



**INVITRO STUDY ON ANTIDIABETIC, ANTIOXIDANT, ANTI-INFLAMMATORY,  
ANTI-OBESITY PROPERTIES OF POLYPHENOL RICH FRACTION FROM THE  
LEAVES OF *ORTHOSIPHON STAMINEUS***

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**ABSTRACT**

*Orthosiphon stamineus* is a medicinal plant including various type of plant used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the “backbone” of traditional medicine. The plant treated to the many diseases including Diabetes, Antioxidant, Inflammatory and Obesity. *O. stamineus* (distilled water, 50% aqueous methanol, methanol, 70% aqueous acetone and chloroform extracts) have been tested for free radical-scavenging activity, using a 1,1-diphenyl-2-picrylhydrazyl *in vitro* model system. The highest activity was found in acetone extract. This showed its anti oxidative potency was comparable with quercetin and butylated hydroxyanisole (BHA). They also proved using different *in vitro* method (superoxide scavenging and xanthine oxidase) that *O. stamineus* extract showed potential antioxidant activity. They were tested for antioxidant activity using inhibition of NO production in LPS-activated. Mostly 60-75% of the medicinal species of *Orthosiphon* have been traditionally used for treatment of inflammation and diseases like arthritis, bronchitis and rheumatoid. The pharmacological activity of the species of genus *Orthosiphon* provides primarily *in vivo* information for anti-inflammatory effects. The polyphenol rich fraction from leaves of *Orthosiphon stamineus* was inhibiting the pancreatic lipase most widely studied mechanism for the identification of potential anti obesity agents.

**KEYWORDS:** Anti oxidative, butylated hydroxyanisole, quercetin, *Orthosiphon stamineus*, polyphenol.

**INTRODUCTION**

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000). These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. The Indian sub-continent has a very rich diversity of plant species in a wide range of ecosystems. There are about 17,000 species of higher plants, of which approximately 8,000 species, are considered medicinal and used by village communities, particularly tribal communities, or in traditional medicinal systems, such as the Siddha and Ayurveda.

The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed by UNESCO, 1996. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and

development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (UNESCO, 1998). During the past decade, traditional systems of medicine have become a topic of global importance. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Medicinal plants are an integral component of research developments in the pharmaceutical industry. Such research focuses on the isolation and direct use of active medicinal constituents, or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds.

**MATERIALS AND METHODS**

**Collection and Preparation of Plant Extracts**

The leaves of *Orthosiphon stamineus* were collected from Government Siddha Medical College Herbal Garden,

Arumbakkam, Chennai-600 106. The plants authenticated identification done by Dr. S. Sankaranarayanan, Asst. professor, Department of Botany, Government Siddha Medical College, Arumbakkam, Chennai-600 106 Tamil Nadu, India.

### Phytochemical Analysis Of Aqueous decoction From The *Orthosiphon Stamineus*

The aqueous decoction of *Orthosiphon stamineus* were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973; Trease and Evans 1983).

### Extraction Preparation

The aqueous extract from the leaves of *Orthosiphon stamineus* (100 g) were crushed using food masher then extracted with sterile water twice at room temperature for 1 h. The extracts partition with ethyl acetate and concentrated under rotary evaporator at 55°C. After evaporation, freeze dryer was applied to remove the moisture from extracts. The dry extracts were stored at -20°C until analysis. The measurements in this study were done in triplicate and the biological activities of food samples were determined at a concentration of 25, 50, 75 and 100 from 1 mg/mL.

### Total Phenolic Content

The total phenolic content (TPC) of aqueous extract from the leaves of *Orthosiphon stamineus* was determined using the method by Gutfinger (1981). The methanol extract (1 mL, 1 mg/mL) was mixed thoroughly with 1 mL of 50% Folin-Ciocalteu reagent and 1 mL of 2% Na<sub>2</sub>CO<sub>3</sub>, and centrifuged at 13400X g for 5 min. The absorbance of upper phase was measured using a

spectrophotometer (ELICO (SL150) UV-Vis Spectrophotometer) at 750 nm after 30 min incubation at room temperature. Total phenolic content was expressed as a catechol equivalent.

