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In Vitro Antidiabetic and Antioxidant Activity Performed in Polyherbal Extract for the Treatment of Women Infertility

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ABSTRACT:
Infertility is the inability to achieve clinical pregnancy over an average period of one year despite adequate regular unprotected sexual intercourse. Several types of infertility include age, smoking, alcohol, STDs, diabetes, stress, radiation therapy. Oxidative stress and carbohydrate (Alfa amylase) also plays major role for infertility and diabetic pathology. The aim of present studies was to evaluate the *in vitro* antioxidant and antidiabetic activity. Various plant extract has inhibitory potential enzymes linked to hypoglycemia and antioxidant activity. Antidiabetic activity was performed using Alfa amylase, Alfa glucosidase and antioxidant activity was performed using 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radicals, ferric reducing/antioxidant power (FRAP) methods, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals and nitric oxide radical inhibition assay. In this study the evaluation of selected plant was performed for *in vitro* potential infertility (antidiabetic & antioxidant) activity. The aqueous extract of Nirgundi seed (*Vitex Nigundu*), Turmeric (*Curcuma Longa*) and Fenugreek (Trigonella foenum graecum) has showed the antidiabetic and antioxidant activity. This combination showed highest polyphenolic compounds (iso flavones and flavonoids), triterpenoid, and phenolic content which exhibited significant antidiabetic and antioxidant activity.

Keyword: Polyherbal Extract; Antidiabetic; Alfa amylase; Alfa Glucosidase; Antioxidant; ABTS; DPPH; FRAP; Nitric oxide radical inhibition assay

INTRODUCTION:

Infertility may defined as the inability of couple to achieve pregnancy for a period of one year or above over an average 35 years of age despite regular unprotected sexual intercourse. There are various causes of infertility including alcohol, smoking, PCOS, obesity, diabetes endometriosis, hormonal imbalance, premature hormonal failure, hyperprolactinemia, and hypothalamic dysfunction etc. Plants rich in polyphenolic compounds (isoflavones and flavonoids), triterpenoid and phenolic content are beneficial for reproductive health in women. (1)These compounds along with regulating the female endocrine pathways, and improving symptoms of menopause, also treat female reproductive disorders such as polycystic ovary syndrome (PCOS), premature ovarian failure (POF), endometriosis, hyperprolactinemia, and hypothalamic dysfunction; moreover, because of their anticancer, antioxidant, and antidepressant properties.[2] They can be used in traditional medicine or in the pharmaceutical industry as safe compounds for

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women's health.

Oxidative stress and diabetes are the factor which is responsible for infertility. Antioxidant products and ROS balance have been shown to be closely related to female subfertility or infertility. Substantial evidence has indicated that some physiological processes, from oocyte maturation to fertilization and embryo development, are particularly sensitive to OS. Diabetes mellitus Type 1 and Type 2 should be considered in the differential diagnosis of menstrual abnormalities and infertility.[5,6] During the reproductive years, diabetes has been associated with menstrual abnormalities, such as oligomenorrhea and secondary amenorrhea. It was found that better glycemic control and prevention of diabetic complications improves these irregularities and increases fertility rates close to those that are seen in the general population. According to literature studies of herbal drug combination of polyherbal extract possess the effective action in infertility.

According to the literature survey on *Vitexnegundo* (VN) L. (Verbenaceae), Fenugreek (*Trigonella foenum graecum*) and Turmeric (*Curcuma Longa*, Zingeberacae) the antioxidant and antidiabetic activity is reported on individual plant but in combination there is no study reported so far.[14,15,16] Besides antidiabetic and antioxidant activity they are also responsible to cure infertility in combination.

MATERIALS AND METHODS:

Materials

Herbal Extract of Vitex Nigundo, Curcuma Longa, and Fenugreek were received as free gift sample from Amsar Pvt. Ltd., Indore. Potato starch was purchased from SD Fine Pvt. Ltd., Mumbai. 3,5-dinitrosalicylic acid, Tris buffer purchased from Hi-Media Pvt. Ltd., Mumbai. α-Amylase and α-Glucosidase enzymes were purchased from SRL Pvt. Ltd., Mumbai. HCl, Mayers reagent, KOH, $FeCl_3$, H_2SO_4, Fehling's solution, NaOH, DPPH, Phenanthroline, Hydrogen peroxide, Potassium persulfate, Potassium ferricyanide, Sodium nitroprusside were purchased from S.D fine chemicals.

