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IN-VITRO XANTHINE OXIDASE INHIBITORY AND ANTI-INFLAMMATORY ACTIVITY OF LEAVES OF *PERSEA AMERICANA* MILL.

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ABSTRACT

The aim of the study is to evaluate the in-vitro xanthine oxidase inhibitory activity and anti-inflammatory activity of the leaves of *Persea americana*. The plant was collected, authenticated and pulverized to coarse powder. Aqueous extract was prepared by maceration method. n-hexane, petroleum ether and ethanolic extracts were prepared by hot continuous extraction using Soxhlet apparatus. They were subjected to phytochemical screening. Xanthine oxidase inhibitory activity was assayed spectrophotometrically and the degree of enzyme inhibition was determined by measuring the increase in absorbance at 290nm. The increase in absorbance is associated with the quantity of uric acid. *In-vitro* anti-inflammatory activity was evaluated by protein denaturation and membrane stabilization method. Extracts showed the presence of tannins, flavonoids, alkaloids, phenols, glycosides and steroids. Among the various extracts, ethanolic extract showed maximum xanthine oxidase inhibitory activity and protein denaturation inhibitory activity. Among the various extracts, n- hexane extract showed maximum membrane stabilization activity.

KEYWORDS: *in-vitro*, membrane stabilization, *Persea americana*, protein denaturation, xanthine oxidase inhibition.

INTRODUCTION

Gout is a heterogenous group of diseases resulting from the deposition of urate (as monosodium urate monohydrate) crystals in supersaturated extracellular fluids. These

crystals cause an acute inflammatory response and can induce a permanent tissue damage which is characterized by the appearance of ulceration of the joint cartilage, marginal osteophytosis, erosive lesions and chronic inflammation of synovial membrane.[1] Gout has a world-wide distribution and affects 1% population. In India, it affects 0.12% - 0.19% of population with a male preponderance.[2] Gout is a significant cause of disability and mortality[3] and hyperuricemia increased the risk of both stroke incidence and mortality. The most common age of onset is >40 years.[4] Urate lowering therapy is the main approach in the treatment of gout. The target level of serum uric acid is < 6.8 mg / dL to dissolve the urate crystals and inhibit gout attack.[5,6] The drugs commonly used in the treatment of gout are, Allopurinol – a xanthine oxidase inhibitor, Probenecid – an uricosuric agent, Colchicines, Corticosteroids, NSAIDs, Corticotrophin. Their prolonged duration of treatment is associated with many adverse reactions such as Stevens-Johnsons syndrome, toxic epidermal necrolysis and teratogenecity when used in pregnancy.

The *Persea americana* of family Lauraceae is native to South Central Mexico. Recent archaeological research published evidence that the avocado was present in Peru for as long as 8,000 to 15,000 years. In recent years, research has focussed on various parts of the plant. The leaves in particular have been shown to possess medicinal properties.[7]

Many pharmacological studies have showed that extracts of *Persea americana* have hypoglycaemic, hypolipidemic, hypotensive, anti-viral, anti-tumour, anti-oxidant, anti-ulcer, anti-bacterial/anti-mycobacterial, wound healing benefits etc.[8]

The anti-gout medications which also possess the anti-inflammatory properties are found to be more effective candidates to treat gout. Hence, in the present study an attempt has been made to screen the xanthine oxidase inhibitory activity and anti-inflammatory activity by in vitro models.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Persea americana* were collected from the hill station, Kodaikanal in Dindigul district of Tamilnadu during the month of August 2017.

The plant was authenticated by Dr. P. Jayaraman Ph.D., The Director (Retd.), Plant Anatomy Research Centre, Pharmacognosy Institute, West Tambaram, Chennai.

Preparation of plant extract

The leaves of *Persea americana* were collected, shade dried and pulverized to a coarse powder. 60 grams of coarse powder was then extracted by various solvents using Soxhlet apparatus. The solvents used were n-hexane, petroleum ether, ethanol and distilled water.

