



ISSN 2278- 4136

ZDB-Number: 2668735-5

IC Journal No: 8192

Volume 2 Issue 2

Online Available at www.phytojournal.com

Journal of Pharmacognosy and Phytochemistry

Comparative Phytochemical and Pharmacological Evaluations of Two Varieties of *Ocimum basilicum* for Antiarthritic Activity

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Tulsi plants (family Lamiaceae) are widely used in the Ayurvedic system of medicine for bronchitis, bronchial asthma, skin diseases, arthritis, inflammation, fever etc. The study was carried out to corroborate the traditional claims for anti-arthritis activity of tulsi. A comparative study of two varieties of *O.basilicum*, *basilicum* and *thyriflora* was done for evaluation of anti-inflammatory and anti-arthritis activity. Carrageenan induced paw oedema for anti-inflammatory activity and Complete Freund's Adjuvant induced arthritis for anti-arthritis activity were the two animal models used for the investigation. The present investigation suggests that *O.basilicum* var.*basilicum* (OSBB) at a dose of 50,100 & 200mg/kg p.o. exhibits decrease in carrageenan induced rat paw oedema. OSBB possess potent anti-arthritis and anti-inflammatory activity in a dose dependent manner. In case of arthritis there was significant inhibition at the dose of 100 & 200 mg/kg (p less than 0.01 and 0.001 respectively). *O.basilicum* var.*thyriflora* (OSBT: p less than 0.05) was unable to show activity at lower dose. Thus higher dose of OSBT may be required to exhibit the same pattern of anti-arthritis and anti-inflammatory activity in lab animals. The study was carried out to corroborate the traditional claims for anti-arthritis activity as well as quantification of three marker compounds ursolic acid, oleanolic acid and eugenol by HPTLC and HPLC and to check interspecies variation.

Keyword: *O.basilicum* var.*basilicum*, *O.basilicum* var.*thyriflora*, Carrageenan, Anti-inflammatory, Complete Freund's Adjuvant, HPTLC, HPLC.

1. Introduction

Ayurveda conceptualizes a category of drug activity known as 'Rasayana'. This word therefore signifies property of the plant that helps to rejuvenate the system, i.e. adaptogenic activity. Rasayanas are a group of non-toxic single or polyherbal preparation to improve the health and longevity^[1,2]. 'Tulsi has been used for centuries in traditional system of medicine for treatment of wide variety of ailments. The antioxidant activity of *Ocimum sanctum* (OS) is evident from its effectiveness in scavenging the free radicals in a dose dependent manner in antioxidant assays. It also possesses significant antipyretic, analgesic

and antiarthritic activity without any noticeable toxicity^[3]. It also possess antihyperglycemic and hypoglycemic activity^[4], Anthelmintic activity^[5], analgesic activity^[6], antiulcer activity^[7], hypotensive activity^[8], radioprotective effect^[9], chemopreventive activity^[10], antistress^[11] anti-inflammatory and antipyretic^[12]. Rheumatoid arthritis is a disease that affects typically the joints, where two bones meet to allow movement of body parts. The definition of rheumatoid arthritis (RA) is sometimes imprecise, but this term is usually used to describe a symmetrical, persistent and destructive polyarthritis often associated with rheumatoid factor and/or positive results for anti-

cyclic citrullinated peptide (anti-CCP) antibodies^[13]. In the Indian community rheumatic disorders are often loosely described as *Vata* and arthritis is addressed as *sandhivata* (*sandhi*, or joint). The most commonly used herbs that are used for arthritis treatment are *Vitex negundo*, *Phyllanthus emblica*, *Camellia sinensis*, *Zingiber officinalis*, *Curcuma longa*, *Commiphora mukul*, *Terminalia chebula*, *Boswellia serrata*, *Azadirachta indica*, *Centella asiatica*, *Desmodium gangeticum* and many more. Many of these plants help in arthritis by acting either as anti-inflammatory, antioxidants, analgesics or else immunomodulators^[14]. In the present study *O.basilicum var.basilicum* and *O.basilicum var.thyrsiflorum* have been studied for anti-inflammatory and anti-arthritis activity. Also their pharmacognostic studies have been carried out along with the quantification of three markers ursolic acid, oleanolic acid and eugenol by HPTLC and HPLC.

