Research Article

A Comprehensive and Comparative Study of Anti-Oxidant Efficacy of Different Extracts of Ionidium Suffruticosum by Mutiple *In-Vitro* Antioxidant Assays

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ABSTRACT

Oxidative stress (OS) caused by generation of reactive oxygen species (ROS) in course of biological activity results in various detrimental effect to the viability of the cell. To counter this effect antioxidants defense mechanisms exist. An imbalance between these necessitates extraneous antioxidant supplementation. Ionidum suffruticosum has been in use in traditional medicine mainly as aphrodisiac among various divergent uses. The plant was subjected to cold extraction method with ethanol, chloroform and aqueous solvents. Ascorbic acid was used as control. The efficacy of this plant as an antioxidant has been analyzed by DPPH free radical scavenging, ABTS free radical scavenging, ferric reducing power assay, superoxide anion radical scavenging, hydrogen peroxide scavenging and hydroxyl radical scavenging activity. Interpretation of IC_{50} values showed maximum efficacy in DPPH assay and for ethanolic extract. The *In vitro* anti-oxidant assay result analysis puts forth, Scavenging properties of the plant as the major mechanism for effective antioxidant property in comparison to reducing pathways. The study found that this plant possess potent antioxidant property capable enough to counter the OS produced due to varied insults to the cell.

Keywords: Ionidium suffruticosum, Extract, Antioxidant, Oxidative stress, Male infertility.

INTRODUCTION

Oxidative stress (OS) denotes the oxidation of biological molecules by Reactive Oxygen Species (ROS). Reactive oxygen species are oxygen ions, free radicals and peroxides that exhibit oxidizing effects. They are produced in the course of metabolic processes in all aerobic cells. Anti-oxidant defense mechanisms exist to counteract the harmful effects of ROS. However, the imbalance between ROS generation and anti-oxidant defense mechanisms results in OS.[1]

OS has been implicated in the etiology and pathogenesis of a multitude of chronic and degenerative conditions like ischemic heart diseases, atheroschlerosis, liver damage, arthritic conditions, diabetic mellitus, cancer, neurodegenerative diseases, aging, male and female infertility and embryological mal development.

ROS play a physiological and a pathological role in the reproductive biology of the sperm.[2] The role of OS in impairment of sperm function was documented as early as 1943.[3] The mechanism by which OS causes sperm damage, which in turn induces infertility has been explained on the basis of protein damage, bio-membrane damage, lipid peroxidation and DNA damage.[4,5] ROS is implicated in the pathogenesis of varicocele, which is one of the leading causes of male infertility.[6,7] Behavioral and lifestyle parameters like nicotine, alcohol and drug exposure, heat and obesity that contribute to male infertility are mediated by the increased generation of ROS.[8] The saturation of the spermatozoa with antioxidants has been reported to confer a protective effect against ROS induced damage.[9] The beneficial effects of antioxidants on improved sperm function has been studied both in-vivo and *in-vitro*.[10-13] There has been a current interest in the identification and utilization of herbal preparations that exhibit anti-oxidant activity.

Active anti-oxidants contain flavonoids, phytosterols that augment the natural anti-oxidant defense mechanisms.[14]

Herbal preparations have been used in traditional, complementary and alternative medicine to treat male infertility.

Animal studies have demonstrated the beneficial effects of plant extracts on sperm characteristics.[15-17]

Ionidium suffructicosum (Hybanthus enneaspermus) is an annual herb belonging to the order Malpighiales and family Violaceae.

The plant is referred to as *Ratanpurush* in Hindi and *Orithalthamarai* or *Orilaithamarai* in Tamil. The plant is found in Africa, Madagascar, India and Sri Lanka and is scattered from South-east China to tropical Australia.[18]

In the Siddha and Ayurveda systems of medicine, the plant and its preparations have been used as a general tonic, aphrodisiac, anti-pyretic, nutritive, demulcent, diuretic and the plant has been noted for its anti-inflammatory, anti-plasmodial and anti-microbial activity.[19-22]

The plant has been used to treat male sterility.

As a preliminary inquiry into the efficacy of the plant in the treatment of male infertility, we proposed to study the anti-oxidant potential of the different extracts of the

plant, Ionidium suffruticosum using various anti-oxidant assay methods.

