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AN ENCAPSULATIVE ANALYSIS ON PREPARATION AND CHARACTERZATION OF FEMALE INFERTILE SYRUP USING HERBALS ON *Cyprinus carpio* (KOI CARP)

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ABSTRACT

Several potential approaches for infertility have been investigated over a long period, including chemical, hormonal and immunological approaches. Infertility is the diminished ability or the inability to conceive and have offspring. It can also be defined as the failure to conceive after a year of regular intercourse without contraception. Numerous plants synthesize substances that are useful in the maintenance of health in humans and animals. Medicinal plants products have a long history of indigenous use in India as well as other countries. Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. The medicinal plants contain several phytochemicals such as Vitamins (A, C, E, and K), Carotenoids, Terpenoids, Flavonoids, Polyphenols, Alkaloids, Tannins, Saponins, Enzymes, and Minerals etc. Herbal therapies have been used since ancient times to treat infertility. Fertility Herbal Combinations for Women, such as Fertilaid for Women, work by the mixture of many potent important fertility herbs. In this present study the fresh leaves, roots and flowers were collected and separated out from the Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia and Lonidium suffruticosum. The collected samples were extracted using Aqueous which is used to prepare the Anti-infertility syrup for women's by standard method. The syrup was named as ***Betalupe*** *and to investigate, the attempt has been made to analyze the secondary metabolites, infertility compounds, nutrients analysis, Antioxidant capacity and heavy metals. And also to evaluate the infertility activity in fish namely Cyprinus carpio (Koi carp) was used to observe the egg production after administration of* ***Betalupe.*** *Hence, decision has been made to use these to prepare an Anti infertility syrup to address the problems pertaining to infertility in female system and the nutrients and antioxidants which induces and balances the immune system and thereby increase the chances of fertility.*

KEYWORDS

Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia, Lonidium suffruticosum, Cyprinus carpio, Betalupe and Infertility.

INTRODUCTION

Several potential approaches for infertility have been investigated over a long period, including chemical, hormonal and immunological approaches. However, no suitable method has emerged that is effective and free from side effects. The World Health Organization (WHO) has constituted a population control programme, which includes studies having traditional medical Practices. Numerous plants synthesize substances that are useful in the maintenance of health in humans and animals **(Sawarkar *et al.*, 2011).** With a view to increasing the wide range of medicinal usages, the present day entails a new drug with more potent and desired activity with less or no side effects against particular disease **(Roy *et al.*, 2009).** Medicinal plants products have a long history of indigenous use in India as well as other countries.

Phytotherapy has a very long tradition, although proper scientific explanation is relatively new. In our country as well as in the world, there are several medicinal plants associated with antifertility properties. Fertility regulation with plants or plant preparations has been reported in the ancient literature of indigenous systems of medicine. A large number of plant species with antifertility effects have been screened in China and India beginning about 50 years ago and were subsequently fortified by national and international agencies **(Chandhra *et al.*, 1998).**

Infertility

Infertility is the diminished ability or the inability to conceive and have offspring. It can also be defined as the failure to conceive after a year of regular intercourse without contraception.

Infertility can be of two types: **primary and secondary.** Primary infertility is a term used to describe a couple who have never been able to conceive, whereas secondary infertility is a term used to describe a couple who have been pregnant at least once, but have not been able to become pregnant again. Infertility may be caused by different factors. A woman is in the fertile phase of the menstrual cycle before and during the ovulation period and in the infertile phase during the remaining period of the menstrual cycle.

MATERIALS AND METHODS

Collection of Plant Material

The dried plants of *Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia* and *Lonidium suffruticosum* were collected in Tamil university, Alakudi village and Kumbakonam at Thanjavur District in TamilNadu.

Preparation of Plant Extract

Fresh leaves, roots and flowers were selected and separated out from the *Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia* and *Lonidium suffruticosum* plants separately. They were washed under running tap water, each plant was allowed for shadow dried at room temperature. The air dried samples were powdered using grinder, homogenized to fine powder and stored in air tight bottles at room temperature.

