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EVALUATION OF ANTIOXIDANT POTENTIAL OF THE AYURVEDIC FORMULATION I.E. ISO-OXITINE

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ABSTRACT

Iso-oxitine is a poly-herbal formulation mentioned in Ayurveda to treat aging and age-associated diseases. Being an anti-aging drug, Iso-oxitine has antioxidants and free radical scavenging activity to minimize free radical-induced damage which is a key cause of aging. The Ethanolic extract of Iso-oxitine was evaluated *in vitro* for total phenolic and tannin content, free radical scavenging activity, hydrogen peroxide scavenging activity, and reducing power. Free radical scavenging activity was measured by Hydrogen Peroxide Scavenging assay. Hydrogen Peroxide scavenging activity assays against ascorbic acid. All studies showed that Iso-oxitine possesses antioxidant activity. The results of this study suggest that the antioxidant and free radical scavenging activity of Iso-oxitine may explain its effect and justify its use as a medicine for age associated diseases.

KEYWORDS: Antioxidant, *Iso-oxitine*, Hydrogen Peroxide, reducing power.

INTRODUCTION

Medicinal plants may be defined as those plants that are commonly used in treating and preventing specific ailments and diseases and that are generally considered to be harmful to humans.[1] These plants are either “wild plant species” those growing spontaneously in self-maintaining populations in natural or semi-natural ecosystems and could exist independently of direct human actions or the contrasting “Domesticated plants species” those that have arisen through human actions such as selection or breeding and depend on management for their existence.[2]

Herbal medicines proved to be the major remedy in traditional system of medicine. They have been used extensively in medical practices since ancient times. This prompts the development in the practices of medicinal plants.

The reasons are because of their biomedical benefits as well as place in cultural beliefs in many parts of world in the development of potent therapeutic agents. During 1950-1970, approximately 100 plants based new drugs were introduced in the USA drug market including deserpidine, reseinnamine and vincristine which are derived from higher plants.

Medicinal plants have provided mankind a large variety of potent drugs to alleviate or eradicate infections and suffering from diseases in spite of advancement in synthetic drugs, some of the plant-derived drugs still retained their importance and relevance. The use of plant-based drugs all over world is increasing.[3] There have been records of advances made in the modern (synthetic) medicine there are still a large number of ailments or infection (diseases) for which suitable drugs are yet to be found. This has brought an urgent need to develop safer drugs (both for man and his environment) for the treatment of inflammatory disorders, diabetes, liver diseases, and gastrointestinal disorder. Through recent researches on herbal plants or medicine, there have been great developments in the pharmacological evaluation of various plants used in traditional systems of medicine. Consequently, plants can be described as a major source of medicines, not only as isolated active principles to be dispensed in standardized dosage form but also as crude drugs for the population.

Modern medicines and herbal medicines are complimentarily being used in areas for health care program in several developing countries such as countries in Africa, Asia and some part of Europe. Due to different outcomes on herbal plants, plant products surfaces all over the world due to the belief that many herbal medicines are known to be free from health and environmental effects. The fear of the masses in the utility of synthetic drug or modern drugs is always accompanied with its single or multiple adverse or health effects.[4,5]

MATERIALS AND METHODS

Collection of the Ayurvedic Formulation i.e. Iso-oxitine

The Iso-oxitine (Ayurvedic formulation) were collected from Dr. Basu clinic i.e. Jagat Pharma. The plant *Zingiber officinale, Allium sativum, Emblica officinalis, Centella asiatica, Calendula officinalis, Vitis vinifera* collected during the month of April 2019. The species

was identified by the local people during the time of collection and later on authentication was made by Department of Pharmacognosy, Invertis University, Bareilly, (U.P.) India.

Chemicals

All chemicals used were of analytical grade. Chemicals and reagents used for the Preparation of buffers, analytical solutions and other experimental purposes are listed in table.

Table 1: Chemicals and reagents used in experimental purpose.