MATERIALS AND METHODS

Plant Collection and Extraction Preparation-Cold Extraction Method

Ionidium suffruticosum was collected from farmlands and wastelands in and around Irungalur, Tiruchirapalli district of Tamuilnadu, India. Before mass collection the plant was subjected to identification and authentication by a botanist from Department of Botany, Holy Cross College, Tiruchirapalli. The collected whole plants were air dried under shade for 15 days, followed by mechanical grinding and pulverisation.

The coarse powdered material of the whole plant was subsequently extracted with sufficient volume of various organic solvents like chloroform, ethanol and water in the order of increasing polarity by cold extraction method. The obtained extracts were subjected to preliminary phytochemical screening.

In vitro Antioxidant Assay

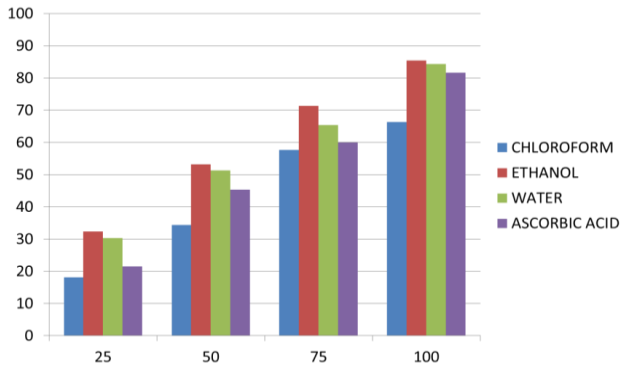
The in vitro antioxidant assay methods used were DPPH free radical scavenging[23], ABTS free radical scavenging[24], ferric reducing power assay[25], superoxide anion radical scavenging[26], hydrogen peroxide scavenging and hydroxyl radical scavenging activity[27]. The antioxidant activity of the extract was expressed as IC_{50} and compared with standard. The IC_{50} value was defined as the concentration of extract that inhibit the formation of radicals by 50%.

RESULTS AND DISCUSSION

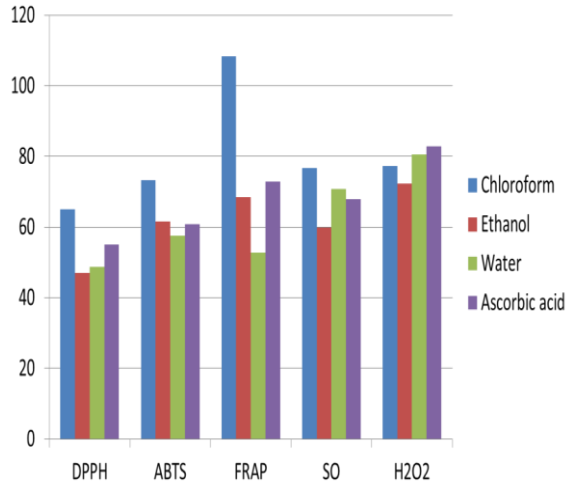
DPPH Activity

1, 1-diphenyl-2-picrylhydrazyl (DPPH) method is a simple and quick method of assaying anti-oxidant activity by colorimetry.

The measurement of loss of the deep violet colour of the molecule is an indicator of anti-oxidant activity. Different fractions of the I. suffruticosum extract exhibited marked DPPH activity (Graph 1) in the order of ethanol> water residue> ascorbic acid > chloro bform as seen by the IC_{50} values. (Graph 2)



Graph 1: Comparison of DPPH activity of different I. suffruticosum extract fractions at differing concentrations



