Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2022; 11(4): 96-105
Received: 26-03-2022
Accepted: 16-05-2022

Garvita Chaudhary Joshi
Research Scholar, Department of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India

Meenakshi Bharkatiya
Associate Professor, Department of Pharmacy, B. N. Institute of Pharmaceutical Science, B.N. University, Udaipur, Rajasthan, India

Corresponding Author:
Garvita Chaudhary Joshi
Research Scholar, Department of Pharmacy, Bhupal Nobles' University, Udaipur, Rajasthan, India

In vitro anti-inflammatory activity determination of a polyherbal formulation containing *Tinosporia cordifolia, Moringa oleifera* and *Allium sativum*

Garvita Chaudhary Joshi and Meenakshi Bharkatiya

Abstract
Some Polyherbal formulations containing *Tinosporia cordifolia, Moringa oleifera* and *Allium sativum extracts* were evaluated for its anti-inflammatory activity by *in-vitro* methods. Qualitative analysis of whole plant extract revealed the presence of alkaloid, flavonoid, saponins, steroid, phenol, tannin, glycoside and terpenoid. *In-vitro* anti-inflammatory activity of polyherbal formulations and individual extracts were estimated using membrane stabilization, antiproteinase activity, albumin denaturation assay, and antilipoxygenase activity at different concentrations. The results showed that polyherbal formulation showed significant results in all activities. Inhibition increased mostly with increasing the concentration. In membrane stabilization (HRBC assay) most active is PHF-3 while in albumin denaturation, Antiproteinase method and Antilipoxygenase assay highest activity is recorded with PHF-1. *In vitro* antiinflammatory activities were evaluated as the first study of inflammation for seeing the activities of polyherbal formulations. The study concluded that the PHF-1 is potent among all the formulations as it showed a better antiinflammatory activity as compared to other formulations.

Keywords: Polyherbal formulation, inflammation, Antiinflammatory activity, *In vitro models*

Introduction
Inflammation is our body's defense response to harmful stimuli like allergens &/or tissue injury; however, a wild inflammatory reaction is the foundation of an extensive variety of disorders which includes: autoimmune diseases, cancer, cardiovascular dysfunctions, allergies, and metabolic syndrome which altogether place a noteworthy financial burden on people and society [1]. There are a variety of medicines for controlling and suppressing inflammatory crunches which includes: nonsteroid anti-inflammatory medications, steroids, and immunosuppressants. All these have some side effects, whereas our goal in practice is to utilize the lowest effective dosage with maximum effectiveness as well as the least side effects. As a result, we must incorporate natural anti-inflammatory components into drug therapy in order to maximise pharmacological efficacy while minimising undesired side effects [1, 2]. Herbal medications are growing in popularity in medicine, and we must continue to learn more about them. Herbal prescription recommendations come mostly from complementary, alternative, and traditional medicine, however contemporary medicine must confirm these principles through scientific means before employing them in practise [3]. "Though the inflammatory response is necessary for host defence, it is a double-sided sword that may result in organ failure and even death" [4]. In the past, nonsteroidal anti-inflammatory medications (NSAIDs), corticosteroids, and opioids were commonly utilised to treat these disorders [5, 6]. However, because of the widespread usage of analgesics and anti-inflammatory drugs, harmful adverse effects are common, especially when treating pain and inflammation with larger doses for longer periods of time [7]. Analgesic and anti-inflammatory drug side effects include gastrointestinal problems, respiratory depression, renal dysfunction [8], potential dependency [5], constipation [7], peptic ulcer, and bleeding [9]. "It is therefore critical to make attempts to integrate novel molecules derived from medicinal plants into the pain and inflammation medication arsenal" [10]. *Tinospora cordifolia* (Family: Menispermaceae) best known, "Amrita" or "Guduchi" is an important drug and has been used in medicine since ancient times. The drug is well known in India for its bitterness and is limited to fever, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhea and diarrhea [11]. The plant has a bitter, spicy and astringent taste, exhibits post-digestive effect and has a strong effect, reduces all three doshas namely, vata, pitta and kapha [12]. It is well known for its anti-inflammatory, antacid. , antipyretic tonic, neuroendocrine, anti stress, antioxidant and immune modulating effect [13]. *Moringa oleifera* is a small native tree in the Himalayan region of Northwest India, which has become a tradition in many parts

of the islands and in South America. Traditionally, in addition to being a daily vegetable among the people of these regions, Moringa is also widely known and used for its health. Among the common people, it has earned its name as the 'tree of wonders' because of its amazing ability to treat various ailments and even incurable diseases. Much research has been done to classify bioactive compounds into different parts of the plant due to its different uses [14]. Therefore, medicinal plants in medicine or phytomedicine are still reliable and widely used [15]. Garlic (*Allium sativum*) is a widely used diet remedy and has been traditionally used in the treatment of fungal and bacterial infections. Numerous studies have shown that garlic exhibits a variety of biological and chemical properties, including prevention of tumorigenesis and antiatherosclerosis and the release of detoxification of pollutants [16, 17].

Materials and Methods

Collection and Authentication of Test Plants

The raw plant's materials of *Tinospora cordifolia* (aerial parts) *Moringa oleifera* (leaves), and *Allium sativum* (cloves) were collected and authenticated from Vikram University Ujjain.

Methodology of Extraction of Plant Materials

The raw materials were air dried for few weeks under shade and pulverized using mechanical blenders until it becomes the coarse powder. Finally, these coarse powders stored in airtight container for future use. Ultimately, by yield, the type of extract was selected for the study.

Sequential Extraction Procedure for Experimental Plant Materials

In this method, freshly collected plant material (*T. cordifolia*, *M. oleifera* and *A. sativum*) were chopped, shade dried and grounded separately for each plant sample. Different extracts were prepared for each plant sample individually

The extraction was conducted by weighing 100 g (*T. cordifolia* dried aerial part, *M. oleifera* dried leaf powder and *A. sativum* clove powder) and then it's extracted using aqueous, methanol, chloroform, petroleum ether in subsequent extraction process by using Soxhlet apparatus. In every step, the marc was dried which results from the previous step of sequential extraction. The extractive was filtered through Whatman No.1 filter paper to eliminate any debris and underivable substances dissolved in the extraction solvent. The resultant extractive solvent removed at their respective temperature by using rotary evaporator under vacuum. Finally, the extracts weighed, and their percentage yield was evaluated using the formula and recorded. Then, the extract was stored in a refrigerator at 4 °C until further use.

