature *bilwa* plant.
2. Characterization study revealed that mature root match with API standards; market sample differed in all characters.
3. Market sample was not of *Bilwa* root but had shown equivalent acute anti- inflammatory activity in carrageenan paw oedema model. It can be used further for clinical trials also.

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