ABSTRACT
Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Antioxidants are substances which prevent oxidation from free radicals and prolong the life of oxidizable materials. Consumers prefer natural agents over the synthetic ones due to their effective and eco-friendly nature. Plants including herbs and spices have many phytochemicals which are potential source of natural antioxidants eg. Phenolic compounds and flavonoids. The objective of present work was to study the antioxidant effect of tamarind seed extract. Seeds of Tamarind –tamarindus indica linn belonging to the family leguminoseae were pulverized and extracted by soxhlation using ethanol as a solvent. Various phytochemical tests of extract was performed which showed presence of flavonoids and phenolic compounds. Peroxide value and Kreis test was performed to detect the antioxidant property of extract. From the results it was concluded that tamarind seeds possesses high content of antioxidants as proved by Kreis test and Peroxide value.

Keywords: Antioxidant, Tamarind, Eco-friendly, Peroxide value, Natural

INTRODUCTION:
Nature has been a source of medicinal plants for thousands of years. Since long, herbs and spices were used in pharmaceutical and cosmetic field as a source of new or complimentary benefits. Oxidation is a chemical reaction that transfer the electrons from the substance to an oxidizing agent. Oxidation reaction can produce free radicals, which starts chain reaction that damage cells. Antioxidants may terminate the chain reaction that damage cell either by removing radicals intermediate or by inhibiting other oxidation reaction by being oxidized themselves.(1)

In general there are two basic categories of Antioxidants natural and synthetic. Synthetic antioxidants are cheaper and are required in lesser amount than natural antioxidants. But now –a-days people are more aware and conscious about natural ingredients rather than synthetics.

Some of the synthetic antioxidants are Butylated hydroxyl anisole (BHA) and Butylated hydroxyl toluene (BHT) are carcinogenic so extensive use of such materials represent potential health risk. (2)

Natural antioxidants can be derived from plant source. Plant products and extracts have many phytochemicals e.g. polyphenols, flavonoids, tannins, alkaloids, terpenes and phenolic acids, which are potential source of natural antioxidants. (3,4)

Tamarind seed extract was used in the present study as a natural antioxidant. Tamarind seed (Tamarindus indica linn) is traditionally a well known herb for its therapeutic property. It is a seasonal herb belonging to family Leguminoseae and widely grown in India. Tamarind seed is an excellent source of phenolic compounds and flavonoids and exhibit good antioxidant activity. Polyphenols are the most significant compounds responsible for antioxidant properties of plant raw material. The antioxidant activity of polyphenols is mainly due to their redox properties.(5). Hence tamarind seed extract can prove to be a beneficial antioxidant.

MATERIAL AND METHODS:
The dried seeds of Tamarindus indica linn were procured from local supplier Nagpur and was further taxonomically identified and authenticated by Department of Botany, RTM Nagpur University.

I ) Extraction of Active from tamarind seed:
1) Tamarind seeds were grinded in small particle size
2) It was packed in the soxhlet apparatus and was extracted with 95% Ethanol to complete extraction.
3) The ethanol was evaporated and whole extract was obtained.
4) Yield was found to be 6.18%

II) Preliminary phytochemical screening of Tamarind seed extract:
1) Alkaloid Test: Dragondroff’s test : In 2ml of extract , 0.2 ml of dil.Hydrochloric acid was added. Then 1ml of dragondroff reagent was added. Orange brown precipitate was formed which indicates the presence of alkaloids.
2) Flavonoid Test: Shinoda Test: Small amount of sample was dissolved in 5ml of ethanol (95%) and treated with few drops of conc.hydrochloric acid and 0.5gm magnesium metal. Pink colour was developed indicating presence of flavonoid.
3) Tannins and Phenol test:

Ferric Chloride test: 5% w/v ferric chloride solution was prepared, few drops of this solution were added to the test solution.
Dark green colour was obtained indicating presence of tannins and phenols.

Lead acetate test: 10% w/v solution of lead acetate was prepared. Few drops of this solution were added to the test solution.
White precipitate was obtained indicating presence of tannins and phenols.

4) Carbohydrate Test:

Molishch’s test: To the test solution few drops of alcoholic alpha naphthnol and few drops of conc. sulphuric acid was added through sides of test tube.
Purple to violet colour ring appears at the junction.

5) Protein test:

Million’s test: To the test solution, 2ml of million’s reagent (mercuric nitrate in nitric acid containing traces of nitrous acid) was added.
White precipitate appears which turned red upon gentle heating.

6) Saponin Test:

To the test solution, 20 ml of water was added in a measuring cylinder. To this 1 ml of alcohol was added and shaken for 15 min.
1cm layer of foam indicated presence of saponin.