Percentage yield of extracts

The formula for the calculation of the percentage of yield: Percentage of yield = (Weight of extract (g) x 100)/ (Weight of raw material (g))

The result expressed w/w

Preliminary Phytochemical Analysis

The determination of phytochemicals was done by the standard methods. These chemical tests can be done by experimenting various phytochemical agents present in the extracts. Phytochemical screening was performed for the analysis of tannins, phenols, alkaloids, flavonoids, glycosides, saponins, terpenoids, carbohydrates and proteins through subsequent standard approaches [18].

Polyherbal Formulation

Three extracts were mixed in following proportion to form polyherbal Churna formulations. The ratio of three different plant extracts is taken as given in following table 1.

Table 1: Details for the Polyherbal Churna Formulations

Polyherbal codes	Proportion of methanolic extracts (*M. oleifera* (MO), *T. cordifolia* (TC), and *A. sativum* (AS)
PHF-1	1:1:1 (1MO:1TC:1AS)
PHF-2	2:1:1 (2MO:1TC:1AS)
PHF-3	2:2:1 (2MO:2TC:1AS)

The crude extracts of drugs were taken and uniformly mixed in above proportions to give three polyherbal formulation PHF-1, PHF-2 and PHF-3

Evaluation of Anti – inflammatory Activity

In vitro Anti-inflammatory activity

Membrne stabilisation method (HRBC method)

Human red blood cell (HRBC) membrane stability was used to assess *in vitro* anti-inflammatory efficacy.

Preparation of blood samples for membrane stabilization assays

In vitro anti-inflammatory activity was determined using the HRBC technique [19].

"1 ml Blood was collected from healthy volunteers and was mixed with equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl).

All the blood samples were stored at 4°C for 24 h before use

This blood solution was centrifuged at 3000 rpm for 5 min and the packed cells were separated.

The packed cells were washed with isosaline solution and a 10% v/v suspension was made with isosaline.

This solution is prepared by adding the 10 grams of pure, noniodized sodium chloride adding 100 ml of distilled water to prepare 10%v/v solution.

This HRBC suspension was used for the estimation of anti-inflammatory property.

Different concentrations of individual extract and all Polyherbal formulations as 200, 300 400mg/ml, reference sample and control were separately mixed with 1mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension.

All the assay mixtures were incubated at 37 °C for 30 minutes and centrifuged at 1300 rpm for 3 minutes. The supernatant liquid was decanted and the hemoglobin content was estimated by a spectrophotometer at 560 nm [19].

The percentage hemolysis was estimated by assuming the hemolysis produced in the control (Distill water) as 100%.

$$\%\text{Inhibition of haemolysis} = (OD2 - OD1/OD3 - OD1) \times 100$$

Where OD_1 = absorbance of test sample in isotonic solution

OD_2 = absorbance of test sample in hypotonic solution

OD_3 = absorbance of control sample in hypotonic solution

Inhibition of albumin denaturation

Sample Extract preparation

The anti-inflammatory effect of *M. oleifera*, *T. Cordifolia*, and *A. sativum*,PHF-1,PHF-2 and PHF-3 was investigated using the inhibition of albumin denaturation approach, which

was minorly modified from originally described by Mizushima Y *et al*. [20] and Sakat *et al*. [21].
In the egg albumin method, the reaction mixture consists of 2.8 mL of phosphate-buffered saline (PBS, pH 6.4), 0.2 mL of fresh hen egg albumin, 2 mL of different *M. oleifera*, *T. Cordifolia* and *A. sativum* dilution,PHF-1,PHF-2,PHF-3 (100,200,300,400 and 500 µg/mL), and Aspirin 0.1% (standard drug) were added in separate test tubes.
The reaction mixtures were incubated for 15 min at 37 ± 2 C, subjected to heating in oven at 70^0C for 10 min, and then allowed to cool.
The absorbance of the reaction mixture was measured at 660 nm by using a UV–Vis spectrophotometer; PBS solution was used as the blank.
The experiment was repeated thrice, and the percentage inhibition of AD was measured using the following equation:
% Denaturation inhibition = (A control — A sample/A control)* 100,
where A sample is Absorbance of Test sample
A control represents the Absorbance of control" [21].

Antiproteinase action
The test was carried out using Sakat *et al*. 2010's modified approach [22].

Procedure
"All the individual extracts and PHF-1, PHf-2 and PHF-3 different concentration solutions were prepared (100,200,300,400 and 500 µg/ml). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 – 500 µg/ml). The mixture was incubated at 37 °C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. (70 ml of perchloric acid plus adding 30 ml of distilled water) Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Anti-lipoxygenase activity [23]
The activity of anti-lipoxygenase was investigated utilising linoleic acid as a substrate and lipoxidase as an enzyme.

Procedure
2 ml of Test samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and added 0.25ml of lipoxidase enzyme solution (20,000U/ml) and incubated for 5 min at 25 °C. After which, 1.0ml of lenoleic acid solution (0.6mM) was added, mixed well and absorbance was measured at 234nm. Indomethacin was used as reference standard. The percent inhibition was calculated from the following equation,

% inhibition= [{Abs control- Abs sample}/Abs control] x 100

A dose response curve was plotted to determine the IC_{50} values. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity. All tests and analyses were run in triplicate and averaged".

Statistical Analysis
Data were presented as "mean± SEM. For continuous variables, student t-test was used to differentiate mean difference. For comparison between more than 2 group, the data were processed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. *P < 0.05 was considered significant. Statistical analysis was performed using SPSS version 21" .

Results and Discussion
***In vitro* anti-inflammatory activity**
Membrane Stabilization method
Extraction of *M. oleifera, T. cordifolia* and *A. sativum*, PHF methanolic extracts at different concentrations (200, 300, 400 mg/mL) showed significant stabilization towards Human red blood cell membranes. The percentage protection of Methanolic extract was found to be increased at higher concentration. The results were tabulated.