Method

Phytochemical analysis

Phytochemical assay of all extracts were performed to determine chemical compound content of extracts qualitatively. Based on an established procedure, was conducted to explore the secondary metabolites, such as: alkaloid, flavonoid, saponin, triterpenoid, polyphenols, steroid, and tannin.

Antioxidant Activity

DPPH Radical Scavenging Activity Assay

Radical scavenging activity of the extracts was measured using the stable radical DPPH such as that determined by with some modifications. In tubes, 2.5 mL of different concentrations of each extract were introduced and 0.5 mL of methanol solution of DPPH (0.2 mM of DPPH, dissolved in methanol) freshly prepared was added. The mixture is vigorously vortexed and left in the dark at ambient temperature for 30 min. Then, the absorbance of the mixture was measured at 425 nm in a spectrophotometer. The antioxidant activity of our extracts is expressed as the percentage of DPPH radical inhibition and the IC_{50} was calculated for comparing the obtained results.[14]

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Hydroxyl Radical Scavenging Activities

The hydroxyl radical scavenging activity was performed as per the method determined by with minor modifications. The reaction mixture was constituted by adding 2.4 ml of phosphate buffer (pH 7.8) into test tubes. To the same test tubes, 90 µl of 1 mM 1, 10 phenanthroline, 150 µl of 0.1 mM hydrogen peroxide, 60 µl of 1 mM iron (III) chloride, and 1.5 ml of the Phytexponent and the standard (L-ascorbic acid) at different concentrations (100%, 10%, 1%, 0.1%, and 0.01%) were added except in the controls, followed by incubation at room temperature for 5 minutes. The increase in absorbance at 425 nm was measured, and radical scavenging activity was calculated using the following formula:[14,16,17]

$$I\ \% = (Ac-As)/Ac\ X\ 100 \quad (1)$$

ABTS Radical Scavenging Assay

The TEAC test (Trolox Equivalent Antioxidant Capacity) or discoloration test of $ABTS^{\cdot+}$ was carried out according to the method described by Sayah *et al.* [19]. Briefly, the cationic radical ($ABTS^{\cdot+}$) was prepared by the reaction between 10 mL of ABTS (2 mM) in H_2O and 100 µL of potassium persulfate ($K_2S_2O_8$) (70 mM). The mixture was incubated in the dark for 16 hours at room temperature. Then, the $ABTS^{\cdot+}$ solution was diluted with methanol to obtain an absorbance of 0.70 at 425 nm . Then, 200 µL of each extract was mixed with 2 mL of the diluted $ABTS^{\cdot+}$ solution and allowed to react for 1 minute. After, the absorbance of the $ABTS^{\cdot+}$ radical is measured at 734 nm. All samples were made in triplicate. The results were represented as Trolox equivalent per gram of extract dry weight (mg TE/edw).

Ferric Reducing/Antioxidant Power (FRAP) Assay

The FRAP test is performed according to the method determined by slight modification. Briefly, 1 mL of each extract (1 mg/mL) was mixed with 2.5 mL of the phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of the 1% potassium ferricyanide aqueous solution. After incubation at 50°C for 20 min, 2.5 mL of 10% trichloroacetic acid were added to the mixture, and then the mixture was centrifuged at 3000 rpm for 10 min. At the end, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of aqueous ferric chloride solution $FeCl_3$ (0.1%, w/v). The absorbance was measured at 425 nm. The results are expressed as ascorbic acid equivalent per gram of extract dry weight (mg AAE/g edw).

Nitric Oxide Radical Inhibition Assay

Nitric oxide (NO) generated from sodium nitroprusside (SNP) was measured according to the method. Briefly, the reaction mixture (5.0ml) containing SNP (5mM) in phosphate buffered saline (pH 7.3), with or without the polyherbal extract at different concentrations, was incubated at 25^{o}C for 180min in front of a visible polychromatic light source (25 Watt tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO^{2-}) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% Nnaphthylethylenediamine dihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was measured at 425 nm The nitrite generated in the presence or absence of the seed extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

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In VitroAntidiabetic Effects