Aqueous Extraction

Take 10 gram of each *Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia,* and *Lonidium suffruticosum* powder was placed in 500ml of mineral water and allowed for boiling at 100°c for 30 minutes. The extract was filter through a sterile funnel containing sterile Whatmann filter paper No.1 then filtered contain preservative and stored in brown bottle at 4°c.

PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS

To screen the phytochemicals in the plants of *Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia,*

and *Lonidium suffruticosum*. Chemical test were carried out on the aqueous extract using standard procedures to identify the phyto constituents as described by Sofowara (1993) Trease and Evans (1998) and Harbone (1973).

PREPARATION OF ANTI INFERTILITY SYRUP

S. No	Ingredients	Percentage (ml)
1	*Withania somnifera*	12
2	*Couroupita guianensis*	9
3	*Ficus religiosa*	12
4	*Aegle marmelos*	8
5	*Tinospora cordifolia,*	8
6	*Lonidium suffruticosum*	6
7	*Aloe vera*	15
8	Sugar base	16
9	Water	15
10	Preservative	0.001

Procedure

- The aqueous plant extracts (*Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia* and *Lonidium suffruticosum*) were prepared.
- Plant extracts *Withania somnifera* (12), *Couroupita guianensis* (9), *Ficus religiosa* (12), *Aegle marmelos* (8), *Tinospora cordifolia* (8), *Lonidium suffruticosum*(6) and *aloe vera* (15), were added in to the 100 ml beaker.
- Add 15% of *Aloe vera* in to the above extracts then adds 16% of sugar base and .then add Reverse Osmosis water (15%), citric acid (0.001%).
- The above ingredients are boiled at 15 minutes for 100°c.
- Finally mix the syrup using blender at 30minutes.
- The prepared syrup was named as **Betalupe**. Then the syrup was stored at brown bottle for 72 hours at 60°c and used to analysis by following methods.

QUANTITATIVE ANALYSIS OF INFERTILITY COMPOUND BY TLC

Thin layer chromatography is one of the valuable and versatile methods for analysis of wide rang biomolecules. TLC is nothing but a modification of paper chromatography. Where the sheet of paper is replaced by thin layer of absorbent material. Therefore the separation in TLC is also due to the differential partition of solutes between the stationary and mobile phases.

Procedure

The syrup solution was used to quantification of lupeol. The sample was spotted in a TLC plate using capillary tubes. The lupeol spots were separated using the solvent mixture toluene, chloroform, ethyl acetate and glacial acetic acid in the ratio of 50:8:5:0.20. The color and R_f value of the separated lupeol was recorded both under Ultra Violet (UV 366 nm) and visible light after spraying with Dragendroff's reagent (Wagner and Bladt, 1996). The ß-sitosterol spots were separated using the solvent mixture toluene, chloroform and methanol solvent mixture in the

ratio of 16:16:4. The color and R_f value of the separated ß-sitosterol were recorded both under Ultra Violet (UV 527 nm) light (Wagner and Bladt, 1996).

TOTAL ESTIMATION OF SECONDARY METABOLITES BY SPECTROPHOMETRIC METHOD

Anti-infertility syrup could be used to estimate the secondary metabolites such as Alkaloid, Flavonoids, and Steroid. Total alkaloid content was estimated by 1, 10-phenanthroline method described by Singh *et al*. (2004). The Flavonoids content was determined by using a method described by Ozsoy *et al*. (2008). The total Steroid was determined by using a method described by (Singh *et al.,* 2003).

NUTRIENTS ANALYSIS

Betalupe syrup used to analyse the nutrients such as Carbohydrate, Protein, Lipid, Calcium, Magnesium, Iron, and Phosphorus. Estimation of Carbohydrate by Anthrone Method (1962), Estimation of Protein by Lowry's Method (1951), Estimation of Lipid by Zak's Method, Calcium and Magnesium estimation by EDTA Method.

HEAVY METAL ANALYSIS

To screen the heavy metals in **Betalupe** syrup such as Mercury, Copper, Nickel, Manganese by AOAC method.

VITAMIN ANALYSIS

The present study shows the vitamins such as Vitamin E, C were analyzed in the prepared infertile syrup **Betalupe**, with commercial products. Determination of Vitamin C by Kemppainen, 2002, Determination of Vitamin E by Nair and Magar., 1954.