### Estimation of Flavanoid

A 1ml aliquot of each aqueous extract from the leaves of *Orthosiphon stamineus* was mixed thoroughly with 1ml of 2% aluminium chloride and 0.5 ml of 33% acetic acid followed by the addition of 90% methanol and the content is thoroughly stirred and allowed to stand for 30 minutes (Delcour and de Varebeke, 1985). The absorbance was measured at 414 nm using a UV-Visible Spectrophotometer. Quercetin was used as a standard.

### The Partial Characterization of Aqueous Extract From The Leaves Of *Orthosiphon Stamineus* by Thin Layer Chromatography

The polyphenol rich extract from the leaves of *Orthosiphon stamineus* were loaded on to pre coated TLC (60 F<sub>2</sub> 54) and it was developed using solvent system in the ratio of Petroleum ether, Chloroform and methanol (1:0.5:0.1, V/V/V) was used for the development of the exudates on silica gel plates silica gel 60 F<sub>254</sub> (10x20 cm, 0.2mm layer). Visible and the non-visible spot given and it is fluorescent with UV light at 360nm and 240nm.

## RESULTS

### Phytochemical Screening of Aqueous Extract From The Leaves Of *Orthosiphon Stamineus*

The phytochemical screening of the aqueous extract from the leaves of *Orthosiphon stamineus* studied presently showed the presence of alkaloids, flavonoids, phenol, Terpenoids, glycosides and saponin, and absence of glycosides and tannin (Table -1 and Fig-2).

**Table-1. Phytochemical screenings of aqueous extract from the leaves of *Orthosiphon stamineus***

Sl. No.	Phytochemical Constituents	Observation	Aqueous extract from the leaves of <i>Orthosiphon stamineus</i>
1	<b>Alkaloids</b> -Dragendorff's test -Mayers test	Orange /red precipitate Cream pie ppt	+ +
2.	<b>Flavonoids</b> -Alkalai Reagent -Lead acetate test	Intense yellow colour Precipitate formed	+ +
3.	<b>Glycosides</b> -Keller-Killiani test	Pink colour (Ammonia layers)	+
4.	<b>Tannin</b> -FeCl <sub>3</sub> test	Blue-black colour	+
5.	<b>Saponins</b> -Frothing test	Foam	-
6.	<b>Terpenoids</b> -Salkowski test	Reddish brown colour ring formed in interface	-
7.	<b>Polyphenols</b> -Ferrozine test	Raddish blue	+
8.	<b>Anthocyanin</b> -Ammonia test	Pink color in ammonia layer	+

+ Positive result; - Negative result



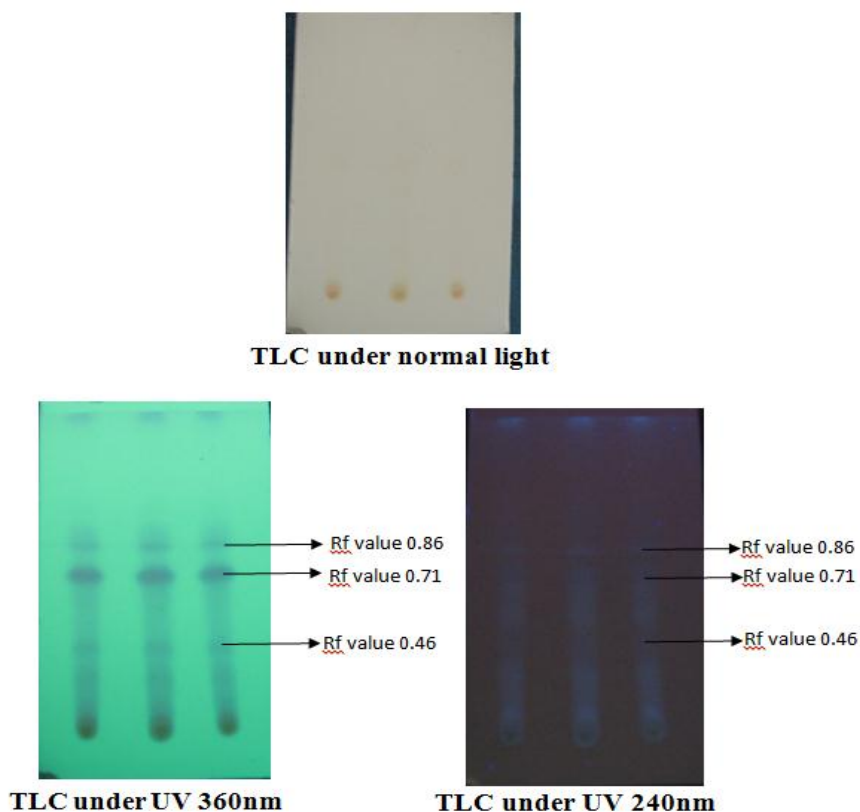
Fig-1. Phytochemical screenings of aqueous extract from the leaves of *Orthosiphon stamineus*.