III) Evaluation of Antioxidant activity by Peroxide value:

Peroxide value was determined by Iodometric titration method.
5.0 gm of sample was weighed and placed in iodine flask, 30ml of 3:2 ratio of acetic acid and chloroform mixture was added and was swirled to dissolve. 0.5ml of saturated potassium iodide solution was added and flask was allowed to stand for 1 minute with occasional shaking. 30ml of distilled water was added slowly and titrated against standard sodium thiosulphate solution using starch solution as an indicator. Blank determination was carried out in similar manner without sample.

Formula:

\[
\text{Peroxide value} = (a-b) \times \frac{10}{W}
\]

Where,

\[a\] - volume of sodium thiosulphate in ml required for sample
\[b\] - Volume of blank sample
\[W\] - Weight of sample in gms

1) Determination of Peroxide value of tamarind seed extract:

Result: The peroxide value of tamarind seed extract was found to be 4

2) Determination of Peroxide value of synthetic antioxidant (BHT):

Result: The peroxide value of BHT was found to be 4.75

IV) Assessment of antioxidant activity by Kreis Test:

Principle: It involves reaction of the fat sample with phloroglucinal ether solution. The samples are then extracted with hydrochloric acid and a red aqueous solution is obtained, if the material is rancid and the compounds responsible for the Kreis color reaction are epoxy aldehyde or their acetals.

Table no.1 Phytochemical testing of Active:

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Phytochemicals</th>
<th>Name of Test</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendoff’s test</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoid</td>
<td>Shinoda test</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Tannin &amp; Phenol</td>
<td>Ferric chloride test and Lead acetate test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrate test</td>
<td>Molishch’s test</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Protein test</td>
<td>Million’s test</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Saponin test</td>
<td></td>
<td>+</td>
</tr>
</tbody>
</table>

Present (+), Absent (-)

Table no.2 Efficacy of antioxidant activity by Kreis Test:

<table>
<thead>
<tr>
<th>Test</th>
<th>Observation</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml of castor oil was shaken with 1 ml of conc.HCL for 1 minute in a test tube. Then 1 ml of 0.1% of phloroglucinol solution in ether was added and shaken continuously for further minute</td>
<td>Red colour was produced</td>
<td>Antioxidant activity was confirmed</td>
</tr>
<tr>
<td>1ml of sample was added to above test solution</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Result: It was found that Tamarind seed extract has shown antioxidant activity.

RESULT AND DISCUSSION

The study reveals the fact that Tamarind seed extract possess antioxidant activity. Further it was reported that the flavanoids and phenols from tamarind seed extract are responsible for their antioxidant activity. Continuous hot extraction of dried seed was carried out using soxhlet apparatus with 95% ethanol. An alcoholic extract obtained was dried and weighed. The yield was found to be 6.18%. Various phytochemical testing were carried out for tamarind seed extract. It was found that the extract passed the phytochemical tests for flavanoids, phenols and tannins as shown in Table no 1.

As per Indian Pharmacopoeia, peroxide value was performed for determination of antioxidant property of seed extract, and it was found to be 4, which was comparable with peroxide value of synthetic antioxidant (BHT) 4.75. The Kries test was performed for assessment of antioxidant property of extract. It was found that extract showed significant antioxidant activity as shown in Table no 2.

CONCLUSION

The above study proves that, the tamarind seed extract contains phenolic compounds which serve as a natural source of antioxidant and the extract of tamarind seed has antioxidant property as proved by Peroxide value and Kreis test.

Further it was compared with standard synthetic antioxidant Butylated hydroxyl toluene (BHT) and was found to be comparable.

REFERENCES:
1) Mitsui T; New Cosmetic Science; (Cosmetic and pharmaceutical agents); Elsevier Publication; 1st edition, 1997; pg no 3
2) Tzu-Feng Tsai, Maw- Rong Lee; Determination of antioxidants and preservation in cosmetics by SPME combined with GCMS in Chromatographi ; March no.5/6; 2008; pg no 67
5) The Wealth of India – A Dictionary of Indian raw material, J-Q; Published by Publication and Information Directorate, Government of India; Vol 4; ph no. 274
6) Buck Up and Samappito; Research journal of medicinal plants; 5 (6); 2011; Pg no. 695-705
7) Y.Sudjaroen,M.Raubner,G.Wurtele,W.E Hull, G.Erben,B.Spieghalder; Isolation and structure elucidation of phenolic antioxidants from Tamarind (Tamarindus Indica Linn) seeds and pericarp; journal of food and chemical toxicology; Volume 43, Issue 11; November 2005; pg no. 1673-1682
8) Kokate C.K, Purohit A.P, Gokhale S.B; Pharmacognosy; Nirali Publication; 34th edition ; September 2006; pg no 593-597
9) Indian Pharmacopoeia; Government of India and Ministry of health and family welfare; Published by the Indian Pharmacopoeia commission Delhi; Volume 2; 1996; Pg no A-52
10) Wilkinson J.B, Moore.R.J; Harry’s Cosmeticology; Chemical publishing company. In; Seventh Edition; 1982; pg no 719