***INVITRO* ANTIOXIDANT ACTIVITY BY REDUCING POWER SCAVENGING ACTIVITY**

To determine the reducing power assay of Plant Sample by **Yildrim *et al.,* Method, (2001).**

1 ml of plant extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50C for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferriccloride (0.5ml, 0.1%) and absorbance measured at 700nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compaired with ascorbic acid standard.

TOTAL MICROBIAL COUNT ON NA MEDIUM

INFERTILITY ACTIVITY

A healthy matured *Cyprinus carpio* (Koi Carp) male and female fish was collected from government pond at Karanthai, Thanjavur Dt. The collected fishes were used to investigate the infertility studies using herbal formulated syrup. The experiments were carried out under laboratory condition. The Prepared syrup was administrated intramuscularly by injecting the predetermined dose in the dorsal musculature between the lateral line and the dorsal fin. All the set of treated fish were maintained separately in identical environment conditions in the laboratory. After administrating the prescribed dose to the selected experimental fish *Cyprinus carpio*, they were continuously monitored to obtain the result.

RESULTS

In this present study the herbal plants namely, *Withania somnifera, Couroupita guianensis, ficus religiosa, Aegle marmelos, Tinospora cordifolia, Lonidium suffruticosum* were used to prepare the infertility syrup for women and to investigate the Anti infertility activity on *Cyprinus carpio* (Koi Carp). The study also intended to analyze the pros and cons in the preparation methods in

syrup making using herbs and its desired results at higher and lower concentration and the nutrients, Anti oxidant activity, Microbial count, Heavy metals and comparative analysis with commercially available products in the market were also done in order to justify whether the prepared product has the potential to have the needed qualities. The results are described as follows.

Phytochemical screening

The present study carried out on the *Withania somnifera, Couroupita guianensis, Ficus religiosa, Aegle marmelos, Tinospora cordifolia* and *Lonidium suffruticosum* leaf, flower and root powder extracts with aqueous, the phytochemical compound screened by qualitative method. In the phytochemical analysis, 4 bio active compounds were present in the *Withania somnifera* , *Aegle marmelos* , *Tinospora cordifolia* and *Lonidium suffruticosum* aqueous extract, but in case *Couroupita guianensis* and *Ficus religiosa* 3 bio active compounds were detected. The phytochemcial active compounds of selected medicinal plants were qualitatively analyzed and the results are recorded.

Quantitative Analysis of Infertility compound Lupeol and Beta Sitosterol by TLC

The present works mainly focus on quantification of anti-infertility compounds such as Lupeol and Beta Sitosterol were isolated from the syrup. Lupeol has no unhealthy impact on surrounding tissue or other organs in the body. The Betalupe contains β sitosterol, Lupeol, were determined as 0.975 and 0.985 respectively. As value of Lupeol is quite high so it can serve as fertility compound. Beta Sitosterol was good for Fertility related alternative medicine supplements and vitamins. So lupeol and Beta Sitosterol Good for Fertility products.

Total Estimation of Alkaloids, Flavonoid and Steroid

The alkaloids are one of the active compounds used to cure sterility and infertility. They act as hormone regulators in the brain, which then helps metabolize both calcium and phosphorus. The high level of alkaloid is found to be 0.125% in the prepared syrup where as the commercial products have in less in when estimated syrup 1 gives 0.022 % and syrup 2 gives 0.024% respectively.

The timing of the release of pituitary hormones regulates menstruation, fertility, and other processes. Hence, an agent that will produce a balance of hormones could be used to regulate these processes. The Betalupe contains Flavonoids 0.145% in the prepared syrup where as the other two commercial syrups namely syrup 1 gives 0.162 % and syrup 2 gives 0.075% respectively.

In a normal female body, testosterones are produced in a small amount. Similar to males, synthetically elevated testosterones due to steroids can affect the hypothalamic-pituitary-gonadal axis. The production and release of LH and FSH gets hampered due to increase in circulating androgens. It may lead to follicle formation, ovulation and an irregular menstrual cycle. Total steroids in Betalupe contains high amount 0.367% in the prepared syrup where as the other two commercial syrups namely syrup 1 gives 0.240 % and syrup 2 gives 0.245% respectively.