S.NO.	Chemical	Supplier/ Manufacturer
1.	Hydrogen peroxide	Pioma Chemtech Inc.
2.	Sodium chloride	Lab Tech
3.	Potassium chloride	Lab Tech
4.	Sodium hydrogen phosphate	Anex Chem Private Limited
5.	Potassium dihydrogen phosphate	Alpha Chemika
6.	Distilled water	Fusion Biotech

Physical evaluation

The physical values like total ash, acid insoluble ash, water-soluble ash, alcohol soluble extractive and water-soluble extractives were determined.[6-17]

(i) Ash values

Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. The ash content of a crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration.

Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information relative to its adulteration with inorganic matter. The total ash of a crude drug reflects the care taken in its preparation. The acid insoluble ash is a part of the total ash which is insoluble in dilute hydrochloric acid. A higher limit of acid insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some analysts favour mixing of sulphuric acid with the powdered crude drug before ashing and this sulphated ash value is normally less fusible than ordinary ash.

(a) Determination of total Ash value

Accurately weighed about 3 gms of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

(b) Determination of acid insoluble ash value

The ash obtained as directed under total ash was boiled with 25 ml of 2N HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

(c) Determination of water soluble ash value

The total ash obtained was boiled with 25 ml. of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 degree Celsius. The weight of insoluble matter was subtracted from the weight of total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

(ii) Loss on drying

Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. About 1.5 gm. of powdered drug was weighed accurately in a tared porcelain dish which was previously dried at 105^0C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

(iii) Extractive values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

(a) Determination of alcohol soluble extractive value

5gms of the air-dried coarse powder of the plant material was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against

loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air dried drug.

(b) Determination of water soluble extractive value

Weigh accurately the 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Preparation of extracts

1 Kilogram of powdered drug was packed in soxhlet apparatus and extracted with different polarity of solvent.

(a) Petroleum ether extract: 1 Kilogram of powdered drug was packed in soxhlet apparatus and extracted with petroleum ether (60-80°C) until the extraction was completed which was confirmed by the colour of the siphoned liquid. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure.

(b) Ethanol extract: The marc left after petroleum ether extraction was dried in hot air-oven below 50°C and packed well in soxhlet apparatus and extracted with ethanol (90%) until the completion of the extraction. The extract was filtered while hot, and the solvents were removed by distillation and the last traces of solvent being removed under reduced pressure. The extracts were weighed and their percentage value was recorded and also the physical appearance and color was evaluated and recorded and thereafter, were stored in refrigerator for further experimental work.

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was evaluated and recorded and thereafter, were stored in refrigerator for further experimental work.

Chemical evaluation

Qualitative chemical tests were performed to determine the presence of alkaloids, Carbohydrates, cardiac glycosides, polyphenols, saponins, tannins and terpenoids.[18,19,20]

1. Test for alkaloids

- **Dragendroff's test:** To 1 ml of the extract, add 1 ml of Dragendroff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.
- **Mayer's test:** To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream colored precipitate indicates the presence of alkaloids.
- **Hager's test:** To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow colored precipitate indicates the presence of alkaloids.
- **Wagner's test:** To 1 ml of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2. Test for saponins

- **Foam test:** Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

3. Test for Glycosides

- **Legal test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.
- **Baljet test:** To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.
- **Keller-Killiani test:** 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with 5ml of chloroform. The chloroform layer is separated in a porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric

chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

- **Borntrager's test:** Add a few ml of dilute Sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinone glycosides.

4. Test for carbohydrates and sugars

- **Fehling's test:** To 1ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.
- **Benedicts test:** To 5ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

5. Test for tannins and phenolic compounds

- **Ferric chloride test**: To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

6. Test for flavonoids

- **Shinoda's test:** The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

7. Test for steroids

- **Libermann-Burchard test:** 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.
- **Salkowski test:** Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

8. Test for triterpenoids

- **Noller's test:** Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, formation of pink colour indicates the presence of triterpenoids.