2. Materials and Methods

2.1 Collection and Authentication of Drug Material

Plants were collected from Jagtap vanasthali plant suppliers, plantation field of *O.basilicum var.basilicum* been situated in a small village called Ranza, Taluka Haveli which is few kms from Pune. *O.basilicum var.thyrsiflorum* was obtained from village called Inamgaon in Shirur. The procured plants were authenticated by Botanical Survey of India, Pune with voucher specimen number SAPOBB1 for *O.basilicum var.basilicum* and OCBASAP2 for *O.basilicum var.thyrsiflorum*.

2.2 Preparation of Extracts

The plant material brought was stored under drying conditions; leaves of the plant were separated. The separated leaves were then dried under shade and were finely powdered. The powder was then subjected to defatting with petroleum ether followed by cold maceration. The extraction by methanol for 48 hrs was followed by drying the extract in an oven at 50°C and under tray dryer at 30°C for 1 hr. Then the extracts were stored at room temperature. These plant extracts were used for quantification of marker compounds

by HPTLC and evaluation of anti-inflammatory and anti-arthritis activity.

2.3 HPTLC

2.3.1 Apparatus:

The spotting device used was Linomat V Automatic Sample Spotter (Camag, Muttenz, Switzerland); the syringe was 100 µl (Hamilton Bonaduz, Switzerland); The TLC chamber was a glass twin trough chamber (20×10×4 cm) (Camag); the densitometer was a TLC Scanner 3 linked to Wincats software (Camag); the HPTLC plates of 20×10 cm, 0.2 mm thickness, precoated with silica gel 60 F254 (E. Merck Kga A, Cat. no. 1.05548, Darmstadt, Germany) were used^[15].

2.3.2 Preparation of Standard Solution of Ursolic Acid, Oleanolic Acid and Eugenol:

A stock solution of ursolic acid was prepared by dissolving 2 mg of accurately weighed ursolic acid in methanol and making up the volume to 10 ml. From this stock solution, standard solutions of 20,40,60,80,100 µg/ml concentrations were prepared.

A stock solution of oleanolic acid was prepared by dissolving 2 mg of accurately weighed oleanolic acid in methanol and making up the volume to 10 ml. From this stock solution, standard solutions of 20, 40, 60, 80, 100 µg/ml were prepared.

A stock solution of eugenol was prepared by dissolving 2 mg of accurately weighed eugenol in methanol and making up the volume to 10 ml. From this stock solution, standard solutions of 10, 20, 30, 40, 50µg/ml were prepared.

2.3.3 Preparation of Sample Solutions

The 2mg of accurately weighed dried extracts were transferred to volumetric flask and the volume was made up to 10 ml with methanol to furnish the final concentration of 2000 µg/ml.

2.3.4 Calibration Curve for Ursolic Acid, Oleanolic Acid

10 µl of each of the standard solutions was applied in triplicate on a TLC plate. The plate was developed in a solvent system Petroleum ether: Ethyl acetate: Acetone (8:2:1.8:0.1 v/v) at 25 ± 2°C temperature and 40% relative humidity up to a

distance of 8 cm. After development, the plate was dried in air and scanned at 530 nm for ursolic acid and oleanolic acid. The peak areas were recorded. Calibration curves of ursolic acid and oleanolic acid were prepared by plotting peak area vs. concentration.

2.4 Estimation of Marker Compounds

2.4.1 Pre-Chromatographic Derivatization

Pre-chromatographic derivatization was done using iodine in chloroform (1%) solution. The solvent is allowed to run upto 1.2 cm. Then the plate is kept in dark for 10min allowing the reaction to complete. The plate is then dipped in the solvent system for 15 min. The plate was then air dried and was viewed under UV lamp at 530 nm for ursolic acid and oleanolic acid. Ursolic acid and oleanolic acid were resolved after derivatization.

2.4.2 Post-Chromatographic Derivatization

For derivatization sulphuric acid in ethanol (10%) was used. After spraying the reagent the plate was kept inside the oven at 120 °C for 3-5 min. Then the plate was scanned under Linomat V, Wincat software^[16].

2.4.3 Calibration Curve for Eugenol

10 µl of each of the standard solutions was applied in triplicate on a TLC plate. The plate was developed in a solvent system Toluene: Ethyl acetate: Formic acid (3:2:0.4 v/v) at 25 ± 2°C temperature and 40% relative humidity up to a distance of 8 cm. After development, the plate was dried in air and scanned at 280 nm for eugenol. The peak areas were recorded. Calibration curve of eugenol was prepared by plotting peak area vs. concentration^[17].