Table 2: *In vitro* anti-inflammatory activity of *M. oleifera*, *T. cordifolia*, *A. sativum* and PHF using membrane stabilsation method

S. N	Extract (mg/ml)	% Protection *M. oleifera*	% Protection T cordifolia	% Protection *A. sativum*	% Protection PHF 1	% Protection PHF 2	% Protection PHF 3
1	Methanolic-200	28.23	25.36	26.92	23.18	26.03	30.26
2	Methanolic-300	30.05	28.55	28.52	28.33	29.11	35.68
3	Methanolic-400	33.88	29.32	29.03	34.25	36.25	42.22

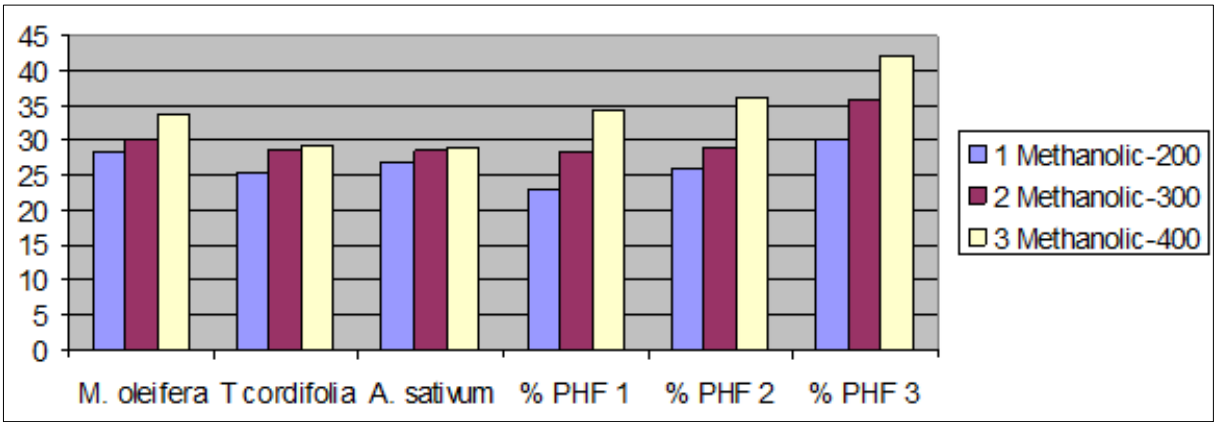

Fig 1: *In vitro* anti-inflammatory activity of *M. oleifera*, *T. cordifolia*, *A. sativum* and PHF using membrane stabilsation method

"The HRBC method was used for *in vitro* anti-inflammatory property evaluation because the erythrocyte membrane is similar to the lysosomal membrane [24], and its stabilisation implies that the extract may also stabilise lysosomal membranes. The stabilisation of the lysosomal membrane is critical for limiting the inflammatory response because it prevents the extracellular release of lysosomal contents of activated neutrophils, such as bactericidal enzymes and proteases, which induce further tissue inflammation and damage. The results showed that the extracts of PHF, *M. oleifera*, *T. cordifolia*, and *A. sativum* have considerable anti-inflammatory properties at varied doses" [25]. In individual extract *M.oleifera* showed the highest activity whereas in Polyherbal formulation PHF-3 has highest activity.

Inhibition of albumin denaturation method

"With minimal adjustments, the inhibition protein denaturation approach was used [21]. 1 mL (0.1 percent) bovine albumin fraction, 1 mL Tris-HCl buffer pH 7.8 solution, and 1 mL test solutions made up the reaction mixture (5 mL). The mixtures were incubated at 37°C for 20 minutes before being heated in a water bath at 70°C for 10 minutes for denaturation" [21].

"The turbidity was measured spectrophotometrically at 660nm after the samples were cooled to room temperature. Positive control and blank solutions were aspirin and buffer, respectively. 1 mL distilled water, 1 mL (0.1%) bovine albumin fraction, and 1 mL buffer solution made up the control solution. The experiment was done in triplicates, and the percent inhibition for protein denaturation was computed. Maximal inhibition of 90.17 percent at a concentration of 500 µg/ml (standard anti-inflammation medicine). The PHF-1 methanol extract had the highest inhibition of 93.76 percent in the methanolic extract. Methanol extract of *M. oleifera* had the highest inhibition of 87.54 percent in the methanolic extract, followed by *A. sativum* (58.65 percent) and *T. cordifolia* (54.49 percent). The presence of flavonoids and phenolic chemicals in the extracts may have caused this inhibition. The presence of alkaloids in methanol extract may be attributed to its high polarity and molecular weight" [26].

Table 3: *In vitro* anti-inflammatory activity of methanolic extracts of *M. oleifera*, *T. cordifolia* and *A. sativum*, PHF plants by inhibition of albumin denaturation method

Name of plant	Percent Denaturation inhibition at Concentration (µg/mL)				
	100	200	300	400	500
Aspirin	35.49±1.91	43.15±0.38	67.71±3.53	85.13±0.99	90.17±1.28
M. oleifera	58.31±0.17	61.53±0.17	66.7±0.17	87.26±0.21	87.54±0.17
T. cordifolia	24.28±0.36	30.39±0.30	48.49±0.25	52.16±0.25	54.49±0.17
A. sativum	52.29±0.30	53.37±0.29	55.86±0.21	56.99±0.39	58.65±0.29
PHF1	62.59± 0.50	69.89±0.87	78.98±0.65	91.65±0.54	93.76±1.78
PHF 2	22.21±0.33	28.98±0.97	51.67±0.78	80.65±0.33	70.98±1.65
PHF 3	21.98±0.86	27.88±0.91	53.78±0.61	79.90±0.31	69.88±0.76