The aqueous extract was prepared by maceration process. 60 grams of the crude drug was soaked in a mixture of 500ml of distilled water and 30ml of chloroform (as preservative) for a period of 7 days. The container was sealed and kept inside the dark chamber at room temperature. It was agitated continuously at regular intervals. Then the soaked plant material was filtered to collect the aqueous extract.

The collected n-hexane, petroleum ether, ethanol and aqueous extracts were concentrated by evaporating them with gentle heat and the residue was stored in air-tight containers.

Preliminary phytochemical evaluation: Anti-gout property of a plant extract depends on its phytoconstiuents. It is already evident that different phytoconstituents are responsible for various pharmacological activities. Content of flavonoids in leaves of *Persea americana* are high. **Flavonoids** are potent water soluble antioxidants and free radical scavengers. They prevent oxidative cell damage, have strong anti-cancer activity and protect against all stages of carcinogenesis. Flavonoids play an important role in the anti-inflammatory, anti-cancer and anti-hypertensive property. **Alkaloids** are therapeutically significant plant secondary metabolite. Isolated pure form of alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic and bactericidal effects. **Phenols** have been extensively used in researches as disease preventives. Phenols detected in the parts of *Persea americana* indicate their ability to act as anti - inflammatory, anti - clotting, anti - oxidants, immune enhancers, etc. **Tannins** are high in leaves when compared to fruits and seeds, which hastens the healing of wounds and inflamed mucous membrane.[9]

The aqueous, ethanolic, petroleum ether and n-hexane extracts of *Persea americana* were qualitatively analysed for alkaloids, glycosides, saponins, tannins & phenolic compounds, flavonoids, terpenoids and steroids as per the standard procedures.[10,11]

***In-vitro* xanthine oxidase inhibitory activity[12]**

$$\text{Xanthine} + H_2O + O_2 \xrightarrow{\text{Xanthine oxidase}} \text{uric acid} + H_2O_2$$

The inhibitory effect on xanthine oxidase was determined spectrophotometrically by following the increase in the absorbance at 290nm. The reaction mixture consisted of 1.9ml of 50mM phosphate buffer of pH 7.5, 1ml of 0.15mM xanthine, 0.1ml of sample solution dissolved in dimethyl sulfoxide and the mixture was incubated at 37°C for 10mins. Then 0.1ml of 0.1- 0.2 U xanthine oxidase enzyme was added and incubated at 37°C for 30mins. The reaction was stopped immediately by addition of 1ml of 1N hydrochloric acid and then absorbance was read at 290nm. Percentage of inhibition was calculated by measuring the absorbance of uric acid from the mixture without test extract (blank samples) compared with the absorbance of mixture of test extract. Controls were analysed in the same manner as the sample group without the addition of enzymes.

% inhibition = (1- B/A) x 100

Where,

A – Absorbance without sample (absorbance with enzyme – without enzyme)

B – Absorbance with sample (absorbance of samples with enzyme – without enzyme)

The absorbance increments of UV absorbance at 290nm indicated the formation of uric acid. For the standard (allopurinol), n-hexane, petroleum ether, ethanol and aqueous extract the dosage for xanthine oxidase inhibitory activities were examined at concentration of 200, 400, 600, 800 and 1000µg/ml respectively.

***IN – VITRO* ANTI – INFLAMMATORY ACTIVITY[13]**

a) Inhibition of protein denaturation

The reaction mixture was consisted of test extract at different concentrations and 1% aqueous solution of bovine serum albumin (BSA) fraction. pH of the reaction mixture was adjusted using small amount of 1N hydrochloric acid. The samples were incubated at 37°C for 20mins and then heated at 51°C for 20mins. After cooling the samples, the turbidity was measured spectrophotometrically at 660nm. The experiment was performed in triplicate. Percentage inhibition of protein denaturation was calculated as follows

$$\textbf{Percentage inhibition} = 100 - \left[\frac{absorbance\ (control) - absorbance\ (sample)}{absorbance\ (control)}\right] \textbf{x100}$$

b) Membrane stabilizing activity

Preparation of Red Blood Cells (RBCs) suspension: Fresh rat blood was collected and transferred to the heparinized centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10

mins and were washed three times with equal volume of normal saline. The volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline.