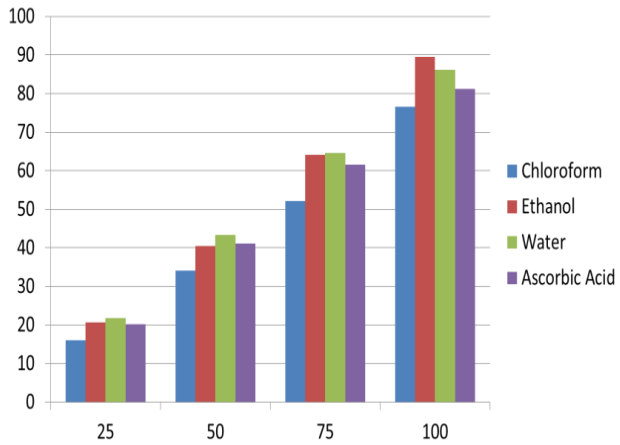
Graph 2: Efficacy of antioxidant activity of different I. suffruticosum extracts by LD_{50} (µg/ml) on assay

ABTS Activity

ABTS· + (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) is reduced to colourless form by the addition of anti-oxidant.

The colorimetric estimation of antioxidant property by this method for I. suffruticosum extract showed maximal activity in the ethanolic, water and ascorbic acid fractions in descending order(Graph 3).

IC_{50} values demonstrate ABTS activity of water residues> ascorbic acid> ethanol > chloroform. (Graph 2)

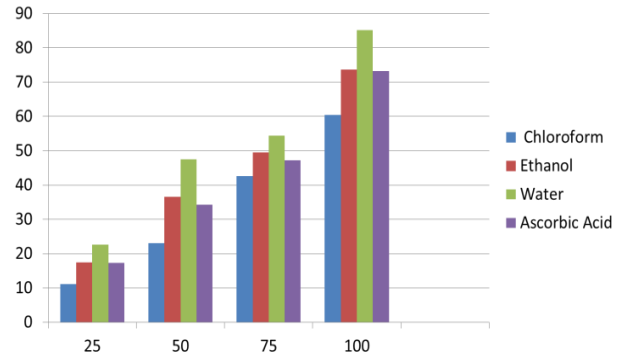


Graph 3: Comparison of ABTS activity of different I. suffruticosum extract fractions at differing concentrations

FRAP Assay

The estimation of Ferric Reducing Anti-oxidant Power measures the ability of the substance to reduce a ferric complex such as ferric 2,4,6-tripyridyl-s-triazine (TPTZ) to its colourless ferrous form.

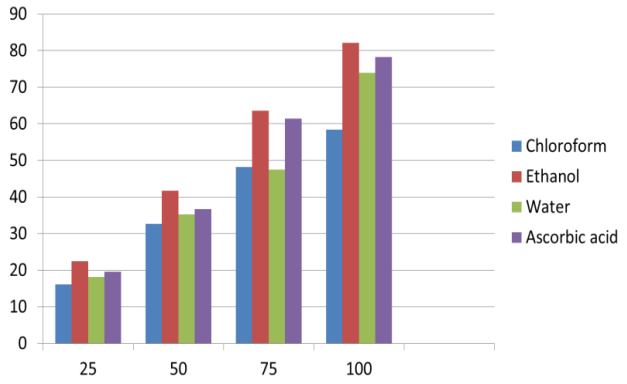
All fractions of the I. suffruticosum extract showed marked reducing activity with the water residue exhibiting the most activity. (Graphs 2, 4)



Graph 4: Comparison of FRAP activity of different I. suffruticosum extract fractions at differing concentrations

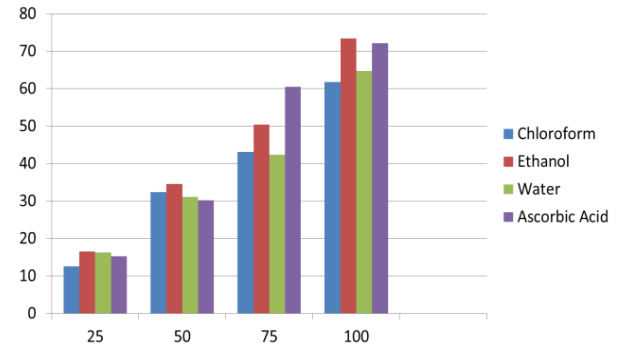
Superoxide (SO) Scavenging Activity

Demonstrable SO scavenging activity was recorded from different fractions of I. suffruticosum extract (Graph 5). The efficacy of the different extracts was maximum for ascorbic acid and ethanolic extracts followed by water residue and Chloroform. (Graph 2)



Graph 5: Comparison of SO scavenging activity of different I. suffruticosum extract fractions at differing concentrations

Hydrogen Peroxide Scavenging Activity



Graph 6: Comparison of H_2O_2 scavenging activity of different I. suffruticosum extract fractions at differing concentrations

Total peroxide scavenging activity was highest in the ethanol fraction (Graph 2) as seen by IC_{50} data. All other fractions showed marked hydrogen peroxide scavenging activity (Graph 6).