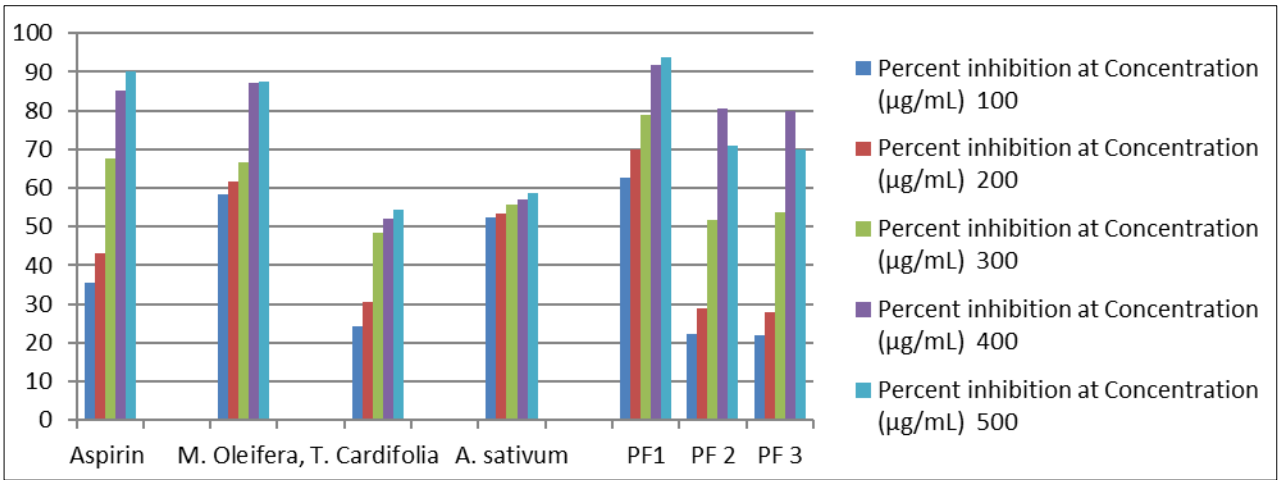

Fig 2: "*In vitro* anti-inflammatory activity of methanolic extracts of *M. oleifera*, *T. cordifolia* and *A. sativum*, PHF plants by inhibition of albumin denaturation method"

PHF-1 has highest activity 93.76 among other formulations and individual extracts comparable to that of standard drug Aspirin 90.17. Protein denaturation can be avoided, which reduces inflammatory situations. The present study shown that methanol extracts of *M. oleifera*, *T. cordifolia*, and *A. sativum* plants and their Polyherbal formulations have anti-inflammatory effect *in vitro* by decreasing protein denaturation. Heat-induced albumin denaturation was effectively inhibited by the extracts.

Antiproteinase action

"The reaction mixture (2 ml) included 0.06 ml trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4), and 1 ml test sample at various concentrations. At 37 degrees Celsius, the reaction mixture was incubated for 10 minutes.

After that, 1 ml of 0.8% (w/v) casein was added. After 20 minutes, the mixture was re-incubated. After incubation, the reaction was stopped with 2 ml of 2M $HClO_4$. The hazy suspension was centrifuged for 15 minutes at 7830 rpm. At 280 nm, the absorbance of the supernatant was measured" As a control, a Tris-HCl buffer was employed. The experiment was carried out three times. Percent inhibition against a range of concentrations was used to determine anti-inflammatory activity. The following formula can be used to compute percent inhibition:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

"The findings of the protease inhibition experiment and the inhibition of protein denaturation method for *M. oleifera*, *T. cordifolia*, *A. sativum* and PHF-1,PHF-2 andPHF-3 are provided in the table. In arthritic processes, proteinases play a crucial role. "Neutrophils are known to be a significant source of serine proteinase, with several serine proteinases localised in their lysosomal granules. It has previously been documented that leukocyte proteinase plays an important role in the development of tissue damage during inflammatory reactions, and proteinase inhibitors give a significant amount of protection" [27]. The proteinase activity was effectively reduced by the methanolic extract. At 500 µg/ml, the typical aspirin medication inhibited the enzyme by 84.79%.

M. oleifera, *T. cordifolia*, and *A. sativum* extracts shown good *in vitro* anti-inflammatory efficacy at 500 g/mL concentration. Many flavonoids and related polyphenols have been found in recent studies to contribute considerably to antioxidant as well as anti-inflammatory actions.

"Methanolic extract of PHF1 Show 95.43% inhibition and *T. cordifolia* showed 91.94% inhibition of protease at 500 µg/mL concentration, the presence of flavonoids and phenolic substances in the leaf extracts could explain this inhibition. At 500 g/mL concentration, the methanol extract of *A. sativum* inhibited protease by 89.06 percent, whereas the methanolic extract of *M. oleifera* inhibited protease by 84.1 percent and that of *T.cordifolia* by 91.94 percent. The presence of glyco-alkaloid solasodine and solasonine may be responsible for the plant's powerful anti-inflammatory activity. These alkaloids found in methanol extract may be due to their high polarity and molecular weight" [27].

Table 4: *In-vitro* anti-inflammatory activity of methanolic extracts of *M. oleifera*, *T. cordifolia* and *A. sativum*, PHF by protease inhibition method

Name of plant	Percent inhibition at Concentration (µg/mL)				
	100	**200**	**300**	**400**	**500**
Aspirin	63.93±1.24	70.99±0.76	78.44±0.47	82.28±0.51	84.79±0.44
M. oleifera	44.17±0.30	64.79±0.20	74.94±0.10	80.86±0.05	84.1±0.02
T. cordifolia	71.24±0.15	81.64±0.07	86.78±0.03	89.35±0.01	91.94±0.01
A. sativum	61.99±0.29	75.53±0.09	82.21±0.04	87.16±0.03	89.06±0.04
PHF1	75.66±0.32	85.76±0.53	90.43±0.23	93.23±0.24	95.43±0.45
PHF2	68.99±0.15	60.11±0.45	72.44±0.55	78.55±0.31	80.56±0.43
PHF3	65.11±0.23	59.15±0.87	71.09±0.54	75.64±0.90	79.98±.09