Heat induced haemolysis

Control: The reaction mixture (2 ml) consisted of 1ml of saline and 1ml of 10% RBCs suspension.

Sample: The reaction mixture (2 ml) consisted of 1ml of test drug solution and 1ml of 10% RBCs suspension.

Diclofenac was taken as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 mins. At the end of incubation, the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 mins and the absorbance of the supernatants was taken at 560nm. The experiment was performed in triplicates. Percentage membrane stabilization activity was calculated as follows:

Percentage membrane

Stabilization activity = 100 – $\left[\frac{absorbance\ (control) - absorbance\ (sample)}{absorbance\ (control)}\right]$**x100**

RESULTS AND DISCUSSION

Percentage yield

The leaves of *Persea americana* collected during the month of August 2017 were shade dried and coarse powdered. The n-hexane, petroleum ether and ethanolic extracts were prepared using Soxhlet apparatus and the aqueous extract was prepared by simple maceration process. Percentage yield was calculated and given in **Table 1** and **Figure 1.**

Table. 1: The percentage yield of various extracts of *Persea Americana.*

Extracts	% Yield (% W/W)
Ethanol	14.26
Aqueous	13.68
Petroleum ether	3.68
n – hexane	3.16

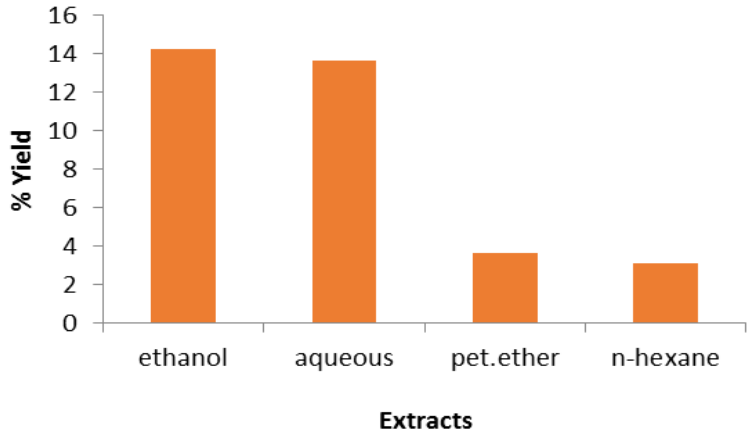

Fig. 1 Percentage yield of various plant extracts.

It is seen that the percentage yield was higher in both ethanolic and aqueous extracts when compared with the n-hexane and petroleum ether extracts.

Preliminary phytochemical evaluation

The n-hexane, petroleum ether, ethanol and aqueous extracts were evaluated qualitatively for various phytoconstituents and the results are tabulated in **Table 2.**

Table. 2: preliminary phytochemical analysis of various extracts of leaves of *Persea Americana.*

Phytochemicals	Extract			
	Ethanol	Petroleum ether	n- hexane	Water
Alkaloids	+	-	+	+
Saponins	+	+	+	+
Tannins	+	+	+	+
Phenols	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	+	+	-
Glycosides	+	+	+	+
Flavonoids	+	+	+	+

'+' denotes – Presence of the constituent; '-' denotes – Absence of the constituent

Table 2 shows that, the phytoconstituents such as alkaloids, saponins, tannins, phenols, terpenoids, steroids, glycosides and flavonoids are present in n-hexane and ethanolic extracts. The alkaloids are absent and all other phytoconstituents are present in petroleum ether extract. Steroids are absent and all other phytoconstituents are present in aqueous extract.

