Anti Oxidant Role of Selected Medicinal Plants

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1. Introduction

Medicinal plants were nature's priceless gift to human. The development in the field of modern medicine temporarily subdued the traditional herbal Medicine. But today herbal medicine renaissance is blooming across the world. Green medicines are healthier, safer and harmless than synthetic ones. The traditional medicine is accepted as an alternative form of health care (Kumar et al., 2015).

Herbal remedies represent one of the most important fields of traditional medicines ' In rural areas ,ethnomedicine is practiced by large group of people for the treatment of several physical , physiological and social ailments(Swarnkar ,*et al.*,2009).Hence there is an urgent need to search new compounds from plant extracts .In the present study, two plants were chosen viz., ,Withania somnifera and Emilia sonchifolia to find out their efficacy in curing male infertility through their antioxidant properties..

Withania somnifera

Withania somnifera (L) is a small , erect evergreen woody shrub that grows up to a height of 1-m and belongs to the family of Solanaceae).This plant is cultivated in India, Bangladesh, South Africa, Congo, Moroco, Jordan, Pakisthan and Afganistan. The plant is reported to have many chemical and pharmacological properties .Hence it is widely used as therapeutic agents in Ayurvedic and Unani systems for treatment(Chopra,1994).

In Ayurveda, *Withania somnifera* (Ashwakanda) is widely claimed for having potent aphrodisiac, rejuvenative, sedative and life prolonging properties. Ayurvedic practitioners are using this plant traditionally to promote youthful vigour, strength, endurance and health. It increases the production of vital fluid, blood, lymph, muscle fat, semen and cells,. (Bhattacharyaand Muruganandam ,2003).

W.somnifera also act as an immunosuppressive agent for the inflammatory disease (Devi ,1996 and Bhattacharya et al., 2000,2001, 2002).Hence the plant deserves attention as herbal therapy to ease or even eliminate many of today's common health problems.

Emilia sonchifolia

Emilia sonchifolia is an annual herbaceous plant which is found mainly in tropical and sub tropical countries and in India and other countries of Asia. E.*sonchifolia* commonly known as Heranakhuri in Hindi belongs to Asteraceae Family. E.*sonchifolia* is a tuberulous slender herb growing 30-40 cm height. It is edible and used as a salad before flowering. Stem and leaves are cooked and eaten as vegetable. E.*sonchifolia is* reported to have anticancer property, anti inflammatory and antioxidant activity. Many studies revealed that the plant contains alkaloids, flavonoids and terpenoids (Shen et al., 2012).Hence this plant is being used in Ayurvedic system of medicine for the treatment of various diseases (Sophia et al,. 2011).

Medicinal plants and male infertility

Recent time's nutrition research on dietary antioxidants and its effects on human health have become a major interest. The synthetic antioxidant leaves a lot of side effects. Many herbals and medicinal plants are powerful antioxidants, due to the presence of phenolic bioactive compounds (Gadallah, 2018). Free radicals are formed as a result of adenosine tri phosphate (ATP) production by mitochondria, when the cells use oxygen to generate energy. Free radicals are generally called as Reactive Oxygen Species (ROS).Based on concentrations they are classified into lower, moderate and high levels. Lower and moderate levels exert beneficial effects in cellular response and immune function (High concentration of ROS generates oxidative stress and damages all cell structure), (Aitken ,1989)

Male infertility is associated with various anatomical abnormalities, environmental factors, life style disorders, inflammation and urino-genital trauma in male reproductive system and oxidative stress (Agarwal *et al.*, 2014). Mammalian spermatozoa membranes are very sensitive to free radical induced damage mediated by lipid peroxidise due its rich poly unsaturated fatty acids component. Reactive Oxygen Species attacks the fluidity of the sperm plasma membrane and the integrity of DNA in the sperm nucleus. Thereby

DNA damage accelerate germ cell apoptosis (Opuwari and Henkel , 2016).

DNA damage, decrease the motility and induce, abnormal morphology which affect the various spermatic physiological processes such as cap acitation, sperm -oocyte fusion and hyper activation (Zini et al., 2009)

Male infertility treatment can be done by the support of antioxidant supplementation (Agarwal *et al*., 201 5).The beneficial effect of antioxidant treatment for the improvement of sperm parameters in men as well as fertilization or pregnancy rates in their partners are reported (Agarwal et al., 2003,2014,2015) .