### Characterization of The Polyphenol Rich Fraction From The Leaves Of *Orthosiphon Stamineus* by TLC

The polyphenol rich fraction from the leaves of *Orthosiphon stamineus* loaded on Pre-coated TLC plates (60 F<sub>2</sub> 54 Merck) and developed with a solvent system of petroleum ether, chloroform and methanol in the ratio of 1:0.5:0.1 were efficient to extract the anti diabetic, antioxidant and anti-inflammatory compound it is used for further studies. The developed plate was viewed under UV 240nm and 360nm (Table-3 and Fig-4).

Table 2: Partial characterization of polyphenol rich fraction from the leaves of *Orthosiphon stamineus* by TLC.

S.No	The polyphenol rich fraction from the leaves of <i>Orthosiphon stamineus</i>		
	UV 240 nm Rf value	UV 360 nm Rf value	Visible Rf value
1.	--	0.86	0.86
2.	--	0.71	0.71
2.	--	0.46	0.46



### Inflammatory Activity on Lipoxigenase Inhibition Activity Of Polyphenol Rich Fraction From The Leaves Of *Orthosiphon Stamineus*

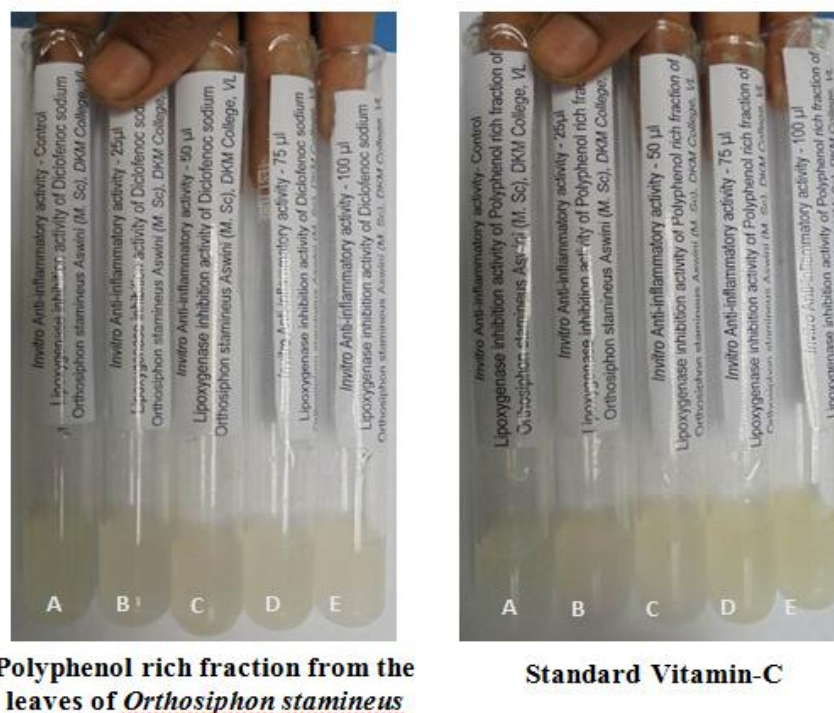
The inhibition of LOX using linoleic acid as substrate was determined for the anti-inflammatory activity of polyphenol rich fraction from the leaves of *Orthosiphon stamineus*. The polyphenol rich fraction from the leaves of *O. stamineus* at 100 $\mu$ l/ml concentration exhibited more inhibition than the other concentration. The inhibition percentage was above 53.40% at 100 $\mu$ l/ml (Table-8 and Fig-12). The standard diclofenac sodium was showed 50.52% inhibition at 100  $\mu$ g/mL. Polyphenol rich fraction from the leaves of *O. stamineus* was showed higher inhibition activity than positive control. Lipoxigenase catalyzes the addition of molecular oxygen to fatty acids containing a *cis*, *cis*-1, 4-pentadiene system. This reaction originates unsaturated fatty acid hydroperoxides. These products are further converted into others that play a key role in inflammatory processes. Hence, polyphenol