***In-Vitro* Studies**

Xanthine oxidase inhibitory activity

Xanthine oxidase is an enzyme which catalyzes the oxidation of hypoxanthine to xanthine and then to uric acid. Uric acid formation is inhibited by inhibiting the enzyme xanthine oxidase. The principle of measurement of the activity of xanthine oxidase inhibition is quantification of uric acid which formed in the reaction catalyzed by xanthine oxidase using UV spectrophotometry at 290nm. Xanthine oxidase metabolizes purines to uric acid.

The results of percentage inhibition of xanthine oxidase by various crude extracts of *Persea americana* Mill by *in – vitro* method was shown in **Table 3 and Figure 2.**

Table. 3: Percentage inhibition of xanthine oxidase by various plant extracts.

Extract	Concentration (µg)	Percentage inhibition
Standard (Allopurinol)	200	72.73±0.78
	400	77.52±0.51
	600	85.98±0.11
	800	86.72±0.31
	1000	88.84±0.24
Ethanolic extract	200	63.21±0.17
	400	69.38±0.29
	600	74.87±0.13
	800	81.82±0.40
	1000	85.86±0.22
Petroleum ether extract	200	55.86±0.67
	400	63.85±0.36
	600	75.38±0.83
	800	81.36±0.39
	1000	84.16±0.31
n-hexane extract	200	37±0.32
	400	51.57±0.39
	600	53.98±0.44
	800	64.76±0.50
	1000	75.69±1.23
Aqueous extract	200	44.69±0.84
	400	62.09±0.66
	600	68.86±0.79
	800	71.97±0.49
	1000	80.66±0.25

The values are expressed as mean ± SEM.

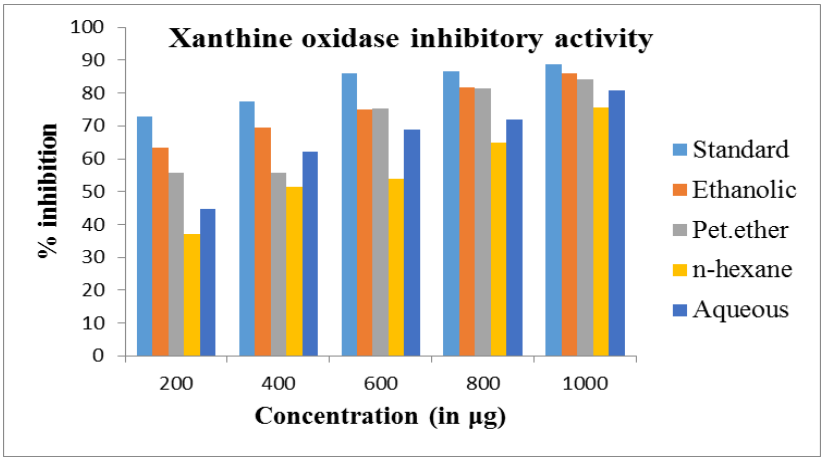

Fig. 2: Percentage inhibition of xanthine oxidase by various extracts of the leaves of *Persea americana* Mill.

Standard (Allopurinol) shows maximum (88.84%) xanthine oxidase inhibitory activity in the concentration of 1000µg. **Ethanolic extract** of the leaves of *Persea americana* Mill. shows dose dependent xanthine oxidase inhibitory activity and the maximum (85.86%) inhibitory action was observed in 1000µg. **Petroleum ether extract** of the leaves of *Persea americana* Mill. shows dose dependent xanthine oxidase inhibitory activity and the maximum (84.16%) inhibitory action was observed in 1000µg. **Aqueous extract** of the leaves of *Persea americana* Mill. shows dose dependent xanthine oxidase inhibitory activity and the maximum (80.66%) inhibitory action was observed in 1000µg. **n - hexane extract** of the leaves of *Persea americana* Mill. shows dose dependent xanthine oxidase inhibitory activity and the maximum (75.69%) inhibitory action was observed in 1000µg. The inhibitory concentration **(IC 50)** values of standard, ethanolic, aqueous, petroleum ether, n-hexane extracts were found to be 140, 160, 320, 180, 490 µg/ml respectively. Among the various extracts, ethanolic extract showed maximum xanthine oxidase inhibitory activity.