An improvement in sperm quality, mainly sperm motility, sperm concentration and in sperm morphology was reported due to the reduction of oxidation treatments (Ko et al., 2014). Drugs with anti oxidative properties had been postulated for the management of male fertility problems(Makker *etal*.,2009) .Reduction of antioxidants, lack of vitamin A and elements such as flavonoids, carnitine, folate, zinc, selenium ,Vit. C and E etc., in diet were the reasons for infertility especially in the cases of oligospermia and asthenospermia in humans.(Lombardo et al., 2011).Recently oxidative stress has become the focus of interest as potential cause of male infertility and non- hormonal treatment is also needed for patients with idiopathic, or non curable oligo-astheno- terato-zoospermia and for non-non zoospermia infertile patients'(Gadallah,2018).

2. Materials and Methods

Fresh leaves of the two plants were collected from Sri Paramakalyani College campus. The plant was authenticated by the Botany Department of the college. The plant leaves were washed thoroughly in tap water, shade dried at room temperature (25^{o} C), powdered and used for solvent extraction. Preliminary phyto chemical screening was done using standard procedures. The powdered samples were packed into a Soxhlet apparatus and were extracted sequentially with methanol and the air dried residue was further extracted with hot water by the method of maceration. The material was dried in a hot air oven at 40^{o}C. The solvents were evaporated using a rotary vacuum-evaporator at 50^{o}C and the remaining water was removed by lyophilisation. The extract recovery in the solvents was expressed as percent of the plant sample dry matter. The freeze-dried extracts thus obtained was dissolved in the solvents at the concentration of 1mg/1ml and used for assessment of antioxidant capacity through various chemical assays.

Determination of total Phenolic and flavonoid contents

The total Phenolic content of leaves was determined by Folin ciocalteu method. The amount of total phenolics and tannins was calculated as gallic acid equivalents (GAE) as described by Shahidi et al., 1992. The total flavonoid content was determined by the method described previously by Pieta, 2000.

Metal chelating activity

The chelating activity of ferrous ions by the extracts of the two plant leaves was estimated by the method described by (Hebbel et al., 1990). Absorbance of the solution was measured spectrophoto metrically at 562nm. The results were expressed as mg ethylene diamine tetra acetic acid (EDTA) equivalent/g extract.

The DPPH radical scavenging activity of the two extracts was measured using the method of Blois (1958). Lc50 value of the extract concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

Antioxidant Activity

Determination of total phenolics in the tested plants

Total phenolic compounds in both the plants were estimated.. Total phenolic activity was high at a concentration of 1000 µg for the plant samples and it was found to be 1.010 nm and 1.030 nm for *Withania sominifera* and *Emilia sonchifolia (*Fig.1). In another study it is reported that the Iranian *Ocimum*, which are often present in Iranian dishes, are strong radical scavengers and can be considered as good sources of natural antioxidants for side dishes, medicinal and commercial uses.(Javanmardi *et al*., 2002).

` Polyphenolic compounds were known to have antioxidant activity (*Raj, 2012)*). This activity was believed to be mainly due to their redox properties, which played an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Sophia, et al.,2012).

Determination of Total Flavonoids in the tested plants.

In terms of flavonoids content, both the plants showed good activity in 1000µg and it was found to be 1.25nm for *Withania sominifera* and 1.60nm for *Emilia sonchifolia.* The total content of flavonoids was influenced by the interaction between varieties and parts of plants. In fact, many medicinal plants contain large amount of antioxidants such as polyphenols (Cibin et al ., 2006) Previous studies had shown that some flavonoids components such as quercetin had anticancer activities and good antioxidant activity .They were able to inhibit cancer cell growth (Lija et al., 2006) . In the present study the antioxidant effects of flavonoids and their role in improving spermatogenesis was evaluated in men with impaired spermatogenesis.

DPPH radical scavenging activity

The Scavenging activity against DPPH assay was

carried out and when there is a scavenging activity, there will be a diminishing character in the colour of the solution showing a better activity. The % inhibition was calculated and both the plants showed the best result in 1000µg and were found to be 70.30 % for *Withania sominifera* and 60.54 % for *Emilia sonchifolia.* Phytochemicals are reported to give a good anti oxidative activity and strong scavenging of DPPH (Damani et al., 2003). Substances capable of donating electrons/hydrogen atoms are able to convert DPPH into their non-radical form 1, 1-diphenyl-2-picrylhydrazine, a reaction which can be followed spectrophotometrically. The reduction capability of DPPH was determined by the decrease in absorbance induced by antioxidants. . Radical scavenging activity of extracts against stable DPPH was determined spectrophotometrically at 517 nm. This assay illustrates a decrease in the concentration of DPPH radical due to the scavenging ability of the soluble phyto constituents present in extracts.The methanolic extract of Emilia sonchifolia exhibited a higher free radical scavenging activity. This was due to the presence of flavonoids which can donate hydrogen atom. Hirano etal., (2001),reported the correlation between flavonoids and antioxidants.