rich fraction from the leaves of *O. stamineus* compounds which are able to inhibit that enzyme can be considered as antioxidants and possessing anti-inflammatory properties.

**Table-3. Inhibition activity of Lipoxigenase of polyphenol rich fraction from the leaves of *Orthosiphon stamineus***

Different concentration ethyl acetate extract	Inhibition percentage of LOX	Diclofenac sodium (+ve control)
25 $\mu$ l/ml	14.39 $\pm$ 2.56	10.99 $\pm$ 0.98
50 $\mu$ l/ml	27.40 $\pm$ 1.49	23.82 $\pm$ 1.24
75 $\mu$ l/ml	42.65 $\pm$ 2.41	39.26 $\pm$ 1.47
100 $\mu$ l/ml	53.40 $\pm$ 1.69	50.52 $\pm$ 0.84
EC <sub>50</sub> value	106.34	112.29

Results are expressed as percentage inhibited Lipoxigenase with respect to control. Each value represents the mean $\pm$ SD of five experiments



**A.Control; B- 25µg/ml; C- 50 µg/ml;D- 75µg/ml;E- 100µg/ml**

**Fig 3: Inhibition activity of Lipoygenase of polyphenol rich fraction from the leaves of *Orthosiphon stamineus*.**

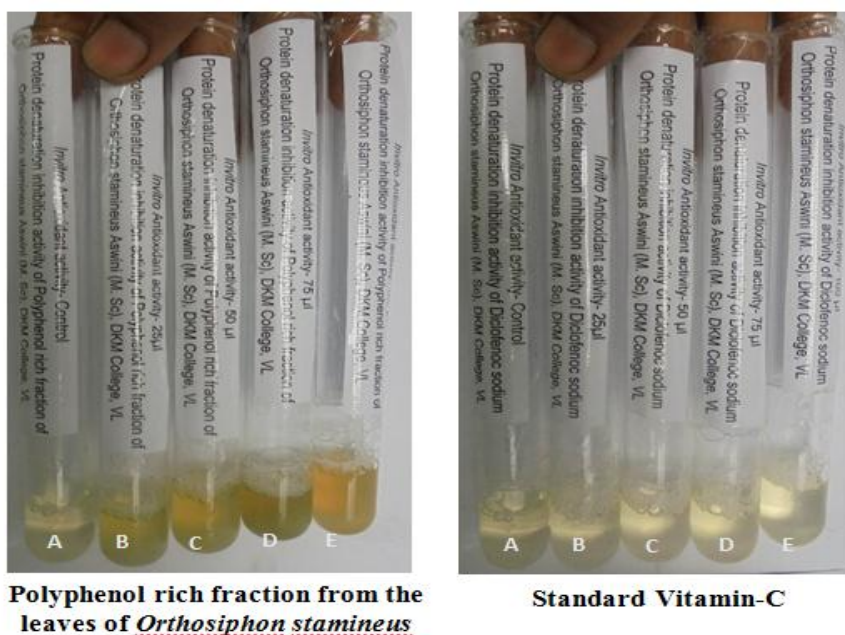
#### **Denaturation Inhibition Of Polyphenol Rich Fraction From The Leaves Of *Orthosiphon Stamineus***