2.5 HPLC

2.5.1 Apparatus

Quantification of ursolic acid, oleanolic acid and eugenol was performed by using isocratic analytical HPLC assay. It was performed on a Jasco 900 instrument and 20µl of supernatant extracts was loaded onto ODS (5µm; hypersil) column (150×4.6 mm). For ursolic acid

Acetonitrile: Water (85:15) was the mobile phase used. It was eluted at a flow rate of 1 mL/min were detected at 230 nm by UV detector (UV-975, Jasco).^[18] Eugenol and oleanolic acid were run simultaneously in mobile phase containing Methanol: Ortho-phosphoric acid:Water (88:0.05:11.95) at a flow rate of 1 mL/min detected at 210 nm by UV detector (UV-975, Jasco).^[19] The peak areas corresponding to ursolic acid, oleanolic acid, and eugenol were integrated by comparison with external standard calibration curves. HPLC assay of different extracts yielded chromatograms with a retention time of 8.9 min for ursolic acid, 3.5 min for eugenol and 12.3 min for oleanolic acid. Validation of quantitative method was performed with samples for three times. The results of the three injections from the same samples at the five concentrations 5-25 µg/mL for ursolic acid, 5-25 µg/mL for eugenol and 25-125 µg/mL for oleanolic acid showed similar retention time.

2.5.2 Preparation of Standard Solution of Ursolic Acid, Oleanolic Acid and Eugenol

A stock solution of ursolic acid, eugenol and oleanolic acid was prepared by dissolving 2mg of accurately weighed ursolic acid, eugenol and oleanolic acid in methanol and making up the volume to 10 mL with methanol. From this stock solution, standard solutions of varying concentrations were prepared by transferring aliquots of stock solution to 10 mL volumetric flasks and adjusting the volume with methanol.

2.5.3 Calibration Curve for Ursolic Acid

20 µl of standard solutions of ursolic acid was injected in triplicate in column. The peaks were detected at 230nm. The peak areas were recorded. Calibration curves of ursolic acid were prepared by plotting peak area vs. concentration.

2.5.4 Calibration curve for Oleanolic Acid and Eugenol

20 µl of standard solutions eugenol and oleanolic acid was injected in triplicate in column. The peaks were detected at 210nm. The peak areas were recorded. Calibration curves of eugenol and

oleanolic acid were prepared by plotting peak area vs. concentration.

2.6 Estimation of Marker Compounds

A standard solution of ursolic acid was introduced into the column using the mobile phase Acetonitrile: Water (85:15). The retention time noted was 8.9 min. Extracts were also introduced into the column individually after been filtered with syringe filter. When the extracts were run guard column was used. The extracts containing ursolic acid were resolved at same retention time as that was the time for the given standard solution of ursolic acid. Wavelength was adjusted at 230 nm under UV detector. Flow rate was 1mL/min. pH adjusted up to 3.5. In case of eugenol and oleanolic acid, their mixtures of standard solutions were introduced into the column using the mobile phase Methanol: Ortho-phosphoric acid: water (88:0.05:11.95).

The retention time noted was 3.7 min and 12.3 min for eugenol and oleanolic acid respectively. Extracts were also introduced into the column individually. The extracts containing eugenol and oleanolic acid were resolved at same retention time as that was the time for the given standard solution of eugenol and oleanolic acid. Wavelength was adjusted at 210 nm under UV detector with a flow rate of 1mL/min.

2.7 Anti-Inflammatory Studies

The anti-inflammatory activity of the *O.basilicum var.basilicum* and *O.basilicum var.thyrsiflorum* was evaluated. The experimental procedures and research protocol used in the various *in-vivo* studies were reviewed and approved by Institutional Animal Ethics Committee (IAEC) constituted as per the guidelines of Committee for Purpose of Control and Safety on Experiments on Animals (CPCSEA No. 47/10.), India. The method described by C.Tsai and C.Lin (1998) was followed to perform this pharmacological activity. Male or female Wistar rats with a body weight between 150 and 180 g were used. The paw volume was measured plethysmographically immediately after injection, again 1, 2, 3 and 4 h, and eventually 24 h after challenge^[20,21].