***In-vitro* anti-inflammatory activity**

Protein denaturation method

Protein denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress (or) compounds such as strong acid or base, a concentrated inorganic salt, an organic solvent (or) heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation in rheumatoid arthritis and gout. In this assay the protein denaturation was induced by heat. The results of percentage inhibition of protein denaturation by various crude extracts of *Persea americana* Mill by *in – vitro* method was shown in **Table – 4.**

Table. 4: Percentage inhibition of protein denaturation by various crude extracts of *Persea americana* Mill.

Extract	Concentration (µg)	Percentage inhibition
Standard (diclofenac)	100	15.02±0.58
	200	28.79±0.48
	400	45.71±0.17
	800	73.49±0.33
	1000	97.76±0.19
Ethanolic extract	100	39.48±0.45
	200	41.82±0.86
	400	47.60±0.73
	800	59.75±0.33
	1000	84.53±0.55
Petroleum ether	100	25.11±0.50
	200	36.19±0.17
	400	43.19±0.34
	800	55.74±0.39
	1000	71.58±0.37
n-hexane extract	100	37.51±0.55
	200	45.87±0.37
	400	51.06±0.61
	800	54.02±0.56
	1000	79.99±0.29
Aqueous extract	100	30.43±0.28
	200	49.30±0.45
	400	51.95±0.64
	800	63.50±0.28
	1000	82.05±0.20

The values are expressed as mean ± SEM.

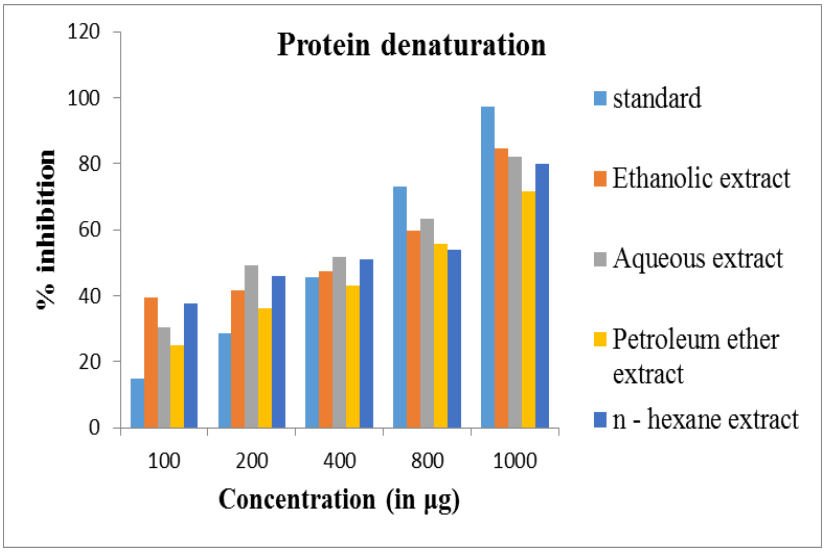

Fig. 3: Percentage inhibition of protein denaturation by various extracts of the leaves of *Persea americana* Mill.

Standard (diclofenac) shows maximum (97.76%) protein denaturation inhibitory activity in the concentration of 1000µg. **Ethanolic extract** of the leaves of Persea americana Mill. shows dose dependent protein denaturation inhibitory activity and the maximum (84.53%) inhibitory action was observed in 1000µg. **Petroleum ether extract** of the leaves of Persea americana Mill. shows dose dependent protein denaturation inhibitory activity and the maximum (71.58%) inhibitory action was observed in 1000µg. **Aqueous extract** of the leaves of Persea americana Mill. shows dose dependent protein denaturation inhibitory activity and the maximum (82.05%) inhibitory action was observed in 1000µg. **n - hexane extract** of the leaves of Persea americana Mill. shows dose dependent protein denaturation inhibitory activity and the maximum (79.99%) inhibitory action was observed in 1000µg. The **IC 50** values of standard, ethanolic, aqueous, petroleum ether, n-hexane extracts were found to be 440, 370, 460, 600, 380 µg/ml respectively. Among the various extracts, ethanolic extract showed maximum protein denaturation inhibitory activity.