Examination of polyphenol rich fraction from the leaves of *O. stamineus* of momentous activity on inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that polyphenol rich fraction from the leaves of *O. stamineus* is proficient of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease. The maximum percentage inhibition of protein denaturation was observed as 81.52% at 100µg/ml which was close to the percentage of inhibition of diclofenac sodium (77.17 %) (Table-9 and Fig-13).

**Table-4. Inhibition activity of protein denaturation of polyphenol rich fraction from the leaves of *Orthosiphon stamineus***

Different concentration ethyl acetate extract	Inhibition percentage of LOX	Diclofenac sodium (+ve control)
25 µl/ml	24.63±2.56	19.70±1.25
50 µl/ml	41.66±1.49	37.68±0.28
75 µl/ml	60.86±1.63	56.52±0.27
100 µl/ml	81.52±1.47	77.17±2.46
EC <sub>50</sub> value	68.34	73.64

Results are expressed as percentage inhibited inhibition of protein denaturation with respect to control. Each value represents the mean+SD of five experiments.



A- Control; B- 25 $\mu$ g/ml; C- 50  $\mu$ g/ml; D- 75 $\mu$ g/ml; E- 100 $\mu$ g/ml

**Fig 4: Inhibition activity of protein denaturation of polyphenol rich fraction from the leaves of *Orthosiphon stamineus*.**

## DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (Rasdi et al., 2010). According to the World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to obtain a thorough knowledge about their properties, safety and efficacy (Hassan et al., 2009). There is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release fluid extra vasation, cell migration, tissue breakdown and repair (Katzung 2004). It is also known that anti-inflammatory effects can be elicited by a variety of chemical agents and that there is little correlation between their pharmacological activity and chemical structure (Vane and Bolting 1995). This associated with the complexity of the inflammatory process makes the use of different experimental models essential when conducting pharmacological trials.

Diabetes mellitus is a metabolic disorder and the key factor for its control is insulin. Lack of insulin in the body of an organism affects carbohydrate, fat and protein metabolism Rajiv Gandhi G et al., (2012). The control over diabetes mellitus without side effects is a challenge to medical community. Synthetic inhibitor causes side

effect such as abdominal pain, diarrhea and soft faces in the colon. The inhibition of alpha-amylase and alpha-glucosidase would delay the degradation of carbohydrate, which causes a decrease in the absorption of glucose; as a result the elevation of postprandial blood glucose level reduces Rhabaso Lhoret et al., (2004). The inhibitors of Alpha-glucosidase retard the digestion of carbohydrates and slow down the absorption. Metformin are known to be the competitive inhibitor of  $\alpha$ -glucosidases and reduces absorption of starch and disaccharides Davis et al (2001). Hence the therapeutic approaches for reducing postprandial blood glucose levels in patient with diabetes mellitus is to prevent absorption of carbohydrate after food intake. Postprandial blood glucose level in diabetic patients gets increased due to the inhibition of two of these two enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidases) Conforti et al., (2005). The  $\alpha$ - amylase inhibitors also act as an anti-nutrients and obstructs the digestion and absorption of carbohydrates. Acarbose as being a complex oligosaccharide delays the digestion of carbohydrates and inhibits the action of pancreatic amylase in breakdown of starch.

Superoxide anion is also very harmful to cellular components. Robak and Glyglewski (1988) reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. As shown in Table-5, the superoxide radical scavenging activities of the polyphenol rich fraction from the leaves of *O. stamineus* and the reference compound are increased markedly with increasing concentrations. The results suggest that the aqueous extract of equal ratio of (roasted and unroasted) three grains are a more potent scavenger of superoxide radical than the standard Vitamin-C.

