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In Vitro Antioxidant Activity Of Oak Gall Oil And *Triphala* Oil

Sharmila S[1*], Mubarak H[2]

[1] Department of Dental Surgery, Government Medical College, Thiruvallur, Tamilnadu, India 610004, Email: drsharmi.29@gmail.com, Contact number: 9442516214*

[2] Senior Medical officer (Siddha), CGHS, Chennai, Tamil Nadu, India.

***Corresponding Author:** Sharmila S*

[*] Department of Dental Surgery, Government Medical College, Thiruvallur, Tamilnadu, India 610004, Email: drsharmi.29@gmail.com, Contact number: 9442516214

Abstract

Traditional Indian literature have evidenced the usage of Oak gall and *Triphala* for common ailments to oral malignancies and other lifestyle disorders. The objective of the study is to examine the antioxidant activity of oak gall oil (OGO) & *Triphala* oil (TPO). Oak gall oil (OGO)) and *Triphala* oil (TPO) were produced with purified oil from coconut in a proportion of 1:5, with one portion of gall of Oak / *Triphala* added to five parts of the oil of coconut. Oak gall & *Triphala* oil were evaluated for antioxidant capabilities using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, the Hydroxyl radical scavenging activity and the Nitric oxide radical scavenge action. In the DPPH experiment, ascorbic acid served as the comparison standard, while gallic acid was utilized to measure nitric oxide activity in reducing radicals and the hydroxyl radical scavenge activity. The percentage inhibition of Oak gall oil (OGO) & *Triphala* oil (TPO) was 75.2%, and 67.4% for DPPH assay,48.4% and 63.3% for Hydroxyl radical scavenging assay, 51.2% and 52.7% for Nitric oxide radical scavenging assay respectively with ascorbic acid (89.1%) and Gallic acid standard (62.9% and 70.4%) used as comparative respectively. The study results show that Oak gall oil (OGO)and *Triphala* oil (TPO) possess antioxidant properties comparable to reference standards. Products derived from native plants, such as oak gall & *Triphala*, if proven to be effective in-vivo, can serve as a simple, safe, cost-effective and culturally acceptable adjuvant in anticancer management.

Keywords:-Triphala, Oak Gall, traditional medicine, DPPH assay, Hydroxyl radical scavenging assay, Nitric oxide radical scavenging assay

1. INTRODUCTION

Free radicals are formed by natural enzymatic and non-enzymatic reactions in our human body. Oxidative damage caused by free radicals is a contributing factor towards a wide variety of illnesses, including

malignancies (1). Antioxidant defences the body as a radical scavenger and counter the destructive impact of free radicals (2). Those sections of plants used in medicine that are abundant in phenolic chemicals like phenolic acids, flavonoids, Gallic acid, ellagic acid and tannins have significant antioxidant properties and are used for various systemic diseases (3). Antioxidants of natural sources protect the health system from oxidative stress and other chronic diseases, thus shows a vibrant role in the fitness care system (4) Review of literature has shown that Oak gall and *Triphala* have the highest amount of polyphenols and their aqueous and alcoholic extracts have proven antioxidant properties(5-10)

Outgrowths known as galls of oak are generated on young branches of the dyer's oak, also known as *Quercus infectoria* Oliv. (*Majphul , Masikai , Aleppo* Oaks) as a consequence of the gall-wasp's eggs being deposited on the tree's surface. *Adleriagallae-tinctoriae* (11).*Tannin*, which accounts for 50–70% of the total, and a trace quantity of free acid called gallic acid are the primary components of the galls produced by *Q. infectoria* (12). It is known to possess high radical scavenging activity (86.8%)(13).

In traditional Indian medicine, a mixture of the three herbs *Terminalia chebula* Retz., *Terminalia bellerica* (Gaertn.)Roxb. and *Emblica officinalis* Gaertn. are together known as *Triphala* and has a long history being utilized as a treatment for a wide variety of illnesses(8)(14). One of the most important aspects of *Triphala* is that it contains both Gallic acid & ascorbic acid (15)Chemotherapeutic measures for various cancers possess potent adverse effects on long-term supplementation (16). Indigenous plants like Oak gall and *Triphala*, if proven to be effective, simple, safe, cost-effective, ethnically satisfactory antioxidants for the supervision of various diseases including cancer. The purpose of this research is to determine the antioxidant effects of OGO and TPO in comparison to the reference compounds gallic acid or ascorbic acid.

