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In Vitro Methods Evaluation And Comparison Antioxidant Activity Of Ethanol Extract Of Medicinal Plants Trigonella Foenum, Acacia Sengeal, Acacia Nilotica, And Pimpinella Anisum.

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ABSTRACT: As a result of ethanol extraction of *Trigonella foenum*, *Acacia Senegal, Acacia nilotica*, and *Pimpinella Anisum* by maceration method using ethanol 80%. Antioxidant activity was determined by DPPH-1,1-diphenyl-2-picrylhdrazyl(α, α-diphenyl-β-picrylhydrazyl), (TBA) Thiobarbituric.Percentage inhibition and IC50 value were calculated using ascorbic acid as a standard curve. The linearity was obtained between 15 to 70 µg/ml concentration for ascorbic acid (R2 =0.9785). *Pimpinella anisum* was tested for antioxidant activity by hydrogen peroxide scavenging assay at 230 nm and showed less activity.

KEYWORDS: Trigonella foenum, acacia Senegal, acacia nilotica, Pimpinella anisum, DPPH, TPA, H_2O_2.

I. INTRODUCTION.

Numerous studies have highlighted the nutritional and health benefits of antioxidants and their inverse correlation with free radicals over the past two and a half decades. Several chronic diseases, such as atherosclerosis, diabetes, cancer, neurodegenerative disorders, cardiovascular disorder, and others are clearly linked to free radicals or reactive species generated by oxidative stress[1]. Oxidative stress is a cellular phenomenon that occurs because physiological antioxidants are outnumbered by oxidants (free radicals or reactive species) by a wide margin[2]. The antioxidants are said to have been overwhelmed by the free radicals. this occurs when the production of free radicals exceeds the level that the body's natural antioxidants defense mechanism can cope with; as a result, a cellular oxidative environment is created that causes DNA, proteins, and lipid to be oxidized[3], resulting in a wide range of diseases. in maintaining health and preventing age-related disease, the interaction between free radicals, antioxidants, and disease is vital[4]. The input of exogenous antioxidants to humane wellness has also been publicized[5]. In fact, it has been suggested that reduced exposure to free radicals and increased intake of antioxidant-rich foods or antioxidant supplements will enhance the body's potential to minimize the risk of free radical-related health problems[6]. Antioxidants such as polyphenols, ascorbic acid, vitamin A, thioredoxin, glutathione, melatonin, β-carotenoids, coenzyme Q, as well as antioxidants enzymes including catalase[7], glutathione peroxidase, glutathione reductases, superoxide dismutase, and glutathione-S-transferase, have been widely investigated for the prevention and treatment of diseases resulting from oxidative damage[8].

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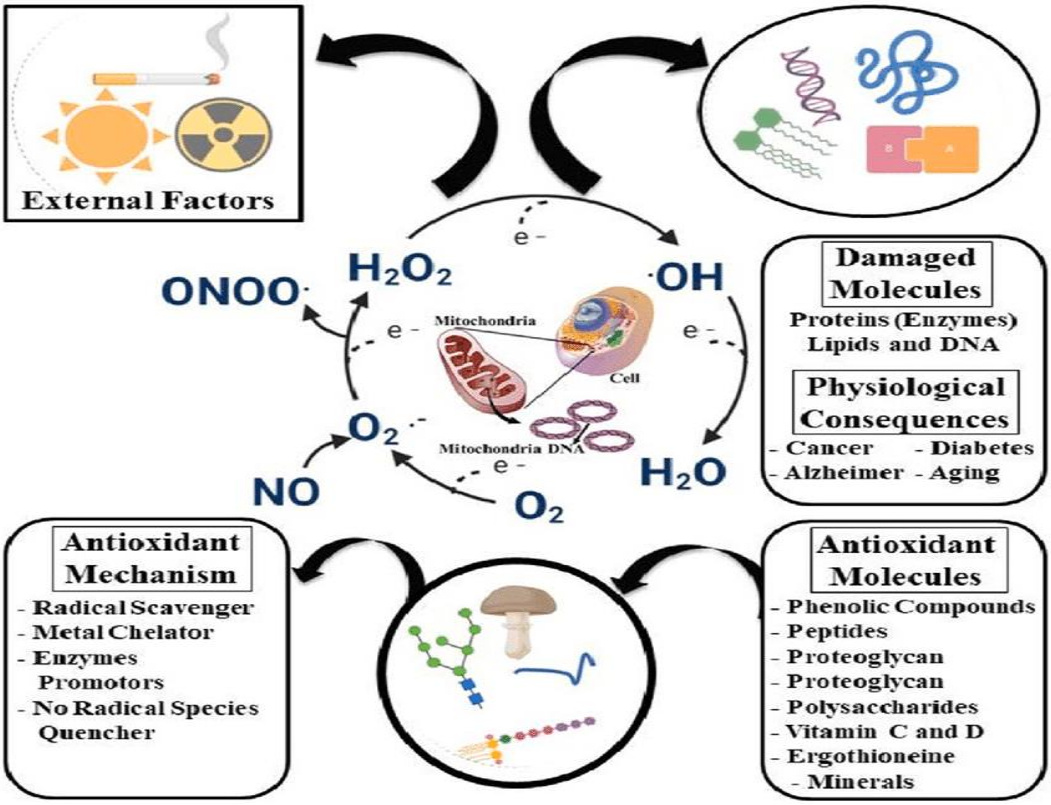