NUTRIENTS ANALYSIS OF SYRUP

The present study depicts the comparative nutrient analysis such as Protein, Carbohydrate and cholesterol were analyzed in the prepared syrup Betalupe, with commercially available products.

Carbohydrates

Carbohydrates are not technically hydrates of carbon. Structurally it is more accurate to view them as polyhydroxy aldehydes and ketones.

Carbohydrate choices also influence fertility. Choosing slowly digested carbohydrates that are rich in fiber can improve fertility. The Betalupe contains 17.27 % of carbohydrate where as the other two commercial syrups namely syrup 1 gives 16.92% and syrup 2 gives 16.05% respectively.

Protein

Proteins are biochemical compounds consisting of one or more polypeptides typically folded into a globular or fibrous form, facilitating a biological function. The protein was the most important nutrient for maintaining the structure of the body. The Betalupe contains high protein level 17.56 % where as the other two commercial syrups namely syrup 1 gives 14.27 % and syrup 2 gives 14.20% respectively.

Cholesterol

Cholesterol is an essential structural component of mammalian cell membranes and is required to establish proper membrane permeability and fluidity. In addition, cholesterol is an important component for the manufacture of bile Acids, steroid hormones, and vitamin D. 0.1 mg of cholesterol is found in this Betalupe where as the other two commercial syrups namely syrup 1 gives 0.07 mg and syrup 2 gives 0.08 respectively.

MINERAL ANALYSIS

Minerals are the electrical transmitters in our body system. The better mobilization of minerals like Calcium, Magnesium, and Phosphorous in the body is very useful for women to counter the problems like calcium depletion due to various reasons. Women, who get desired iron irrelevant of any sources, have the greatest protection against infertility.

Calcium

Calcium is a mineral that plays an important role in the development and maintenance of the bones. The high level of calcium is present in the Betalupe has 40 mg where as very low calcium in commercial syrups namely syrup 1 gives 28 mg and syrup 2 gives 18 mg respectively.

Magnesium

Magnesium is the fourth most abundant mineral in the body and is essential to good health. The maximum level of magnesium was present in the Betalupe has 27.5 mg where as very low in commercial syrups namely syrup 1 gives 26.5 mg and syrup 2 gives 16.3 mg respectively.

Iron

Iron is a mineral it is Essential for transfer of oxygen between tissues in the body. An important nutrient for pregnancy, iron also helps with conception and with the health of your respiratory system. The iron content of Betalupe has 7.72 mg where as very low in commercial syrups namely syrup 1 gives 40.94 mg and syrup 2 gives 8.87 mg respectively. The high amount of iron could be attributed to varieties, stages of incubation and concentration of ingredients.

Phosphorous

The Phosphorus is the most abundant mineral in the body. It Builds bones and teeth, energy production, acid-base balance, necessary in metabolism and cell membranes, calcium absorption. The high level of phosphorous is present in 0.1mg of Betalupe where as very low in commercial syrups namely syrup 1 gives 0.07mg and syrup 2 gives 0.08 mg respectively.

VITAMIN ANALYSIS

Vitamins are essential for the normal growth and development of a multicellular organ. Vitamins and minerals boost the immune system; support normal growth and development, and help cells and organs do their function. All of the vitamins are essential during the pre-conceptual period. Women who have trouble conceiving increases fertility and vitamins have been found to improve low sperm counts. The present study shows the vitamins such as Vitamin E, C were

analyzed in the prepared infertile syrup Betalupe, with commercial products.

Vitamin C

Vitamin C is one of the safest and most effective nutrients it is have rich in antioxidants**.** Vitamin C is also an antioxidant, and studies show that vitamin C enhances sperm quality, protecting sperm and the DNA within it from damage. Vitamin C also appears to keep the sperm from clumping together, making them more motile. 43.10 mg of vitamin C is present in the Betalupe Syrup where as very low in commercial syrups namely syrup 1 gives 8.62 mg and syrup 2 gives 24.56 mg respectively.