Iron can stimulate lipid peroxidation by the Fenton reaction ( $\text{H}_2\text{O}_2 + \text{Fe}^{2+} = \text{Fe}^{3+} + \text{OH}^- + \text{OH}^\cdot$ ) and can also accelerate lipid peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can perpetuate the chain reaction (Halliwell, 1991). Metal chelating capacity is significant since it reduces the concentration of the transition metal that catalyzes lipid peroxidation (Duh *et al.*, 1999). According to the results, the polyphenol rich fraction from the leaves of *O. stamineus* are not as good as the standard EDTA; but the decrease in concentration-dependent color formation in the presence of the extract indicates that it has iron chelating activity.

It is well known that nitric oxide has an important role in various inflammatory processes. Sustained levels of production of this radical are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Tylor *et al.*, 1997). The toxicity of NO increases greatly when it reacts with superoxide radical, forming the highly reactive peroxy nitrite anion ( $\text{ONOO}^-$ ) (Huie and Padmaja, 1993). The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by directly competing with oxygen in the reaction with nitric oxide. The present study proved that the polyphenol rich fraction from the leaves of *O. stamineus* studied has more potent nitric oxide scavenging activity than the standard Vitamin-C.

LOXs are sensitive to antioxidants as antioxidants are involved in inhibition of lipid hydro peroxide formation due to scavenging of lipid hydro peroxide or lipid peroxy-radicals. This could lead to less availability of lipid hydro peroxide substrate required for LOX catalysis (Rackova *et al.*, 2007). Another hypothesis proposed indicated that inhibition by antioxidant could be attained via chelation of its non-heme bound iron or by reduction of its ferric form (Lin *et al.*, 2001), suggesting a competitive kind of inhibition as reported for *Mahonia aquifolium* (Rackova *et al.*, 2007). The present work would like to speculate that LOX inhibition could be due to antioxidant properties of the polyphenol rich fraction from the leaves of *O. stamineus* with the mechanism of action to be elucidated.

In the present study polyphenol rich fraction from the leaves of *O. stamineus* showed good anti-LOX activity 78.69% at 100  $\mu\text{g/ml}$  with an  $\text{EC}_{50}$  value 82.23. LOX inhibition was used to evaluate anti-inflammatory activity of a few medicinal plants used in Limousin country. LOX inhibition was used to evaluate anti-inflammatory activity of a few medicinal plants used in Limousin country. *Filipendula ulmaria* (Meadowsweet) recorded LOX inhibition with  $\text{IC}_{50}$  of 60  $\mu\text{g/ml}$  and *Urtica dioica* (Nettle) methanolic extract inhibited LOX with  $\text{IC}_{50}$  of 348  $\mu\text{g/ml}$  (Trouillas *et al.*, 2003). In another

study, eight methanolic extract out of 18 undomesticated plants of South Africa showed significant inhibition of 5-lipoxygenase (5-LOX) activity. *Bidens pilosa* extract exhibited  $\text{IC}_{50}$  of 21.8  $\mu\text{g/ml}$  and *Emex australis* extract recorded  $\text{IC}_{50}$  of 81.4  $\mu\text{g/ml}$  for LOX inhibition (Akula *et al.*, 2008).

Denaturation of proteins is a well-documented cause of inflammation. The inflammatory drugs (salicylic acid, phenylbutazone etc) have shown dose dependent ability to thermally induced protein denaturation. Similar results were observed from many reports from plant extract (Sakat *et al.*, 2010). From the above study it was concluded that the polyphenol rich fraction from the leaves of *O. stamineus* had maximum albumin denaturation protection property as compared to the aqueous extract of the same plant and it was comparable to the standard drug Diclofenac Sodium. The extracts may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The precise mechanism of this membrane stabilization is yet to be elucidated; it is possible that the polyphenol rich fraction from the leaves of *O. stamineus* produced this effect by reducing the surface area/volume ratio of the cells, which could be brought about by an expansion of membrane or the shrinkage of cells and an interaction with membrane proteins. The above findings also confirmed that polyphenol rich fraction from the leaves of *O. stamineus* possessed maximum protection activity of RBC membrane.