The α-amylase inhibition assay

Alpha amylase inhibitory test Iodine solution was prepared by dissolving 0.254 g 12 and 4.0 g KI in 1L of distilled water. Starch solution was prepared by dissolving 1g of starch in 10 ml of distilled water, gently boiling, cooling and completing to 100 ml with distilled water. Amylase solution was prepared by transferring 6 µl of the standard porcine pancreatic amylase suspension (40 mg/ml) to 8 ml of phosphate buffer (pH 6.9). Alpha amylase inhibitory activity was based on the starch-iodine method described by Hansawasdi with some modifications. Briefly, control and test solutions were prepared as follows: 0.3 ml of amylase solution were transferred to a sample tube containing 0.3 ml of the extract to be tested (substituted by the solvent of extraction in the case of control) and 0.6 ml phosphate buffer (pH 6.9). The mixture was incubated at 37ºC for 15 minutes. 0.4 ml aliquots of that incubate were transferred to sample tubes containing 3 ml starch (1g%) and 2 ml of phosphate buffer (pH 6.5) and the mixture was re-incubated for 45 minutes. At zero time and at the end of the incubation period 0.1 ml of the reaction mixture was withdrawn from each tube after mixing and discharged into 10 ml of iodine solution. Solutions were thoroughly mixed and the absorbance measured immediately at 425 nm. Percentage inhibition was calculated according to the formula:

$$[(A_0-A_t)_{control} -(A_0-A_t)_{sample}/(A_0-A_t)_{control}] \times 100\% \quad (2)$$

where, A_0 and A_t are the absorbance values at zero time and at the end of the incubation, respectively. Each experiment was repeated three to four times and the average value was used for obtaining the relevant plots.

Inhibitory assay of alpha glucosidases enzyme

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1µ/ml) to it followed by incubation. Then, the reaction mixture was heated in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method.

Calculation of 50% Inhibitory Concentration (IC_{50})

Calculation of 50% Inhibitory Concentration (IC
50
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The concentration of the plant extracts required to inhibit 50% of the enzyme (IC
50
) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and As is the absorbance of the sample.

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calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by equation 1.

RESULTS AND DISCUSSION

Phytochemical Analysis

After the phytochemical screening of polyherbal extract it was found that tannins, glycosides, flavonoids, polyphenols, triterpenoids and saponin are present. The data is given in Table 1.

Table 1: Phytochemical analysis of polyherbal extract

S. No.	Phytochemical Test	Inference
1	Alkaloids	Negative
2	Tannins	Positive
3	Glycosides	Positive
4	Flavanoids	Positive
5	Pholyphenols	Positive
6	Triterpenoids	Positive
7	Resins	Negative
8	Saponin	Positive

Antioxidant Activity

The antioxidant activity **(IC_{50})** of polyherbal extract of *Vitex Nigundu*, *Curcuma Longa* and *Trigonella foenum graecum* is given in Table 2.

Table 2: Antioxidant activity (IC_{50}) of the Polyherbal Extract of *Vitex Nigundu, Curcuma Longa* and *Trigonella foenum graecum*

S.No	Polyherbal Extract	DPPH	Hrdroxy radical scavenging	ABTS	FRAP	Nitric Oxide radical inhabitation assay
1	50	5.0	10.2	22.00	38.12	30.78
2	100	17	22.0	27.03	41.45	35.12
3	150	42	34.09	32.01	48.12	43.67
4	200	54	46.08	42.06	51.78	49.98
5	250	65	51.01	53.07	58.45	53.56
6	300	71	65.77	61.11	61.45	58.23
7	350	86	77.9	74.01	68.45	60.76
8	400	88.02	89.02	87.09	74.56	68.23
9	450	91	94.7	92.09	80.89	79.23

Antidaibetic Activity

The antidaibetic activity of polyherbal extract of *Vitex Nigundu*, *Curcuma Longa* and *Trigonella foenum graecum* is given in Table 3.

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Table 3: Antidaibetic activity (IC_{50}) of the Polyherbal Extract of *Vitex Nigundu*, *Curcuma Longa* and *Trigonella foenum graecum*

S.No	Alfa-Amylase	Alfa- Glucosidase
1	44. 00	22.08
2	52.07	35.04
3	61.00	42.06
4	67.08	51.08
5	73.04	61.07
6	81.09	74.08
7	88.07	84.09
8	93.06	94.08
9	98.09	99.01

CONCLUSION

Antioxidant supplementation has been shown to improve insulin sensitivity and restore redox balance in patients with PCOS, infertility, menopause, aging, hairfall, dysmonneria Polyherbal extract mixture of Vitex Nigundu, Turmeric (Curcuma Longa) and Fenugreek (Trigonella foenum graecum) mixture. It had high amount of polyphenols, terpenoids, flavonoids and saponins so it may be good for relieving stress and treat diabetes. Thus offers a potential for greater efficacy infertility that are associated with diabetic and free radical causing stress.

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