Vitamin E

Vitamin E is another powerful antioxidant and has been shown to increase fertility when given to both men and women. Men going for IVF treatment with their partners have been given vitamin E, and fertilization rates have increased. The Betalupe syrup has 328.18mg of vitamin E where as very low in commercial syrups namely syrup 1 gives 204.24 mg and syrup 2 gives 157.14 mg respectively.

HEAVY METAL ANALYSIS OF SYRUP

In this present investigation the results shows the amount of heavy metals present in the prepared syrup are very low in concentration.

***IN VITRO* ANTIOXIDANT ACTIVITY BY POWER REDUCING ASSAY**

The antioxidant property of the syrup was analyzed by Power reducing assay. Health cells = healthy fertility. Antioxidants can provide protection from these free radicals which keeps the DNA intact thus results in a healthy eggs for conception.

Power reducing assay

The syrup showed potent free radical scavenging activity showed the presence of phenolic including flavonoids as a major class of compounds. The Betalupe syrup contains 64.71% of antioxidant where as very low concentration in commercial syrups namely syrup 1 gives 49% and syrup 2 gives 24% respectively. The syrup contains good antioxidant property. The results are tabulated in Table 7 (**FIG 15**).

MICROBIAL ANALYSIS OF SYRUP

The microbial analysis was done in syrup. The fungus colonies are absent and the overall microbial colonies were very low numbers present in Nutrient media. The low numbers of microbial colonies were present in due to the addition of herbal plants.

ANTI-INTERTILITY ACTIVITY OF BETALUPE ON *CYPRINUS CARPIO*

The oral administration of Betalupe for *Cyprinus carpio* to evaluate the anti infertility activity of syrup. The prepared syrup could serve as a herbal source to isolate the promising bioactive compounds which are later used as an catalyst to induce the ovaries for egg production. The positive response of female *Cyprinus carpio* fish with single simultaneous injection of Syrup was very significant. In an ideal normal condition the chances of egg production in the particular taken fish is about 40, 000 eggs. Where as it was estimated that the production of eggs evident slight escalation ranges about 5000 eggs than the normal condition when administered with the syrup **BETALUPE.** So the study reveals that the isolated bioactive compound has played a significant role in the egg production from its early stages. But a detailed analysis has to be done to enhance the feeding pattern according to the sizes and the ages of the fishes. So as to make use of the compounds for the female reproductive system balance to overcome the irregularities in the egg production.

CONCLUSION

Most of the fertility drugs prescribed to infertile women are made from hormones and functions by stimulating the reproductive system. The present open study was conducted to evaluate the safety and efficacy of syrup, a polyherbal uterine tonic, in the treatment of infertility. Results of this study indicated that syrup promotes conception and helps to correct menstrual, uterine disorders and ovulatory defect in infertile women without causing any adverse effects. Thus, syrup was found to be safe and effective in the treatment of infertility in women. So we conclude, in this study that the two infertile compound was enriched in this syrup. This indicates that in future it leads a path for developing a new medicine to sterile humans.

Fig 1- Collection of Experimental Samples

Fig 2-IDENTIFICATION OF LUPEOL& BETA SITOSTEROL

Table 1: NUTRIENTS ANALYSIS OF SYRUP

S. No	Nutrients Analysis	Betalupe	Commercial Syrup	
			S1	S2
1.	Protein (mg)	17.56	14.20	14.27
2.	Carbohydrate (mg)	17.27	16.05	16.92
3.	Lipid (mg)	91.1	83	24
4.	Calcium (mg)	40	36.5	38
5.	Magnesium (mg)	27.5	16.3	18
6.	Iron (mg)	7.72	8.87	6.94
7.	Phosphorous (mg)	0.1	0.08	0.07
8.	Vitamin E (mg)	43.10	24.56	8.62
9.	Vitamin C (mg)	328.18	167.14	204.24