2.8 Animals

Female wistar rats weighing approximately in a range between 150-180 g were brought from National Institute of Biosciences. The animals were identified by marking with picric acid on head, tail or back.

2.9 Groups and Treatment Schedule

Through sub-plantar route, carrageenan was injected to each animal before the administration of drug. The methanolic extract of leaves *O.basilicum var.basilicum* and *O.basilicum var.thyrsiflorum* was emulsified with CMC (carboxyl methyl cellulose) and distilled water separately.

The animals were grouped into eight, each group consisting of six animals.

- **Group I:** Control group, received only the vehicle consisting of CMC in distilled water.
- **Group II:** Standard group received diclofenac sodium i.e. 10mg /kg, as standard drug for comparison.
- **Group III:** The animals were administered with methanolic extract of leaves of *O.basilicum var.basilicum* i.e. 50mg /kg, where in each of the animal was given individually 0.5ml of the extract.
- **Group IV:** The animals were administered with methanolic extract of leaves of *O.basilicum var.basilicum* 100mg/kg, 0.5ml of extract per animal was given.
- **Group V:** The animals were administered with methanolic extract of leaves of *O.basilicum var.basilicum* 200mg/kg, 0.5ml of extract per animal was given.
- **Group VI:** The animals were administered with methanolic extract of leaves of *O.basilicum var.thyrsiflorum* 50mg/kg, 0.5ml of extract per animal was given.
- **Group VII:** The animals were administered with methanolic extract of leaves of *O.basilicum var.thyrsiflorum* 100 mg/kg 0.5ml of extract per animal was given.
- **Group VIII:** The animals were administered with methanolic extract of leaves of

O.basilicum var. *thyrsoiflorum* 200 mg/kg, 0.5ml of extract per animal was given.

The animals were starved overnight. To ensure uniform hydration, the rats received 5 mL of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats were challenged by a subcutaneous injection of 0.05 mL of 1% solution of carrageenan into the plantar side of the left hind paw. The paw were marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection, again 1, 2, 3 and 4 h, and eventually 24 h after challenge.

2.10 Statistical Analysis

The results are expressed as mean \pm S.E.M. The statistical analysis was performed by analysis of variance (ANOVA) by Dunnett's Multiple Comparison test.

2.10.1 Anti-Arthritic Study

The no. given by the (IAEC) for performing this pharmacological activity was 46/10. The method described by R.T. Narendhirakannan et al (2007) was used for performing this activity. The severity of the induced adjuvant disease was followed by measurement of the non-injected paw (secondary lesions) with a plethysmometer^[22,23].

2.12 Animals

Female Wistar rats weighing approximately in a range between 250-300gm were brought from National Institute of Biosciences and kept in aluminium cages in groups of 6 animals per cage in the departmental animal house, at a constant temperature of 24-25°C with a light-dark cycle of 12/12hrs. They were supplied with rat food obtained from Amrut feed Center, Kolhapur (India) and water *ad libitum*.

2.13 Groups and Treatment Schedule

Through Sub plantar administration, Complete Freund's Adjuvant (CFA) was injected to each animal before administration of drugs. The methanolic extracts of leaves of *O.basilicum*

var. *basilicum* and *O.basilicum* var. *thyrsoiflorum* were emulsified with CMC (carboxyl methyl cellulose) and distilled water separately. The animals were grouped into eight similar to the groupings done for anti-inflammatory activity.

2.14 Statistical Analysis

The results are expressed as mean \pm S.E.M. The statistical analysis was performed by analysis of variance (ANOVA) by Bonferroni's test.

3. Results and Discussion

3.1 HPTLC

Standard ursolic acid and oleanolic acid were run simultaneously with the methanolic leaf extract of other *ocimum* species in one solvent system which showed the resolution factor at 0.23 and 0.57 respectively. Calibration curves were prepared using standard solution in range of 20-100ug/spot for ursolic acid and oleanolic acid. Pre-chromatographic and post-chromatographic derivatization was done for separation of ursolic acid and oleanolic acid. The pre-chromatographic derivatization process was found to improve the selectivity of separation substances with the same or similar chromatographic properties by exploiting their differing chemical behavior. The equation obtained by the calibration curve is $y = 25.371x + 254.52$ and correlation coefficient was $R_2 = 0.9998$, for ursolic acid. The equation obtained by the calibration curve is $46.352x + 43.8$, and correlation coefficient $R_2 = 0.9993$, for oleanolic acid. Standard eugenol was run simultaneously with the methanolic leaf extract of other *ocimum* species in one solvent system which showed the resolution factor at 0.78. Calibration curve was prepared using standard solution in range of 10-50ug/spot for eugenol. The equation obtained by the calibration curve of eugenol was $y = 576.19x - 4936.5$ and the correlation coefficient was $R_2 = 0.998$.