2.MATERIALS AND METHODS

2.1. Preparation

Oak gall & *Triphala* dried fruit were purchased from a neighbourhood vendor. In Chennai, at the Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, legitimacy was confirmed. (F.No.1-24/sample testing/CSMRADDI/2016-17/360) Certificate of Authenticity. Both groups' herbal formulations were developed by a Siddha physician, and the OGO & TPO were prepared by an authorized pharmacist following SOP. In a ratio of one-part powdered Oak gall to five parts pure coconut oil, dried galls of Oak are cleaned, crushed, & sieved before being combined with the oil to form Oak gall oil (OGO). In a similar fashion, the *Triphala* fruit was sun dried, cleaned, powdered, sieved, and finally combined with pure oil from coconut at a ratio of 1-part *Triphala* powdered to 5 parts pure oil from coconut to form *Triphala* oil(TPO).

2.2. Antioxidant Assays

This research used the DPPH (1,1-diphenyl-2-picryl hydrazyl) test, the Hydroxyl free radical scavenging test, or the Nitric oxide scavenging assay for its antioxidants tests. The principle involved in these assays is when the concentration of the drug increases, the absorbance decreases and percentage inhibition increases.

2.1.1. *DPPH (1, 1-Diphenyl -2-Picrylhydrazyl) Assay*

The DPPH assay is a basic evaluator of radical scavenging activity (17). After the introduction of an antioxidant, a reduction in the amount of light absorbed by the DPPH solution could be seen at 517 nm. Ascorbic acid concentration that was employed as an example was 10 mg/ml DMSO. The DPPH test is based on the idea that 1,1-diphenyl-2-picrylhydrazyl is a persistent free radical that has a pink hue and becomes yellow as it is scavenged. The DPPH test is performed to show that this characteristic has a role in scavenging free radicals. When DPPH is combined with an antioxidant, the resulting reaction could be represented as DPPH + [H-A] DPPH-H + (A).

Interaction of antioxidant molecules with DPPH, results in the conversion of DPPH to DPPH-H and a subsequent reduction in absorbance. The degree of discolouration provides information about the hydrogen-donating capacity of the antioxidant substances or extract being tested. The 0.1mM solution of DPPH was made by dissolving 4 milligram of DPPH in 100 ml of ethanol to make the solution. After bringing the total volume of the samples to a total of 40 l with DMSO, 1.48 l of a DPPH (0.1 mM) solution were added. The sample volumes ranged from 1.25 l to 20 l. The mixture was kept in the incubator at room temperature and kept in the dark for twenty minutes. After waiting for twenty minutes, the absorption of the combination was determined to be 517nm. It was decided to utilize 3 milliliters of DPPH as the control (18).

2.1.2. *Hydroxyl (OH) Radical Scavenging Activity*

This test determines a value for the decomposition product of 2 deoxyribose by condensing it with TBA (19). The system consisting of Fe3+, ascorbate, EDTA, and H2O2 was the one that produced the hydroxyl radical. (The response of Fenton) ... 2 deoxy-2 ribose (2.8mM), KH2PO4—KOH buffer (20 mM pH 7.4), FeCl3 (100m), EDTA (100m), H2O2 (1.0mM), ascorbic acid (100m), and different quantities of the test sample are included in a reaction mixture that has a final volume of 1 ml. Following a one-hour incubation at 37 degrees Celsius, one milliliter of a solution containing 2.8% TCA and one milliliter of a solution containing 1% TBA in aqueous medium were each added to the mixture, and it was then heated to ninety degrees Fahrenheit for fifteen minutes so that the color could develop. After cooling down an appropriate blank solution was used to take the reading for the absorbance at 532 nm.