Fe^{2+} Chelation activity

Iron chelation activity was high in 1000 µg concentration and it was found to be 86.64% for *Withania sominifera* and 75.25% for *Emilia sonchifolia* .For both plants. Chelation therapy reduced iron-related complications and thereby improves quality of life and overall survival .

Ferro zinc can quantitatively form complexes with Fe2+. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Measurement of colour reduction, therefore, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe2+ possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals .The main strategy to avoid ROS generation that is associated with redox active metal catalysis involves chelating of the metal ions reaction which is implicated in many diseases. The chelating effect on the ferrous ions by methanolic extract of *Emilia sonchifolia*a and *Withania sominfera* extract, the positive control exhibited the ability to chelate metal ions but in varying capacities. The chelating effect of the *Emilia sonchifolia* extract increased with the increase in the concentration. This may be due to the increase in the concentration of the secondary metabolites in the extracts. Thus the results suggest that they are capable of scavenging the free radicals and prevent the initiation of free radicals by stabilizing them to participate in any deleterious reactions.

The methanolic extracts of the two plants tested for various phytochemical analysis, indicated the presence of alkaloids, phenolic compounds, flavonoids etc., (Table 1 and 2). Total phenolic compounds and flavonoids are in high proportion.

The in vitro examination of DPPH scanning activity showed a good result in 1000µg dose (70.30%) for *W.somnifera* and 60.54% for *Emila soncifolia (Fig.1).* DPPH scavenging is an indication for antioxidant activity. The flavonoid content in the plant extracts has exerted the DPPH scavenging role and both the plants have good antioxidant maintaining components.

Iron chelation ($Fe^{+}2$) activity of the two plants were found good. At a dose of 1000µg *W.somnifea* and *E.sonchifolia* are able to make iron chelation 86.64% and 75.25% respectively.

From the total Phenolic and flavonoids content and subsequent testing of free radical scavenging assay (DPPH assay and metal chelating activity, the two tested plants are found to have a good antioxidant potential. The administration of the plant drugs to infertile male will help to reduce free radical stress during spermatogenesis. There by the male infertility can be corrected.

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Figure 1. Determination o f total phenol in the tested plants.

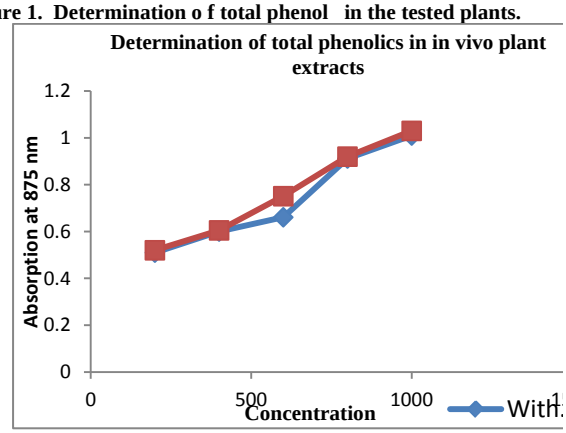

Fig. 2. Determination of total flavonoids in the tested plants.