With more people avoiding chemical drugs for the management of overweight and obesity, due to the fear of health adverse side effects, tendency is now towards natural-based products; thus the development of new anti obesity molecules from natural products has become a necessity. This seems doable because, in traditional herbal medicine, several plants are used for their weight-reducing effects. The plant bioactive constituents are expected to act as natural inhibitors of digestive lipases (Kazemipour *et al.*, 2012). Antioxidant and in vitro porcine pancreatic lipase, PPL, inhibitory tests were conducted on seventy-six plants, of which thirty-nine species with weight-reducing or related potential were used in Palestinian traditional medicine, to find new crude anti obesity products from natural sources. The present study polyphenol rich fraction from the leaves of *O. stamineus* possessed maximum inhibition activity lipase its promising control obesity.

#### SUMMARY

- ✓ The phytochemical screening of polyphenol rich fraction from the leaves of *O. stamineus* studied presently showed the presence of all metabolites except Glycosides.
- ✓ In the partial characterization of the polyphenol rich fraction from the leaves of *O. stamineus* by TLC, which showed 7 UV fluorescent compounds respectively.

- ✓ The highest total phenolic content yield in polyphenol rich fraction from the leaves of *O. stamineus*.
- ✓ The reducing power activity was assessed on average and high free radical-scavenging values were found in polyphenol rich fraction from the leaves of *O. stamineus*.
- ✓ Maximum inhibition of lipid peroxidation induced by ferrous sulfate in egg yolk homogenates was recorded polyphenol rich fraction from the leaves of *O. stamineus* 84.45% than standard vitamin-C
- ✓ The polyphenol rich fraction from the leaves of *O. stamineus* showed highest scavenging activity for superoxide radicals activity in the percentage of 73.06% when compared to positive control.
- ✓ The polyphenol rich fraction from the leaves of *O. stamineus* reduced the greenish blue color complex immediately and showed the highest chelating activity 77.62% than positive control.
- ✓ The highest nitric oxide inhibition activity polyphenol rich fraction from the leaves of *O. stamineus* was 86.68%.
- ✓ The present study was to evaluate the anti-inflammatory activity and their inhibitions way were investigated by polyphenol rich fraction from the leaves of *O. stamineus*.
- ✓ The strategies of invitro lipoxygenase inhibitory activity were used to evaluate the efficacy of these compounds as anti-inflammatory properties (53.04%).
- ✓ The polyphenol rich fraction from the leaves of *O. stamineus* was studied, effective inhibition of protein denaturation (81.52%).
- ✓ The maximum percentage of lipase inhibition was shown by polyphenol rich fraction from the leaves of *Orthosiphon stamineus* (63.13 %).

## CONCLUSION

Polyphenols are valuable plant constituents for the scavenging of free radicals because of their phenolic hydroxyl groups. This, together with the obtained results, suggests that as the amount of polyphenolic compounds increases, the antioxidant and anti-obesity activity also increases. In conclusion, the present study demonstrates that the polyphenol rich fraction from the leaves of *Orthosiphon stamineus* can protect the body from oxidative stress from ROS, which may be due to the phytochemicals in the form of polyphenols that occur in the plant. The polyphenol rich fraction from the leaves of *Orthosiphon stamineus* should be great source for natural treatments of inflammation that are safe and effective and can be provided as dietary supplements, added to multiple vitamins, and incorporated into food products to create functional foods. In addition, the novel bioactive identified in the polyphenol rich fraction from the leaves of *Orthosiphon stamineus* when fully characterized, could prove to be promising new drug leads for Lipoxygenases as well as triple inflammatory enzyme inhibitors for treatment of a range of inflammatory, Obesity and diabetic that are safe and efficacious.

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