2.1.3. *Nitric Oxide (NO) Scavenging Activity*

In addition to its role in neurotransmission & vascular homeostasis, NO additionally possesses antibacterial & anticancer properties. Despite NO's potential advantages, evidence suggests it also contributes to damage from oxidation. This is due to the fact that NO can react with O2– to generate the highly reactive oxidant peroxynitrite anion, that in turn can break down into hydroxyl (OH) & nitric oxide (NO). The nitrite ions could be detected via the Griess reagent, and the approach relies on the fact that nitric oxide is formed when sodium nitroprusside is dissolved in water at a physiological pH. Reducing the production of nitrite ions is the outcome of nitric oxide scavengers competing with oxygen. Spectrophotometric analysis was used to evaluate the activity of scavenging for nitric oxide. Different quantities of the extract (125-2000g mL-1) made in methanol were combined with sodium nitroprusside (5mmolL-1) in phosphate-buffered saline (pH 7.4) & kept at 25°C for a period of thirty minutes. The control sample was created by omitting the test chemical and replacing it with a quantity of methanol that was quantitatively comparable. After incubation for 30 minutes, 1.5 milliliters of the remaining was mixed with 1.5 milliliters of the Griess reagent, which consisted of 1% sulphanilamide, 2% phosphoric acid, & 0.1% N-1-naphthyl ethylene diamine dihydrochloride. At 546 nm, the absorption of the chromophore that was produced through the the diazotization of nitrates with sulphanilamide and afterward coupled with N-1 naphthyl ethylenediamine dihydrochloride was determined, and the resulting percentage of scavenging activity that was detected was evaluated compared to the reference (20-22)
The formula that follows was used to determine the proportion of inhibition for each assay:
The absorption of the control subtracted from the absorption of the test sample divided by the absorption of the control multiplied by one hundred

2.1.4. *Statistical analysis*

The outcomes were stated using mean values together with standard deviation (SD) values. The amount of medication response curve was used to get the 50% inhibitory concentration (IC_{50}), which was then plotted against concentrations to give the percentage of inhibition. A one-way analysis of variance (ANOVA) was followed by a post hoc Tukey HSD test when comparing the antioxidant activity of the various categories utilizing SPSS statistical software version 19.

3. RESULT AND DISCUSSION

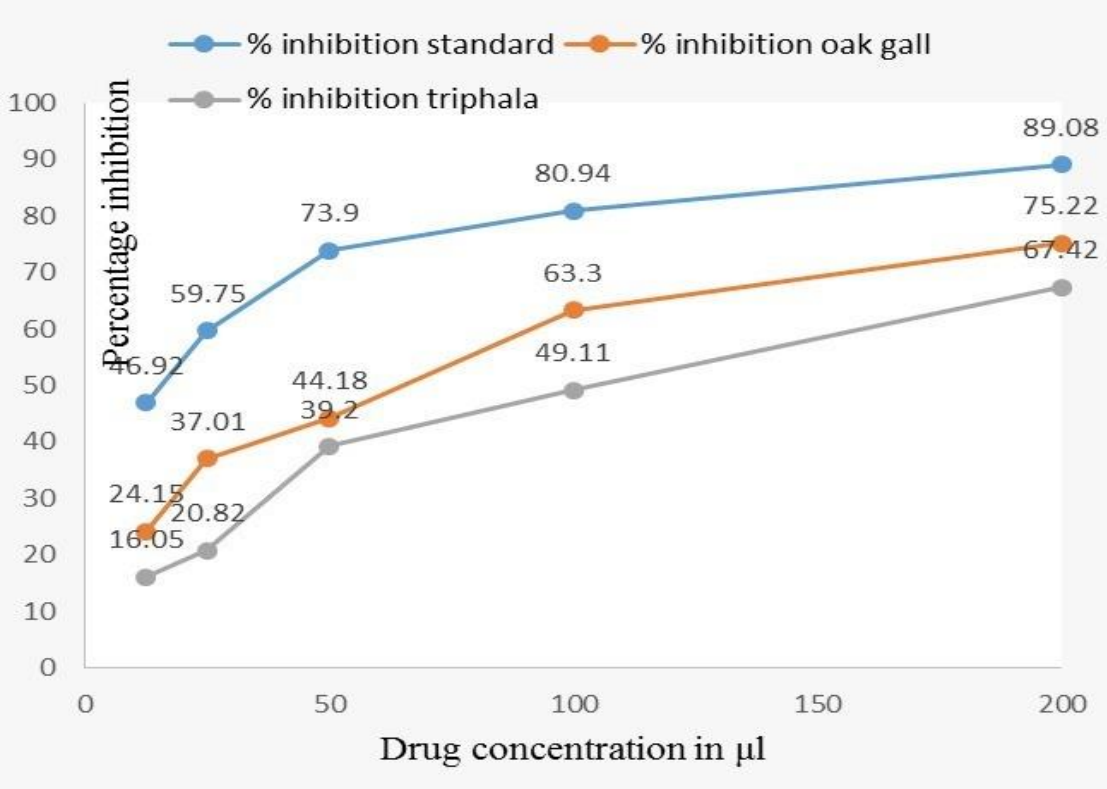
The significance that free radicals & reactive oxygen species (ROS) play in a variety of pathophysiological disorders, such as cancer, has been shown by a large number of studies in recent years. Antioxidants, both non-enzymatic and enzymatic, counteract the negative effects of ROS. Oak gall and *Triphala* contains significant polyphenols that can scavenge free radicals and thus can serve as a potent antioxidants.