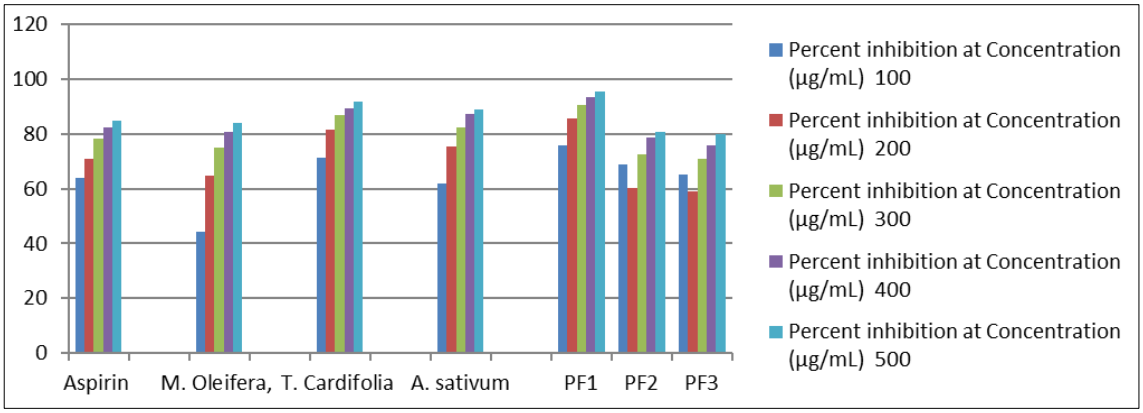

Fig 3: "*In-vitro* anti-inflammatory activity of methanolic extracts" of *M. oleifera*, *T. cordifolia* and *A. sativum*, PHF by protease inhibition methode

Anti-lipoxygenase activity

"Linoleic acid was used as a substrate and lipoxidase as an enzyme to test anti-lipoxygenase activity. Test samples were dissolved in 0.25ml of 2M borate buffer pH 9.0 and incubated for 5 minutes at 25 °C with 0.25ml of lipoxidase enzyme solution (20,000U/ml). Following that, 1.0ml of lenoleic acid solution (0.6mM) was added, thoroughly mixed, and absorbance at 234nm was measured. The reference standard was indomethacin. The percent inhibition was calculated from the following equation,

% inhibition= [{Abs control- Abs sample}/Abs control] x 100

To calculate the IC_{50} values, a dosage response curve was plotted. The IC_{50} concentration is defined as the concentration required to achieve 50% of the maximum scavenging capability. All tests and analyses were performed in triplicate and the results were averaged" .

Table 5: Effect of *M. oleifera* on lipoxygenase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance at 234 nm	% inhibition of lipoxygenase action
Control	-	0.40±0.02	-
M. oleifera	100	0.36±0.03	09
M. oleifera	200	0.33±0.01	17
M. oleifera	300	0.30±0.01	26
M. oleifera	400	0.21±0.07	48
M. oleifera	500	0.15±0.02	62
Indomethacin	100	0.06±0.05	86

"Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant; *p<0.05, considered significant; ns p>0.05, non significant".

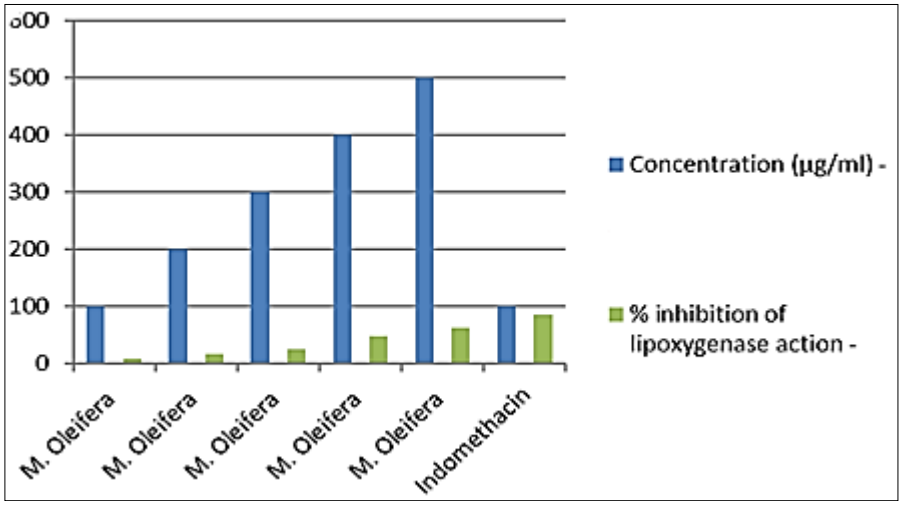

Fig 4: Effect of *M. oleifera* on lipoxygenase inhibitory action

Table 6: Effect of *T. cordifolia* on lipoxygenase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance at 234 nm	% inhibition of lipoxygenase action
Control	-	0.31±0.02	-
T. cordifolia	100	0.22±0.01	30
T. cordifolia	200	0.16±0.09	48
T. cordifolia	300	0.14±0.04	54
T. cordifolia	400	0.12±0.07	61
T. cordifolia	500	0.07±0.02	75
Indomethacin	100	0.15±0.01	51

"Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant; *p<0.05, considered significant; ns p>0.05, non significant".

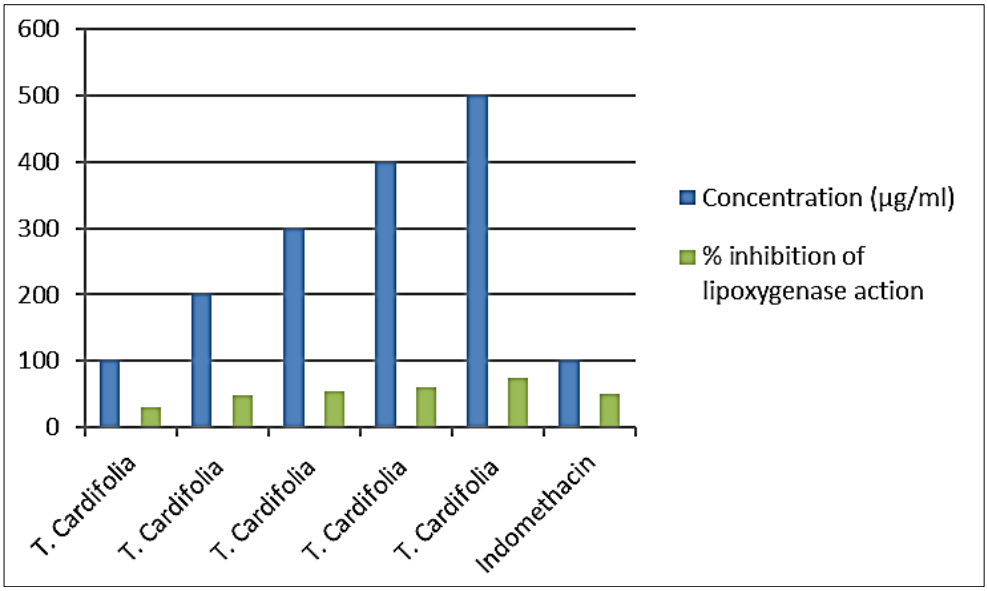

Fig 5: Effect of *T. cordifolia* on lipoxygenase inhibitory action

Table 7: Effect of *A. Sativum* on lipoxygenase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance at 234 nm	% inhibition of lipoxygenase action
Control	-	0.30±0.03	-
A. Sativum	100	0.24±0.08	21
A. Sativum	200	0.21±0.07	30
A. Sativum	300	0.19±0.05	36
A. Sativum	400	0.17±0.01	43
A. Sativum	500	0.15±0.03	51
Indomethacin	100	0.09±0.06	71

"Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant; *p<0.05, considered significant; ns p>0.05, non significant".