Anti-oxidant Activity

In –Vitro Model for evaluation of Antioxidant activity

Hydrogen Peroxide Scavenging Assay

A solution of Hydrogen Peroxide (43mM) is prepared in phosphate buffer (1M pH7.4). Different concentration of Ayurvedic formulation i.e. Iso-oxitine capsules (2-10mg/ml) was added to Hydrogen Peroxide solution (0.6ml, 43m M). Absorbance of Hydrogen Peroxide at 230nm was determined after 10 minute against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard. The free radical scavenging was determined by evaluating percentage inhibition.

The percentage of Hydrogen Peroxide scavenging is calculated as follows:

$$\% \text{ scavenged } (H_2O_2) = [(A_i - A_t) / A_i] .100$$

Where A_i is the absorbance of control and A_t is the absorbance of test.

RESULT

Morphological evaluation:

i) Colour : Yellow- Brownish

ii) Taste : Tasteless

iii) Odour : Odourless

Microscopological evaluation

The microscopic studies; shows cuticle, epidermis, cortex, endodermis and scattered vascular bundles. Epidermis consists of narrow thin walled continuous single layered with rectangular cells, surrounded by cuticle. The cortex region have parenchymatous and homogenous cell having large prismatic crystals of calcium oxalate and vascular bundles, abundant starch grains, vessels with pigmented cells; thin- walled cork cells, abundant parenchymatous cortical cells filled with starch; and some oleoresin- bearing cortical cells.

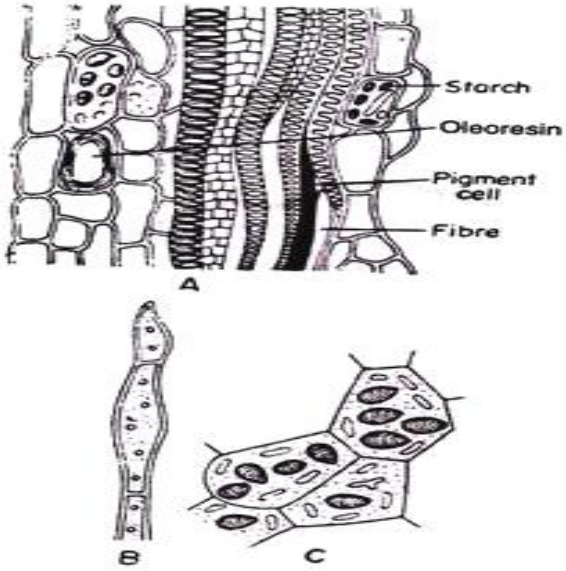


Fig. 1.6 : (A) L.S. of the rhizome of Z. officinale (B) Isolated fibre showing segmentation (C) Parenchymatous cells containing starch grains

Physical evaluation

Table 2

S. No.	Experimental Studies	Observations
1.	Total Ash Value	11.25%
2.	Water Soluble Ash Value	9.25%
3.	Water Soluble Extractive Value	68.75%
4.	Ethanol Soluble Extractive Value	43.21%

Chemical evaluation

Chemical analysis of Iso-oxitine capsules revealed the presence of alkaloid, glycoside, sterols, phenols, tannins, flavonoids, terpenes, saponins, flavonoids, tannins, carbohydrates, proteins, phenolic compounds, and Phytosterol.