Antioxidant properties of OGO

DPPH (1, 1-Diphenyl -2-Picrylhydrazyl) Assay:

Throughout the course of this test, levels of ascorbic acid that varied from 20 to 100 g/ml were employed as the normative control. The level of discolouration that occurs during the DPPH test is indicative of the antioxidant's free radical scavenging activity . OGO is able to neutralizing the DPPH free radicals by 75.23% at levels of 200 ul/ml, which was equivalent to ascorbic acid, which demonstrated an amount of inhibition of 89.05% at 200 ug/ml. OGO was effective in inhibiting the DPPH free radicals in a manner that was equivalent to ascorbic acid. (Fig.1).

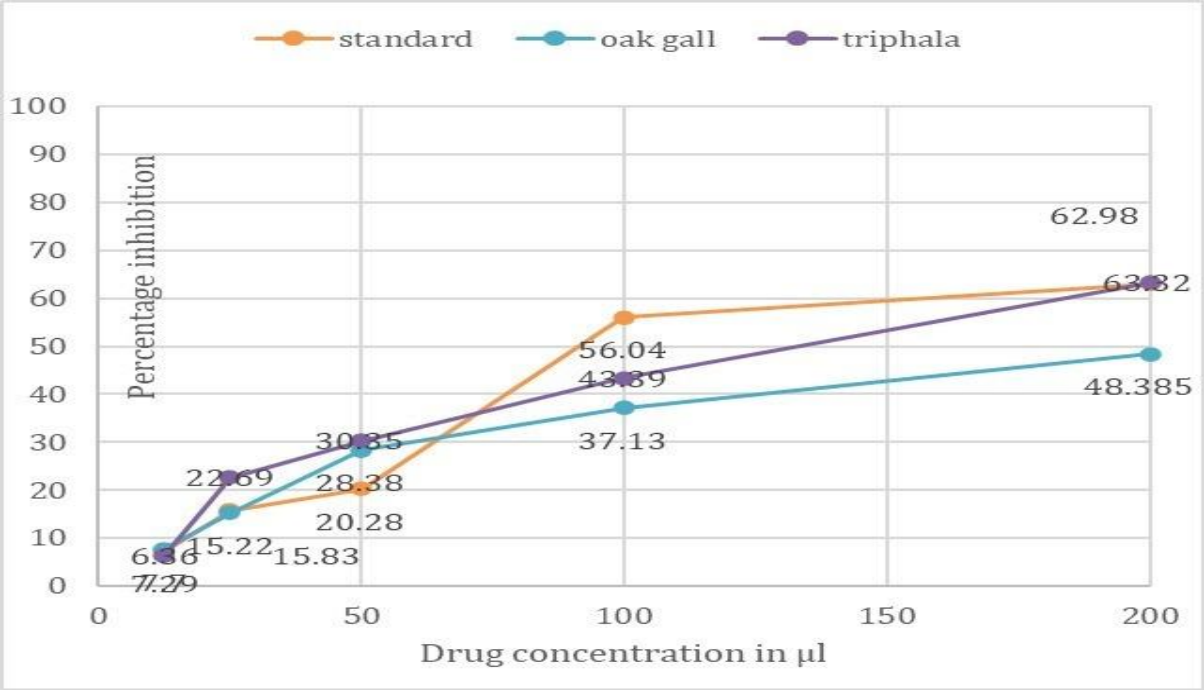
Fig.1 Comparison of percentage inhibition of OGO and TPO with Ascorbic acid standard (DPPH assay)