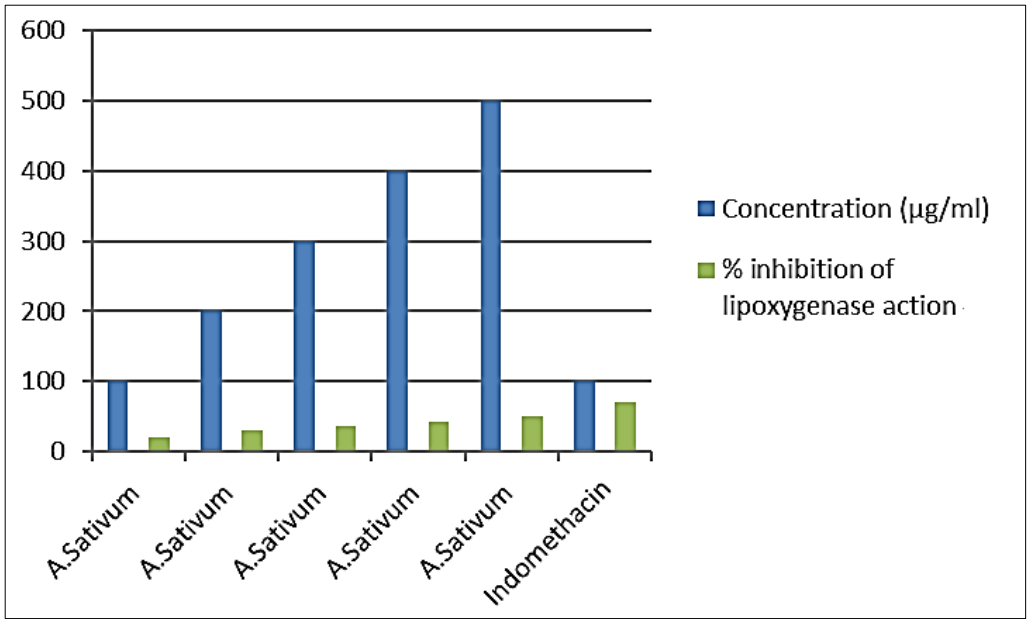

Fig 6: Effect of *A. sativum* on lipoxygenase inhibitory action

Table 8: Effect of PHF1 on lipoxygenase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance at 234 nm	% inhibition of lipoxygenase action
Control	-	0.65±0.03	-
PHF1	100	0.34±0.08	51
PHF 1	200	0.37±0.07	67
PHF 1	300	0.40±0.05	75
PHF 1	400	0.49±0.01	79
PHF 1	500	0.59±0.03	80
Indomethacin	100	0.09±0.06	85

"Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant; *p<0.05, considered significant; ns p>0.05, non significant".

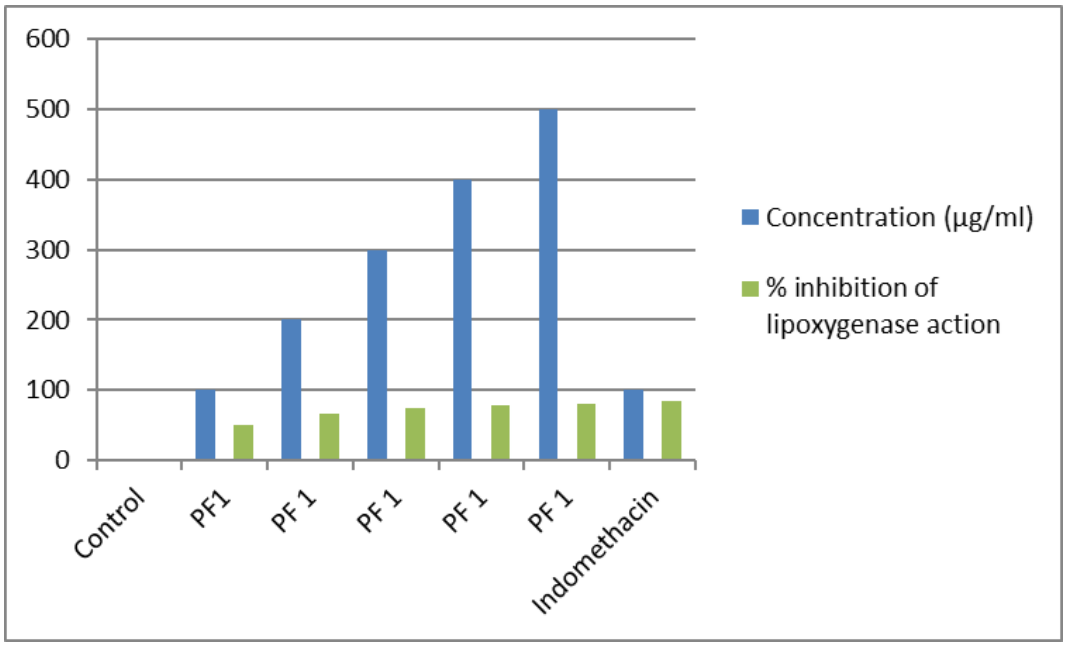

Fig 7: Effect of PHF1 on lipoxygenase inhibitory action

Table 9: Effect of PHF2 on lipoxygenase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance at 234 nm	% inhibition of lipoxygenase action
Control	-	0.55±0.01	-
PHF2	100	0.24±0.01	41
PHF 2	200	0.17±0.05	57
PHF 2	300	0.30±0.06	55
PHF 2	400	0.29±0.07	69
PHF 2	500	0.39±0.08	60
Indomethacin	100	0.06±0.07	55

"Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant; *p<0.05, considered significant; ns p>0.05, non significant".