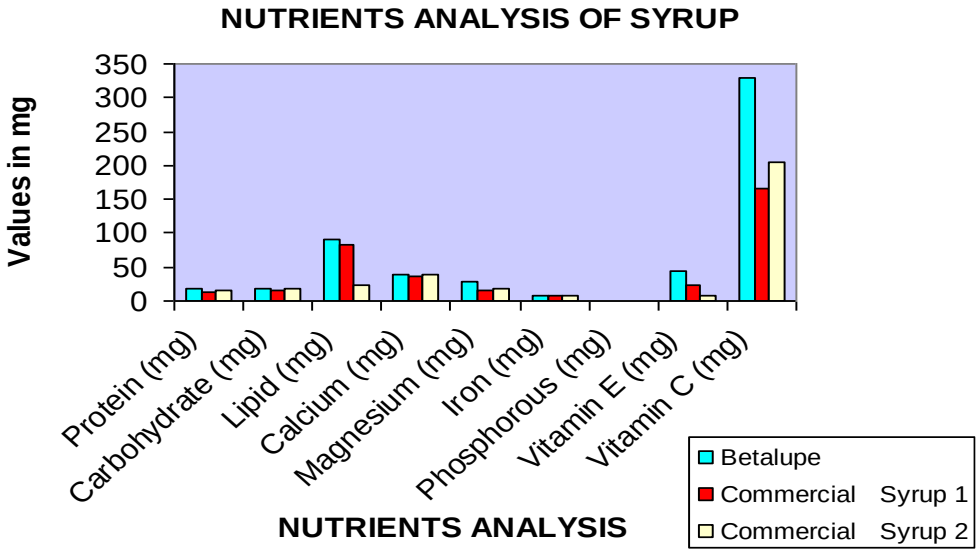

Table 2: *In-Vitro* Anti oxidant activity of Syrup by Power reducing assay

S.NO	Sample	Values in %	
		0.5 ml	1 ml
1.	Betalupe	44.71	64.71
2.	Commercial Syrup 1	11.9	24.9
3.	Commercial Syrup 2	24.1	45.2

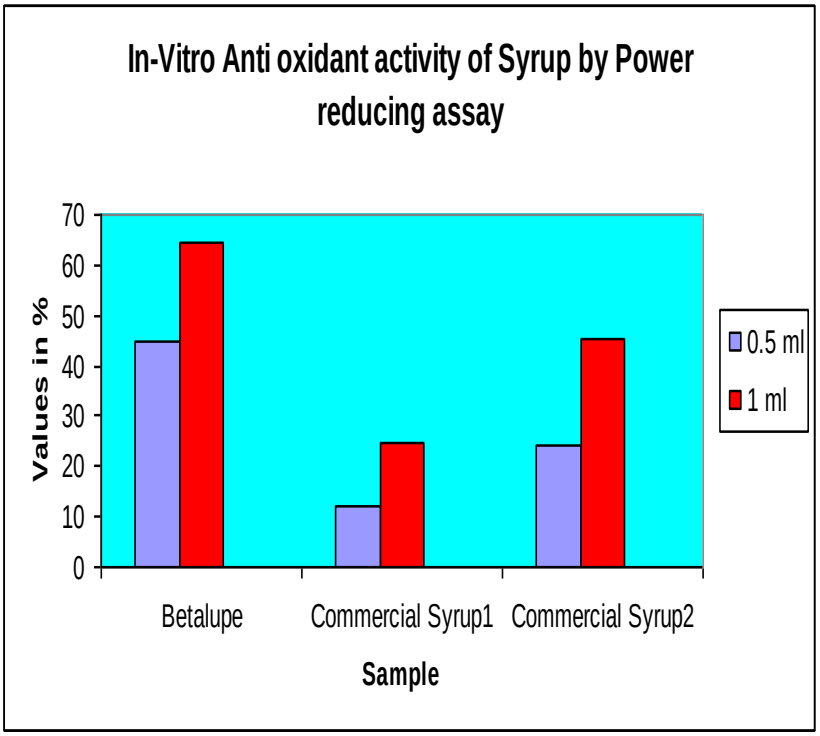

Fig 3- INVESTIGATION INFERTILITY ACTIVITY OF BETA LUPE ON *Cyprinus carpio*

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