Membrane stabilization method

The erythrocyte membrane resembles to lysosomal membrane and as such, the effect of drugs on the stabilization of erythrocyte could be extrapolated to the stabilization of lysosomal membrane. Therefore, as membrane stabilizes there would be a decrease in the release and/or action of mediators like histamine, serotonin, prostaglandins, leukotrienes etc. The principle involved in this assay is stabilization of rat red blood cell membrane against hypotonocity induced membrane lysis. The results of membrane stabilizing activity by various crude extracts of *Persea americana* Mill by *in – vitro* method was shown in **Table – 5.**

Table. 5: Membrane stabilizing activity by various crude extracts of *Persea Americana.*

Extract	Concentration (µg)	Percentage stabilizing activity
Standard (diclofenac)	100	16.07±0.09
	200	25.49±0.17
	400	45.99±0.09
	800	74.73±0.26
	1000	98.13±0.05
Ethanolic extract	100	24.16±0.13
	200	30.76±0.14
	400	59.06±0.36
	800	89.53±0.21
	1000	97.31±0.16
Petroleum ether extract	100	37.58±0.16
	200	58.29±0.20
	400	62.37±0.24

	800	76.82±0.19
	1000	89.63±0.22
n-hexane extract	100	39.19±0.12
	200	58.01±0.11
	400	62.31±0.37
	800	94.76±0.19
	1000	98.21±0.19
Aqueous extract	100	21.38±0.28
	200	52.06±0.14
	400	61.41±0.10
	800	94.27±0.15
	1000	97.81±0.07

The values are expressed as mean ± SEM.

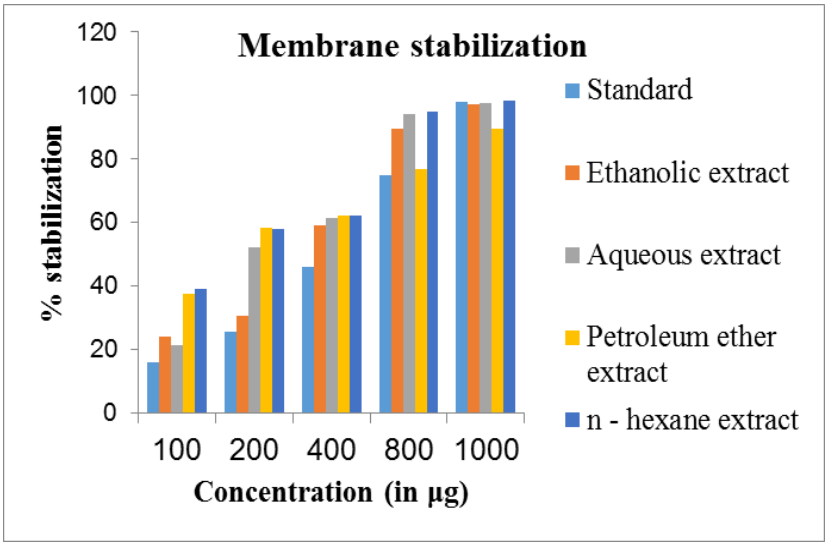

Fig. 4: Membrane stabilization action of various extracts of the leaves of *Persea americana* Mill.

Standard (diclofenac) shows maximum (98.13%) membrane stabilization activity in the concentration of 1000µg. **Ethanolic extract** of the leaves of *Persea americana* Mill. shows dose dependent membrane stabilization activity and the maximum (97.31%) stabilization action was observed in 1000µg. **Petroleum ether extract** of the leaves of *Persea americana* Mill. shows dose dependent membrane stabilization activity and the maximum (89.63%) stabilization action was observed in 1000µg. **Aqueous extract** of the leaves of *Persea americana* Mill. shows dose dependent membrane stabilization activity and the maximum (97.81%) stabilization action was observed in 1000µg. **n - hexane extract** of the leaves of *Persea americana* Mill. shows dose dependent membrane stabilization activity and the maximum (98.21%) stabilization action was observed in 1000µg. The **IC 50** values of standard, ethanolic, aqueous, petroleum ether, n-hexane extracts were found to be 440, 320,

300, 310, 230µg/ml respectively. Among the various extracts, n- hexane extract showed maximum membrane stabilization activity.