Table 3

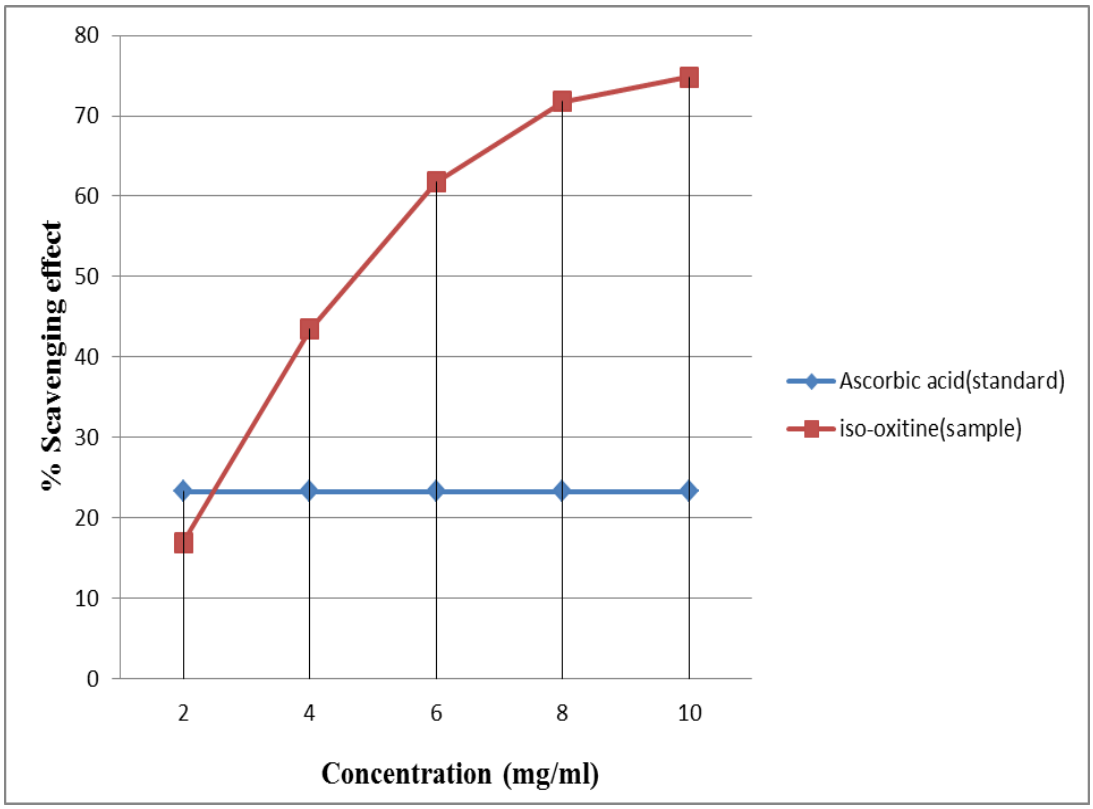
S.NO	CONSTITUENT	TEST	FORMULATION
1	Alkaloids	Mayer's Test Dragendroff's Test Hager's Test Wagner's Test	+ve +ve -ve +ve
2	Carbohydrates	Fehling's Test Benedict's Test Barfoed's Test	-ve +ve +ve
3	Proteins	Biuret's test Millon's Test	+ve -ve
4	Saponin	Foam test	-ve

5	Phenols	Ferric chloride test	+ve
6	Tannins	Bromine water test	+ve
7	Flavonoids	Shinoda's test	+ve

Table 4: Hydrogen Peroxide Scavenging Activity of Iso-oxitine formulation.

Concentrations (mg/ml)	Wavelength (nm)	% Scavenging effect of Ascorbic acid (Standard)	% Scavenging effect of the sample (Iso-oxitine)
2	230	23.265	16.89
4	230	23.265	43.45
6	230	23.265	61.72
8	230	23.265	71.78
10	230	23.265	74.82

Antioxidant Activity



Graph 1: Hydrogen Peroxide Scavenging Activity of Iso-oxitine formulation.

CONCLUSION

The results of in-vitro antioxidant tests suggests that the iso-oxitine capsules possess strong free radical scavenging activity that is analogous to a well in own standard anti-oxidant ascorbic acid, which could exert beneficial action against pathological alterations. Their broad range effects in biological systems have drawn the attention of many experimental works. Antioxidants used in food products, either natural or synthetic, can interact among

themselves and results in synergistic, additive, and antagonistic interactions. This indicates that the drug is significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

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