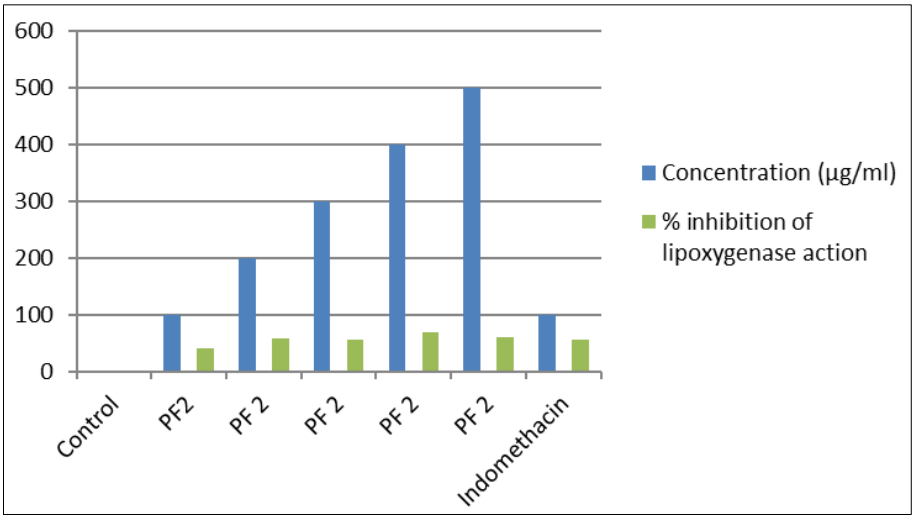

Fig 8: Effect of PHF2 on lipoxygenase inhibitory action

Table 10: Effect of PHF3 on lipoxygenase inhibitory action

Treatments	Concentration (µg/ml)	Absorbance at 234 nm	% inhibition of lipoxygenase action
Control	-	0.45±0.05	-
PHF3	100	0.14±0.07	31
PHF 3	200	0.12±0.09	37
PHF 3	300	0.20±0.09	25
PHF 3	400	0.21±0.01	39
PHF 3	500	0.19±0.03	30
Indomethacin	100	0.16±0.02	25

"Each value represents the mean ± SD. N=3, Experimental group were compared with control **p<0.01, considered extremely significant; *p<0.05, considered significant; ns p>0.05, non significant".

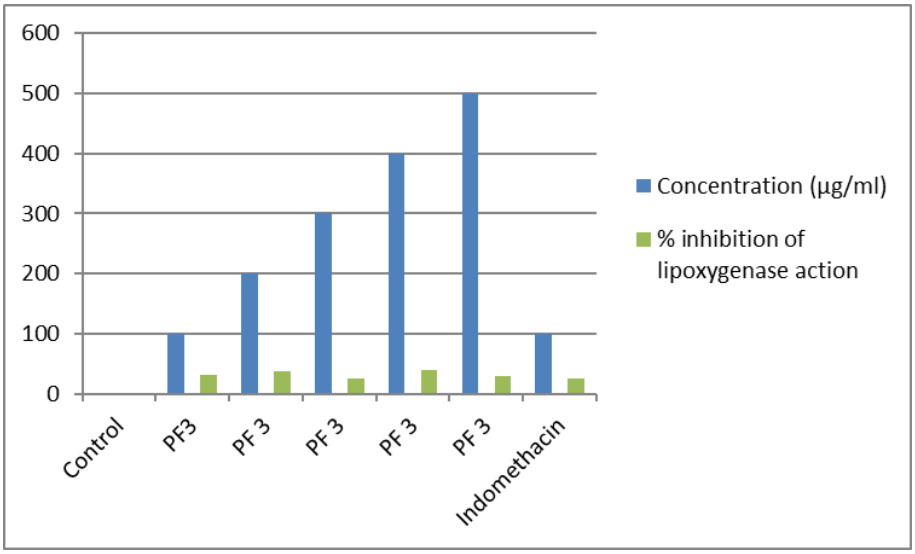

Fig 9: Effect of PHF3 on lipoxygenase inhibitory action

The introduction of new *in vitro* test technologies has increased plant screening in order to "uncover leads for the development of novel medications. The plant lipoxygenase route is similar to animal 'arachidonic acid cascades' in many ways" [28]. As a result, *in vitro* lipoxygenase inhibition is a "promising paradigm for screening plants with anti-inflammatory potential" [29]. "LOXs are susceptible to antioxidants, and the majority of their effect may consist in inhibiting the generation of lipid hydroperoxides by the scavenging of lipidoxy or lipid peroxy-radicals produced during enzyme peroxidation. This can reduce the availability of lipid hydroperoxide substrate, which is required for the LOX catalytic cycle" [29].

M. oleifera was tested at "100, 200, 300, 400, and 500 µg/ml" and demonstrated antilipoxygenase inhibition of 9, 17, 26, 48, and 62 percent, respectively. *T. cordifolia* was tested at "100, 200, 300, 400, and 500 µg/ml" concentrations and demonstrated 38, 48, 54, 61, and 75 percent antilipoxygenase inhibition, respectively. *A. sativum* was also tested at "100, 200, 300, 400, and 500 µg/ml" and demonstrated antilipoxygenase inhibition of 21, 30, 36, 43, 51, and 71%. Based on these findings, the strongest inhibition was observed at a concentration of 500 µg/ml. At a concentration of 100 µg/ml, the standard Indomethacin inhibited the enzyme by 86 percent.

Our findings on PHF and *T. cordifolia* suggest that they may have anti-inflammatory properties. The lipoxygenase enzyme activity was reduced by *T. cordifolia* preparations. This suggests that the plant *T. cordifolia* is more effective in research of inflammation and other relevant physiological investigations, ageing, and diseases such as cancer, neurological disorders, and so on.