Hydroxyl radical scavenging assay

As free radicals have a harmful role in the induction of carcinogenesis, radical scavenging activity is a very important assay for anticancer preparations. The results reveal that the hydroxy radicals are eliminated with an increasing dose response increase in the capacity to quench hydroxy radicals for all the observed concentrations. OGO had an effective radical scavenging activity in a concentration-dependent manner. At a concentration of 200 ug/ml, the free radical scavenging activity of OGO was 48.39% in comparison with standard gallic acid was 62.98% (Fig.2).

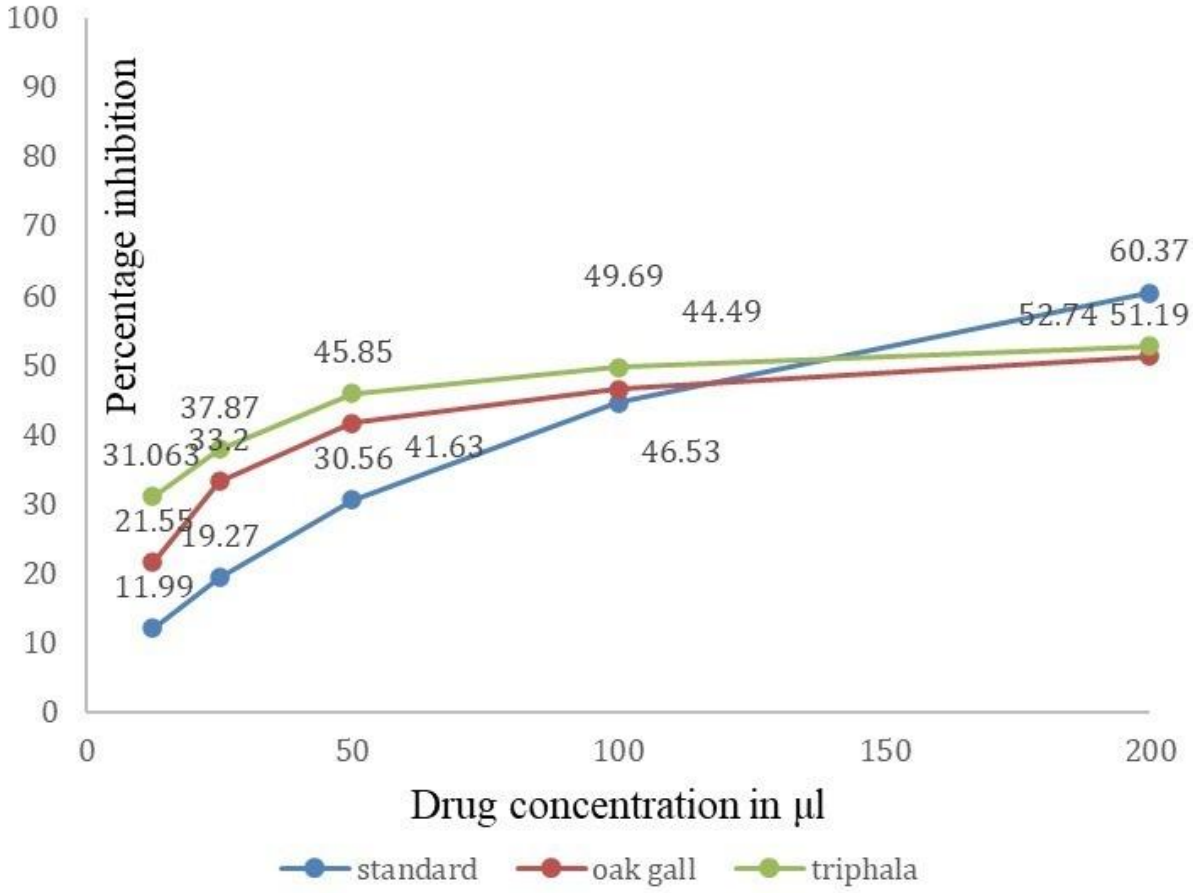
Fig.2 Comparison of percentage inhibition of OGO and TPO with Gallic acid standard (Hydroxyl radical scavenging assay)



Nitric oxide radical scavenging assay

As the quantity of OGO was raised, so too was the amount of inhibition it provided. The percent of inhibitor at the concentration of 200 l was found to be 51.19%, while the percentage of inhibition of the gallic acid reference was 60.37%. In the DPPH test, the IC_{50} value for OGO was recognized to be 8.23 l, while the value for the radical hydroxyl scavenging assay was 214.3 l, & the value for the nitric oxide scavenging test was 163.3 l. (Fig.3).