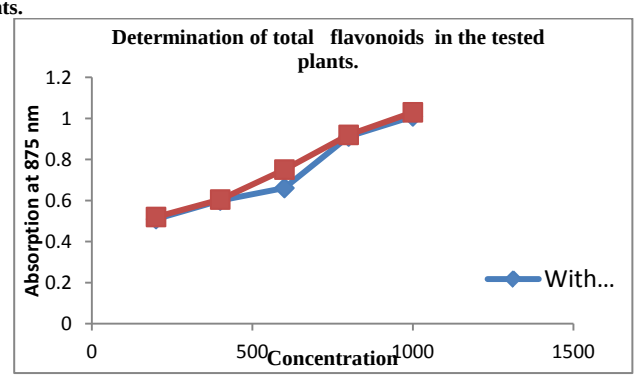

Figure 3. DPPH radical scavenging activity in the tested plant

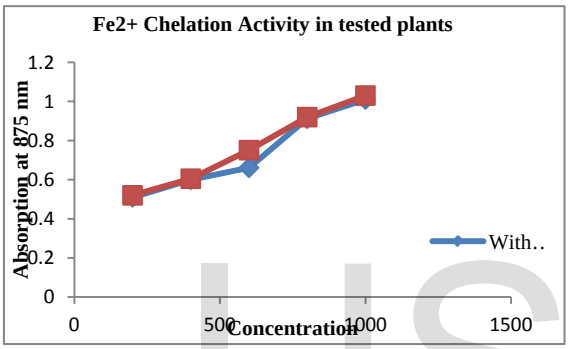

Figure 4 .Fe2+ Chelation Activity in tested plants

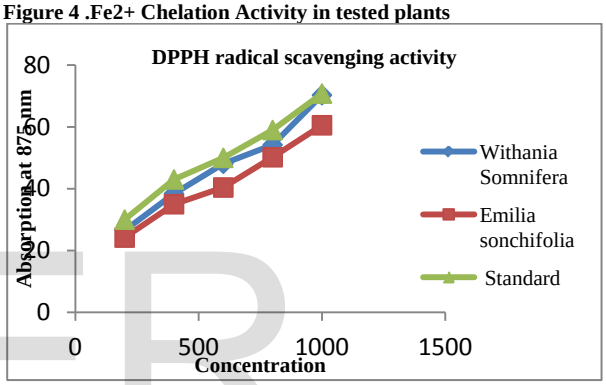

Table 1.Preliminary Phyto chemical Screening of *Withania sominifera*

S.No	Reagent	Nature of colour change	Inference	Phytochemical changes
1	Methanol extract+ 5.0ml Fehling's solution	Yellow or red	Presence	Presence of carbohydrates
2	Methanol extract+ 2ml glacial acetic acid+ $FeCl_2$+ H_2SO_4	Brown ring	Presence	Presence of Glycosides
3	Powdered sample+ Dis. H_2O+ Olive oil	Frothing present	Presence	Presence of Saponins
4	Powder	Oil strain	Presence	Presence of Oils and Fats
5	Methanol extract+ 2ml chloroform+ con.H_2SO_4	Reddish brown	Absence	Absence of Terpenoids
6	Methanol extract+ Acetic acid+ Dragendorff's reagent	Orange red precipitate	Presence	Presence of Alkaloids
7	Methanol extract+ 2ml acetic anhydride+ 2ml H_2SO_4	Violet- blue green	Absence	Absence of Steroids and Sterols
8	Methanol extract+ 5ml Dil. Ammonia soln+ Con.H_2SO_4	Yellow colour	Presence	Presence of Flavonoids
9	Powdered sample+ 2ml water+0.1 % $FeCl_2$	Brownish green / blue colouration	Presence	Presence of Tannins
10	Methanol extract+ alcohol+ $FeCl_2$	Blue green or red colour	Presence	Presence of Phenolic compounds
11	Methanol extract+ 2 % ninhydrin	Blue colour	Presence	Presence of proteins
12	Methanol extract+ Sodium hydroxide	Blue green or red colour	Presence	Presence of Quinones

Table 2. Preliminary Phytochemical Screening of *Emilia sonchifolia*

S.No	Reagent	Nature of colour change	Inference	Phytochemical changes
1	Methanol extract+ 5.0ml Fehling's soln	Yellow or red	Presence	Presence of carbohydrates
2	Methanol extract+ 2ml glacial acetic acid+ $FeCl_2$+ H_2SO_4	Brown ring	Presence	Presence of Glycosides
3	Powdered sample+ Dis. H_2O+ Olive oil	Frothing present	Presence	Presence of Saponins
4	Powder	Oil strain	Presence	Presence of Oils and Fats
5	Methanol extract+ 2ml chloroform+ con.H_2SO_4	Reddish brown	Absence	Absence of Terpenoids
6	Methanol extract+ Acetic acid+ Dragendorff's reagent	Orange red precipitate	Presence	Presence of Alkaloids
7	Methanol extract+ 2ml acetic anhydride+ 2ml H $_2SO_4$	Violet- blue green	Absence	Absence of Steroids and Sterols
8	Methanol extract+ 5ml Dil. Ammonia soln+ Con.H_2SO_4	Yellow colour	Presence	Presence of Flavonoids
9	Powdered sample+ 2ml water+0.1 % $FeCl_2$	Brownish green / blue black colouration	Presence	Presence of Tannins
10	Methanol extract+ alcohol+ $FeCl_2$	Blue green or red colour	Presence	Presence of Phenolic compounds
11	Methanol extract+ 2 % ninhydrin	Blue colour	Presence	Presence of proteins
12	Methanol extract+ Sodium hydroxide	Blue green or red colour	Presence	Presence of Quinones