In case of leaf extract of *basilicum* variety markers eugenol and ursolic acid were found in higher quantity compared to *thyrsoiflorum* variety whereas oleanolic acid content was found to be higher in *thyrsoiflorum* variety. *Basilicum* variety showed higher efficiency in pharmacological activities like

analgesic, anti-inflammatory and anti-arthritic activity in comparison to *thyrsoiflorum* variety. Thus results suggest that higher quantity of the markers may be responsible for higher activity in *basilicum* variety.

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Table 1: Percentage content of ursolic acid, oleanolic acid and eugenol in both the species in reference to the standards given

Leaf Extracts	Ursolic acid	Oleanolic acid	Eugenol
<i>O.basilicum var.basilicum</i>	0.00332	0.00731	0.05706
<i>O.basilicum var.thyrsoiflorum</i>	0.00319	0.00055	0.00947

3.2 HPLC

Linear regression revealed good relationship between the concentration of standard solutions and the peak response within the concentration range of 5 to 25 µg/ml with a correlation coefficient (r²) of 0.999 ± 0.02 (Y= 8446.3X+45418) for ursolic acid. Linear regression revealed good relationship between the concentration of standard solutions and the peak response within the concentration range of 25 to 125 µg/ml with a correlation coefficient (r²) of 0.997 ± 0.02 (Y=1665x + 4693.6) for oleanolic acid. The peak response within the concentration range of 5 to 25 µg/ml with a correlation coefficient (r²) of 0.9981 ± 0.02(Y=15819x + 6200.5) for eugenol.

In case of leaf extract of *basilicum* variety two markers eugenol and ursolic acid were found in higher quantity compared to *thyrsoiflorum* variety. *Basilicum* variety shows higher efficiency in pharmacological activities like analgesic, anti-inflammatory and anti-arthritic activity in comparison to *thyrsoiflorum* variety. Thus results suggest that higher quantity of these markers may be responsible for higher activity in *basilicum* variety.

Table 2: Percentage content of ursolic acid, oleanolic acid and eugenol in both the species in reference to the standards given

Leaf Extracts	Ursolic acid	Oleanolic acid	Eugenol
<i>O.basilicum var.basilicum</i>	1.5901	0.0073	6.0706
<i>O.basilicum var.thyrsoiflorum</i>	0.1970	0.7032	0.1870

3.3 Anti-inflammatory Study

The present investigation suggests that *O.basilicum var.basilicum* (OSBB) at a dose of 50,100 & 200mg/kg p.o. exhibits decrease in carrageenan induced rat paw oedema in a dose dependent manner. It is well known that carrageenan induced rat paw oedema is characterized by inflammation by a plethora of inflammatory mediators. The first hour inflammation is caused due to histamine. The second hour is caused due to bradykinin and 3rd and 4th hour inflammation is caused due to PG. The drug OSBB inhibits the elevation of rat paw at 3rd and 4th hour at a dose of 100 & 200 mg/kg.p.o.(p less than 0.01 and 0.001 respectively).This suggests that the drug inhibits rat paw oedema in a dose dependent manner. However *O.basilicum var.thyrsoiflorum* (OSBT) exhibits the elevation of rat paw at 3rd and 4th hour (p less than 0.05). This suggests that at lower doses OSBT is unable to exhibit anti-inflammatory activity.