Fig.3 Comparison of percentage of OGO and TPO with Gallic acid standard (Nitric oxide radical scavenging assay)



Antioxidant properties of TPO

DPPH (1, 1-Diphenyl -2-Picrylhydrazyl) Assay

Triphala oil was capable of neutralizing the DPPH free radicals by 67.42 % at a concentration of 200 ul/ml whereas ascorbic acid standard showed the percentage inhibition of 89.05% at a concentration of 200 ug/ml (Fig.1).

OH radical scavenging activity

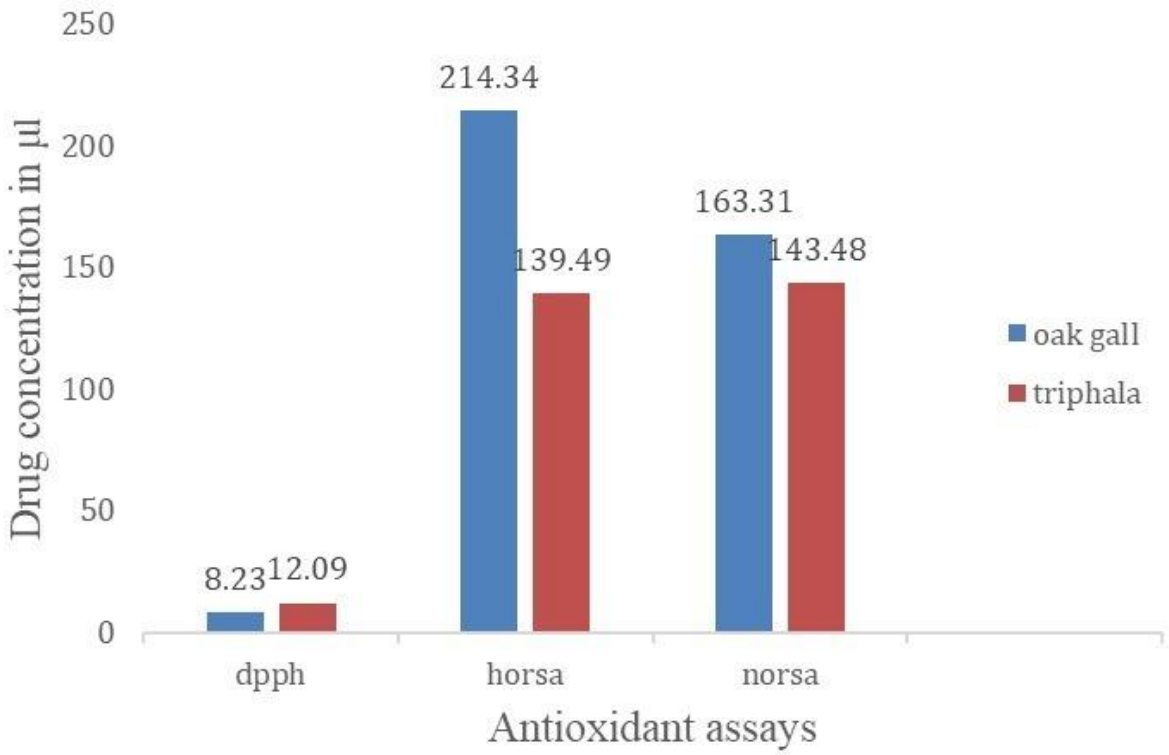
OH scavenging activity of TPO increased as the concentration of the oil increased and at a concentration of 200 ul/ml, the percentage inhibition was 63.32% which was comparable to that of gallic acid standard at the same concentration and was 62.98% (Fig.2).