Conclusion

The results obtained from the present study polyherbal formulations have shown a potential anti-inflammatory activity. These activities may be due to the strong occurrence of phenolic compounds alkaloids, flavonoids, tannins and steroids. The overall results of the study indicate that the polyherbal formulation containing *Tinosporia cordifolia*, *Moringa oleifera* and *Allium sativum* is a good source of biologically active compounds with antiinflammatory potentials. Best among these is PHF-1 with promising results as compared to standard. However, further research regarding *in vivo* study using animal models to check their effect on animal cells is suggested.

References

1. Bagad AS, Joseph JA, Bhaskaran N, Agarwal A. Comparative evaluation of anti-inflammatory activity of curcuminoids, turmerones, and aqueous extract of Curcuma longa. Advances in pharmacological sciences, 2013
2. Ghasemian M, Owlia S, Owlia MB. Review of anti-inflammatory herbal medicines. Advances in pharmacological sciences, 2016.
3. Muangman P, Muffley LA, Anthony JP, Spenny ML, Underwood RA, Olerud JE, *et al*. Nerve growth factor accelerates wound healing in diabetic mice. Wound Repair and Regeneration. 2004;12(1):44-52.
4. Mookherjee N, Brown KL, Bowdish DM, Doria S, Falsafi R, Hokamp K, Hancock RE. Modulation of the TLR-mediated inflammatory response by the endogenous human host defense peptide LL-37. The Journal of Immunology. 2006;176(4):2455-2464.
5. Shojaii A, Motaghinejad M, Norouzi S, Motevalian M. Evaluation of anti-inflammatory and analgesic activity of the extract and fractions of Astragalus hamosus in animal models. Iranian Journal of Pharmaceutical Research: IJPR. 2015;14(1):263-269.
6. Yong SL. Protective Analgesia Using Bupivacaine and Ibuprofen for Postoperative Dental Pain. The University of Manchester (United Kingdom). Ann Stomatol (Roma). 2011 Jul-Dec; 2(3-4 Suppl):3-24.
7. Köksal M, Gökhan N, Küpeli E, Yesilada E, Erdogan H. Analgesic and antiinflammatory activities of some new Mannich bases of 5-nitro-2-benzoxazolinones. Archives of pharmacal research. 2007;30(4):419-424.
8. Munir F, Yarker J, Haslam C, Long H, Leka S, Griffiths A, *et al*. Work factors related to psychological and health-related distress among employees with chronic illnesses. Journal of occupational rehabilitation. 2007;17(2):259-277.
9. Niemi TT, Taxell C, Rosenberg PH. Comparison of the effect of intravenous ketoprofen, ketorolac and diclofenac on platelet function in volunteers. Acta anaesthesiologica scandinavica. 1997;41(10):1353-1358.
10. Vittarao A, Shanbhag T, Kumari K. "Evaluation of anti-inflammatory and analgesic of alcoholic extract of Kaemperia Galagna in rats. Indian Journal of Physiology & Pharmacology. 2011;55(1):13–24.
11. Guevara AP, Vargas C, Sakurai H, *et al*. An antitumour promoter from *Moringa oleifera* Lam. Mutat Res, 1999;440:181-8.
12. Abalaka ME, Olonitola OS, Onaolapo JA, *et al*. using wistar rats to determine safety level and usefulness of the plant ethnochemotheraphy. Int J Appl Sci. 2009;3:1-6.
13. Misra B, Bhava Prakash Nighantu, Vol. 1, (Hindi commentary by K C Chunekar), (Chowkhamba Vidya Bhavan, Varanasi), 1969, 269.
14. Singh SS, Pandey SC, Shrivastava S, Gupta VS, Palio B. Chemistry and medical properties of T. Cordifolia. Indian J. Pharmacol. 2003;35:83-99.
15. Thatte VM, Roa SG, Dahanukar SA. Tinospora *cordifolia* induces colony-stimulating Shivraj & Author activity in serum, j. post, grad, med, Bombay. 1994;40:202-3.
16. Agarwal KC. Therapeutic actions of garlic constituents. Med. Res. Rev. 1996;16:111-124.
17. Fukushima S, Takada N, Hori T, Wanibuchi H. Cancer prevention by organosulfur compounds from garlic and onion. J. Cell. Biochem. 1997;27(Suppl.):100–105.
18. Kokate A. Phytochemical methods. Phytotherapy Research. 1999;78:126-129.
19. Azeem AK, Dilip C, Prasanth SS, Junise V, Hanan Shahima. Anti-inflammatory activity of the glandular extracts of Thunnus alalunga. Asia Pac J Med. 2010;3(10):412-20.
20. Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum preoteins, especially with some biologically active proteins. J Pharma Pharmacol. 1968;20:169-173.
21. Sakat S, Tupe P, Hule A, Juvekar A. Anti-inflammatory potential of flavonoid fraction of Tamarindus indica Linn (seeds). Planta Medica. 2010;76(12):SL_20.
22. Das SN, Chatterjee S. Long term toxicity study of ART-400. Indian Indg Med. 1995;16(2):117-123.
23. Biomed. Sci. 2011;2:256-259. 23. Kumaraswamy, MV., Satish, S. Antioxidant and Anti-Lipoxygenase Activity of

Thespesia lampas Dalz & Gibs. Adv. Biol. Res. 2008;2:56-59.
24. Abad MJ, Bermejo P, Villar A. The activity of flavonoids extracted from *Tanacetum microphyllum* DC. (Compositae) on soybean lipoxygenase and prostaglandin synthetase. General pharmacology. 1995;26(4):815-819.
25. Vileges JH, DeMarchi E, Lancas EM. Phytochemical studies of some medicinal plants. Anal. 1997;8:266-70.
26. Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, *et al*. High molecular weight plant polyphenolics (tannins) as biological antioxidants. Journal of agricultural and food chemistry. 1998;46(5):1887-1892.
27. Widsten P, Cruz CD, Fletcher GC, Pajak MA, McGhie TK. Tannins and extracts of fruit byproducts: antibacterial activity against foodborne bacteria and antioxidant capacity. Journal of agricultural and food chemistry. 2014;62(46):11146-11156.
28. Sparg S, Light ME, Van Staden J. Biological activities and distribution of plant saponins. Journal of ethnopharmacology. 2004;94(2-3):219-243.
29. Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: a review. Phytochemistry Reviews. 2010;9(3):425-474.