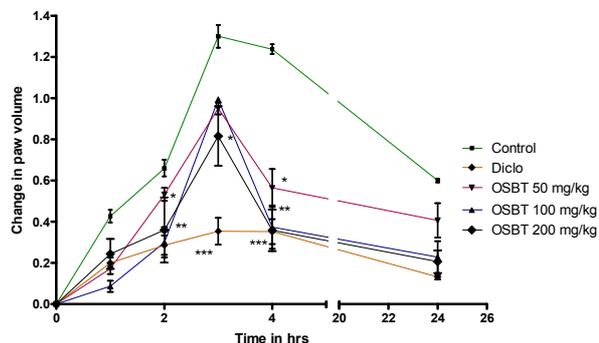


Fig.1. Anti-inflammatory activity of *Ocimum basilicum var.thyrsoiflorum*

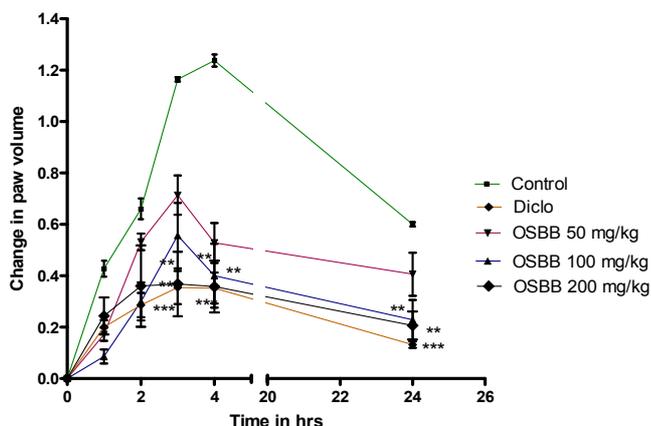


Fig 2: Anti-inflammatory activity of *Ocimum basilicum var. basilicum*

3.4 Anti-arthritis Study

CFA induced arthritis is characterized by biphasic responses in rats when sub plantar administration of CFA is subjected. The present investigation suggests that OSBB is capable of inhibiting the biphasic responses of inflammatory process in the experimental animals. The inhibition was exhibited in a dose dependent fashion. The initial increase in paw volume on the 7th day was maximum in control group of animals. But in animals treated with OSBB it was not elevated upto that extent. The inhibition was dose dependent. There was significant inhibition at the dose of 100 & 200 mg/kg (p less than 0.01 and 0.001 respectively). The similar pattern was exhibited in the second phase as well which peaked on 14th day. The other parameters like elevation in joint diameter, arthritis score were also exhibited in dose dependent manner. At a dose of 200 mg/kg and 100 mg/kg p.o. OSBB demonstrated an inhibition of both the phases of inflammation. However it should be noted that the inhibition of disease state was more in first phase than the second phase. This reflects the fact that the drug possesses potent anti-inflammatory response and a faint immunomodulatory activity. In the animals treated with OSBT the exhibition in various in vivo arthritic markers was less when compared to the action of OSBB treated animals. This response was exhibited in dual phases of P.G induced inflammation and immunity induced inflammation. This suggests that the drug OSBB is

mild anti-arthritis drug. The changes in paw volume, elevation in joint diameter were exhibited but elevation was less when compared to the control group. The present investigation suggests that at dose of 100 & 200 mg/kg.p.o. OSBB possesses potent anti-arthritis activity but OSBT possesses weak anti-arthritis activity at 200mg/kg.p.o.