NO radical scavenging assay

Percentage inhibition of Gallic acid standard at a concentration of 125, 250, 500, 1000, and 2000µg was 11.99, 19.27, 30.56, 44.49, and 60.37% respectively. Percentage inhibition of TPO at a concentration of 12.5, 25, 50, 100 and 200µl was 31.06, 37.87, 45.85, 49.69, and 52.74% respectively (Fig.3).

The IC_{50} for TPO was found to be 12.09µl in DPPH assay, 139.48µl in hydroxyl Radical Scavenging assay and 143.48µl for nitric oxide scavenging assay (Fig.4).

Fig.4 Comparison of IC_{50} values for DPPH, Horsa-Hydroxyl radical scavenging, Norsa- Nitric oxide radical scavenging Assays



DISCUSSION

The difference between OGO, TPO and the standard controls was statistically insignificant (p›0.05) for the DPPH (p=0.06), OH radical scavenging (p=0.89), and NO radical scavenging assay (p=0.71) as determined by one-way ANOVA.

The utilization of conventionally used therapeutic plant components and substances produced from plants has opened up a new area of study in the study of cancer. However, the scientific evidence for their usefulness in managing and preventing cancer is insufficient. Antioxidant-rich medicinal herbs can be quite helpful in the treatment of numerous malignant situations. Traditional remedies can be used to treat pre-malignant lesions and disorders at an earlier stage, which would not only lower the cost of treating the disease but also significantly extend life expectancy and improve the quality of life for the patient. Studies examining the antioxidant abilities of conventional medications in oil formulations are few. The present study shows the percentage inhibition of OGO at a concentration of 200µl as 75.22% (89.1%), 48.39% (62%) and 51.2% (70.4%) for DPPH, OH radical and NO radical scavenging assay respectively. The percentage inhibition of TPO at a concentration of 200µl was 67.4% (89.1%), 48.39% (62%) and 52.7% (70.4%) for DPPH, OH radical scavenging, NO radical scavenging assay respectively.

Effects of extraction with solvent on *Quercus infectoria* Oliv.has shown antibacterial and antioxidant qualities (23) and has proven that aqueous extract of Oak gall showed highest radical scavenging activity (94.55%). The free radical scavenging action of *Quercus infectoria* Oliv. has shown IC_{50} value for DPPH assay as 52.90µl/ml (24). According to research conducted on *Quercus infectoria* Oliv., this species has demonstrated to have high free radical scavenging activity of 86.8% for the DPPH test(13).Similarly it was proven that *Q. Infectoria* galls have antioxidant properties, which inhibits oxidative stress's effect on functional Changes in murine macrophages (6)

According to the findings of another study, both *Triphala* as a whole and each of its constituent components have the ability to neutralize free radicals produced by DPPH, Superoxide, & Nitric oxide (25) . According to

a previous research, the action of *Triphala* against a number of different free radicals can be recognized due to the herb's high phenolic and polyphenolic content (26). Using a variety of in vitro designs, researchers were able to find that the antioxidant as well as free radical scavenging activities of *Triphala* had an IC_{50} of 5.94 g/ml for DPPH & 32.59 g/ml for Nitric Oxide radicals scavenge assays correspondingly (27).
Thus, OGO and TPO have comparable antioxidant properties which are consistent with the results of the previous studies. Coconut oil also possesses inherent antioxidant properties and has been used in traditional medicine since age memorial. Thus, the antioxidant properties of the oil formulation may also have a trivial contribution from the coconut oil (28).

CONCLUSION

OGO and TPO possess significant antioxidant properties which are comparable with that of Standards (Ascorbic acid and Gallic acid). Antioxidant activities of Oak gall & *Triphala* will serve as a simple, safe adjuvant in the management of various free radical releasing precancerous conditions and prevent its malignant transformation thus reducing the economic burden of treating cancer. Oil form of preparation can be directly applied over several oral malignant disorders. Further large scale clinical testing is advised in to find the effectiveness of OGO and TPO for its antioxidants .

AUTHOR'S CONTRIBUTIONS

Both the authors have made substantial contributions to the conception and design, invitro study analysis and data interpretation. They contributed in drafting the article, revising it for journal submission and gave final approval before submission to the current journal.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects

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