CONCLUSION

In the present study, results indicate that the ethanolic extract of leaves of *Persea americana* possess potent xanthine oxidase inhibitory activity and maximum percentage inhibition of protein denaturation when compared to n-hexane, petroleum ether and aqueous extracts. Maximum percentage of membrane stabilization was seen in n-hexane extract. These activities may be due to the occurrence of phytoconstituents such as alkaloids, tannins, flavonoids, steroids, terpenoids and phenols. Results of present study suggest that ethanolic extract of *Persea americana* leaves has the most potent activity in inhibiting xanthine oxidase activity, indicating its potential to be developed as an agent for treating hyperuricaemia and gout.

REFERENCE

1. Vaidehi N. Sarvaiya et.al,Evaluation of anti-gout activity of Phyllanthus emblica fruit extracts on potassium oxonate induced gout rat model, Veterinary world, 2015; 8: 1230-1236.
2. EUR Smith, Epidemiology of gout: an update, Best Practice & Research: Clinical Rheumatology, 2010; 24: 811-827.
3. Kim SY, Guevara JP, Kim KY, Choi HK, Heitijan DF and Albert DA, Hyperuricemia and risk of stroke: a systemic review and meta – analysis, Arthritis & Rheumatology, 2009; 61(7): 885-892.
4. Karissa Y, Ralph Schumacher, Elke Hunsche, Albert and Sheldon X.Kong, A literature review of the epidemiology and treatment of acute gout, Clinical therapeutics, 2003; 25(6): 1593-1617.
5. Stamp L.K., O' Donnell J.L and Chapman P.T., Emerging therapies in the long – term management of hyperuricaemia and gout, Internal Medicine Journal, 2007; 37(4): 258-266.
6. Falasca G.F., Metabolic diseases, Gout. Clin. Dermatol, 2006; 24(6): 498-508.
7. Raymond I. Ozolua, Ogochukwu N. Anaka, Stephen O. Okpo and Sylvester E. Idogun, Acute and sub – acute assessment of the aqueous seed extract of Persea americana Mill (Lauraceae) in rats, African Journal of Traditional, Complementary and Alternative Medicines, 2009; 6(4): 573-578.

8. Mohammed Yasir, Sattwik Das and M.D. Kharya, The Phytochemical and pharmacological profile of Persea americana Mill, Pharmacognosy Reviews, 2010; 4(7): 77-84.
9. Amadi B.A.et.al., Chemical composition of Persea americana leaf, fruit and seed, International Journal of Research and Reviews in Applied Sciences, 2012; 11(2): 346-349.
10. C. K. Kokate, A. R. Purohit, B. Gokhale, Pharmacognosy, 45th edition A-1 to A-6.
11. Rahman Gul, Syed Umer Jan, Syed Faridullah, Samiullah Sherani and Nusrat Jahan, Preliminary Phytochemical screening, Quantitative analysis of alkaloids and anti-oxidant activity of crude plant extracts from Ephedra intermedia Indigenous to Balochistan, The Scientific World Journal, 2017; 2017: 162-167.
12. Muthiah PL, In-vitro xanthine oxidase inhibitory activity of leaves, fruits and peel extracts of Citrus aurantium, Citrus limetta and Citrus limon, Internatinal Journal of Phytopharmacy, 2012, vol . 2 (3): 77-80.
13. Sakat S, Juvekar AR, Gambhire MN, In-vitro anti-oxidant and anti-inflammatory activity of methanol extracts of Oxalis corniculata Linn. International Journal of Pharmaceutical Sciences, 2010; 2(1): 146-156.