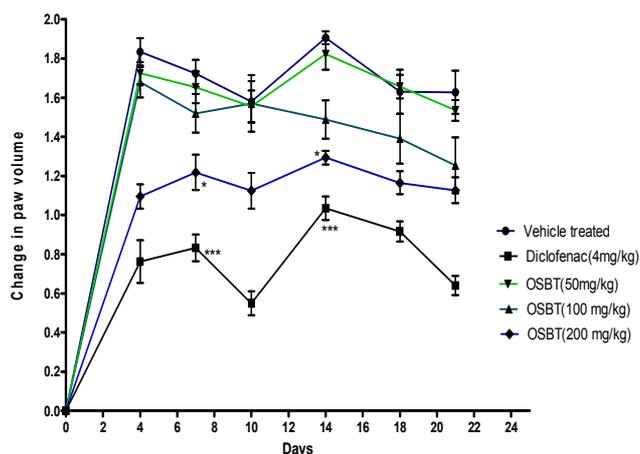


Fig 3: Graph of effect of *Ocimum basilicum var. thrysiflorum* on paw oedema

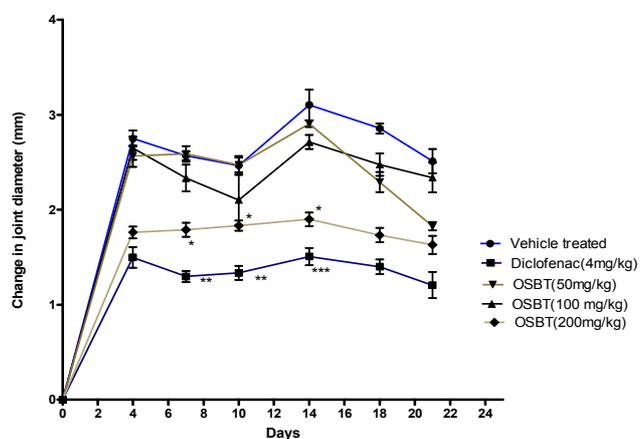


Fig 4: Graph of effect of *Ocimum basilicum var. thrysiflorum* on joint diameter

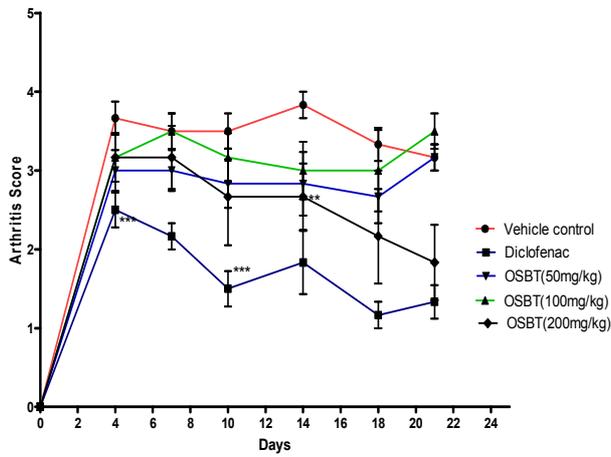


Fig 5: Graph of effect of *Ocimum basilicum var.thrysiflorum* on arthritis score

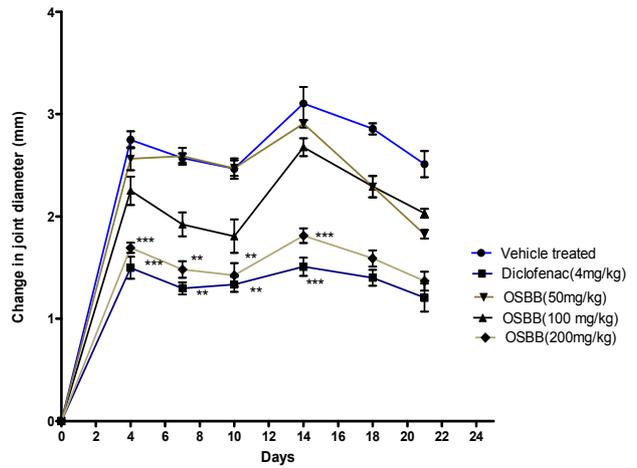


Fig 8: Graph of effect of *Ocimum basilicum var.basilicum* on joint diameter

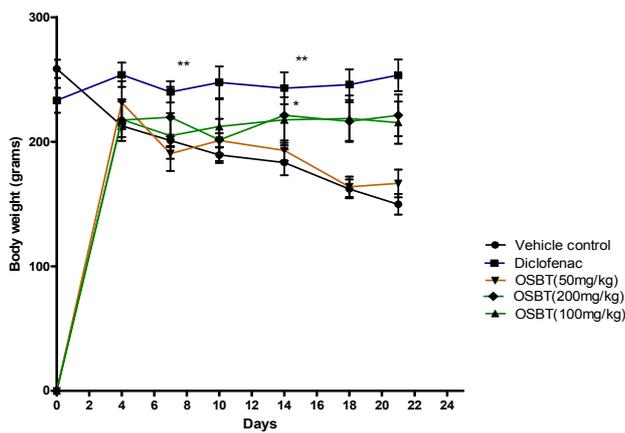


Fig 6: Graph of effect of *Ocimum basilicum var.thrysiflorum* on body weight

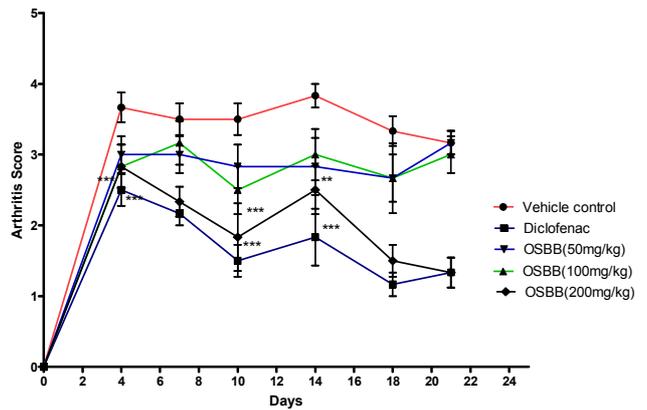


Fig 9: Graph of effect of *Ocimum basilicum var.basilicum* on arthritis score

3.5 Anti-arthritic activity of *Ocimum basilicum var.basilicum*

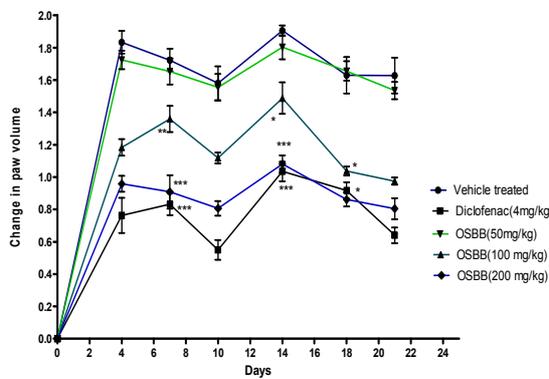


Fig 7: Graph of effect of *Ocimum basilicum var.basilicum* on paw oedema

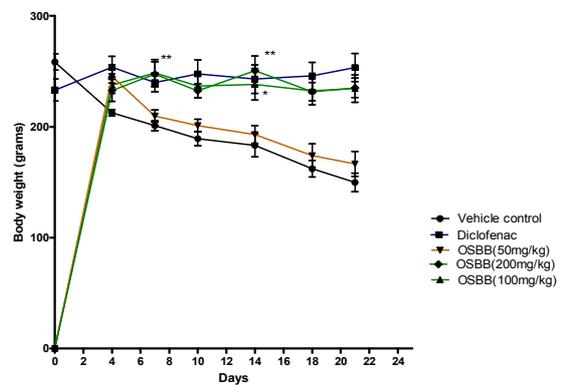


Fig 10: Graph of effect of *Ocimum basilicum var.basilicum* on body weight

4. Conclusion

The phytochemical investigation was done mainly for detecting and comparing the three marker constituents eugenol, ursolic acid and oleanolic acid by HPTLC and HPLC. Ursolic acid and oleanolic acid were estimated simultaneously by HPTLC and eugenol estimated individually. The pharmacological studies such as anti-inflammatory activity, anti-arthritic activity suggest that *O.basilicum var.basilicum* (OSBB) possess significant anti-arthritic and anti-inflammatory activity in a dose dependent manner whereas higher dose of *O.basilicum var.thrsiflorum* (OSBT) is required to exhibit the same pattern of anti-arthritic and anti-inflammatory activity in lab animals.

The results have shown that *O.basilicum var.basilicum* have higher amount of chemical markers, (eugenol and ursolic acid) as compared with *O.basilicum var.thrsiflorum*. Also in the pharmacological studies carried out it is seen that the efficacy of leaf extract of *O.basilicum var.basilicum* is higher as compared with *O.basilicum var.thrsiflorum*. If compared to *Ocimum sanctum*, then it is seen that *basilicum* and *thrsiflorum* variety has higher content of eugenol and oleanolic acid in comparison to *Ocimum sanctum*. Ursolic acid content is nearly similar in all the three varieties. In case of formulation (Himalaya Tulsi capsules), all the three markers are present in a quantity less than *basilicum* but more than *thrsiflorum*. Thus it can be concluded that *basilicum* variety can be a better substitute for *Ocimum sanctum*. Hence, on the basis of the results of phytochemical studies it may be concluded that *basilicum* variety is more effective than *thrsiflorum* variety.

Findings show potential of variety *basilicum* for treatment of arthritis. This necessitates further detailed and systematic evaluation of the plants for the search of new lead molecule.

5. Acknowledgments

Ms. Sapna Phadtare is thankful to Dr. K.R.Mahadik and Dr. Vaibhav Shinde for their kind support and guidance.

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