Scavenging and Reducing Property

Analysis of the scavenging and reducing properties of I. suffruticosum extract by interpretation of IC_{50} values, shows that efficacy was maximum in DPPH assay and for ethanolic extracts. (Graph 2)

Oxygen, the vital element that determines cellular viability turns detrimental to cellular existence, when the level of by-products like ROS increases in the cellular milieu.[28] Among the varied ROS, superoxide (O2-•) anion, hydrogen peroxide (H_2O_2), peroxyl (ROO-) radical and the very reactive hydroxyl (OH-•) radical exhibits greater impact on reproductive cellular functions. This accumulation of ROS leads on to OS resulting in reduced fertility potential by means of dysfunctional metabolism, morphology, motility of the spermatozoan.[29]

It has been proposed that there are three different sources for the generation of ROS in reproductive cells leading on to their dysfunction. They are Leukocytes[30], immature and morphologically abnormal sperms[31], and life style factors like smoking, alcoholism and pollutants.[32] This scenario of OS induced infertility in males, triggered a lot of research for effective antioxidants that can counter the effects of ROS.

Plants are a significant source of natural antioxidants and this has resulted in a spurt of research work to identify indigenous and herbal based antioxidants.[33,34] The plant Ionidium suffruticosum has been recommended in traditional medicine for treating a multitude of diseases, among which male infertility is a current significant global condition.[19-22] Now a days, there is increased use of traditional medicinal herbs that lacks scientifically proven efficacy. This necessitates inquiry and analysis about the phytochemical and antioxidant property of these medicinal herbs.[35,36]

Divergent results exist regarding the effective extraction solvent for the plant Ionidium suffruticosum. Literature reports claim that among commonly used ethyl acetate, methanol, ascorbic and aqueous extracts, the methanol extract was comparatively effective.[37,38] In contrast to these previous studies, this study found that the effective extract of the whole plant that exhibited antioxidant property was ethanol solvent. Also the aqueous extract showed almost equal anti-oxidant potential.

Previous studies had shown the in vitro antioxidant property of whole plant or individual parts of the plant was tested by different extracts like DPPH scavenging assay, Iron chelating activity, phenol content analysis, ABTS scavenging assay, reducing power assay, nitric oxide scavenging assay.[37-39]

The availability of numerous methods and their inability to evaluate the total antioxidant activity and *in vitro* antioxidant screening singly or as a dual combination, necessitated employment of as many as five diverse methods in this present study.

A significant indicator of antioxidant property is the reducing capacity of the compound and this forms the principle of scavenging and reducing power assays.[40,41] While the commonly used DPPH assay has merits like stability, sensitivity, simplicity, and feasibility,[42-44] other assays measure alternate pathways of neutralizing ROS. Among the antioxidant assays in this study, DPPH scavenging and SO activity exhibited more potent activity when compared to other three assays. This put forth that scavenging pathway plays a central role in ROS neutralization as compared to nitric and ferric ion reducing pathway in exhibiting antioxidant property of this plant.

The proportion of the active constituents like tannins, flavonoids, phenols present in medicinal plants determines the intensity of antioxidant activity of the plant.[45,46] Further studies need to be done to identify and isolate the constituents which are responsible for the antioxidant property of the plant. Moreover, the possibility of mismatch between *in-vitro* antioxidant properties and *in-vivo* antioxidant potential needs to be considered for further therapeutic research.[47]

CONCLUSION

This study concludes that the effective solvent of extraction of the plant Ionidium suffructicosum is ethanol. And by the results of varying antioxidant assays, this study found that the scavenging property of the plant Ionidium suffruticosum is the main factor in determining its antioxidant effect.

This effective antioxidant potential is capable of counteracting the ROS generated at cellular level due to varied etiology.

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