Figure:1 Mechanism of antioxidants

II. Materials And Methods:

Chemicals

DPPH, hydrogen peroxide, and Thiobarbituric acid were purchased from Everon Life Sciences-Delhi. Reference Number (125INV-2023). Plant materials were purchased from FNA Botanicals reference Number (120-DP) -Delhi.

Extraction by maceration method[9]**.**

Powdered plant material (*Trigonella foenum, acacia Senegal, acacia nilotica, Pimpinella anisum)* are soaked in ethanol 80% for 2 days with constant shaking. The extract was filtered and dried.

Figure: 2 Plant extraction

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In vitro, antioxidants study

a-DPPH scavenging activity [10]

1g of plant extract *(T-foenum,A-Senegal,A -nilotica, P-anisum*) where dissolved in 80% methanol. Concentration 01,0.2,0.4,0.6 and 0.8µg/ml of the above extract was transferred in a 10ml conical flask. Dissolve 2mg of DPPH (0.5mM) in 100 ml of 98% methanol solution. Keep the DPPH solution in a dark place. After 30 min, add 0.1 ml of DPPH to each conical flask containing plant extract. Make the volume up to 10 ml with D/W. DPPH is characterized as a stable free radical by virtue of the delocalization of spare electrons over the molecule[11], so that the molecule does not dimerize, the absorbance is measured at 517 nm. The percentage of the DPPH radical scavenging is calculated using the equation given below.

$$\%\ Inhibation\ of\ DPPH\ radical = \frac{(Abr - Aar)}{Abr} X100$$

where A_{br} is the absorbance before reaction and Aar is the absorbance after the reaction.

b- hydrogen peroxide (H_2O_2) assay[12].

The ability of plant extract to scavenge hydrogen peroxide could be estimated according to the method of Ruch et al. A solution of hydrogen peroxide (40mM) is prepared in phosphate buffer (50mM pH 7.4). The concentration of hydrogen peroxide is determined by absorption at 230 nm using a spectrophotometer. (*T-foenum,A-Senegal,A -nilotica, P-anisum*) extract 10,20,40,60 and 80 µg/ml in distilled water is added to hydrogen peroxide and absorbance at 230 nm is determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

$$\%\ scavenged\ (\mathrm{H2O2}) = \frac{(Ai - At)}{Ai} X100$$

where Ai is the absorbance of the control and A_t is the absorbance of the test.

c-Thiobarbituric acid (TBA) method[13]

2 ml of 20 % trichloroacetic acid and 2 ml of 0.67 % of thiobarbituric acid were added to 1 ml of (*T-foenum,A-Senegal,A -nilotica, P-anisum*) sample solution. The mixture was placed in a boiling water bath for 10 min and then centrifuged after cooling at 3000 rpm for 20 min. The absorbance activity of the supernatant was measured at 552 nm and recorded after it has reached its maximum.

III. RESULTS

Table: 1 Antioxidants activity of plant extracts.

S.No	Plant	DPPH Assay	% H_2O_2 Assay	% TPA Assay
1	Trigonella foenum	0.30404	4.7	2.8
2	Acacia senegal	0.03346	15.5	1.8
3	Acacia nilotica	0.04522	1.8	0.9
4	Pimpinella anisum	0.04522	3	1.1

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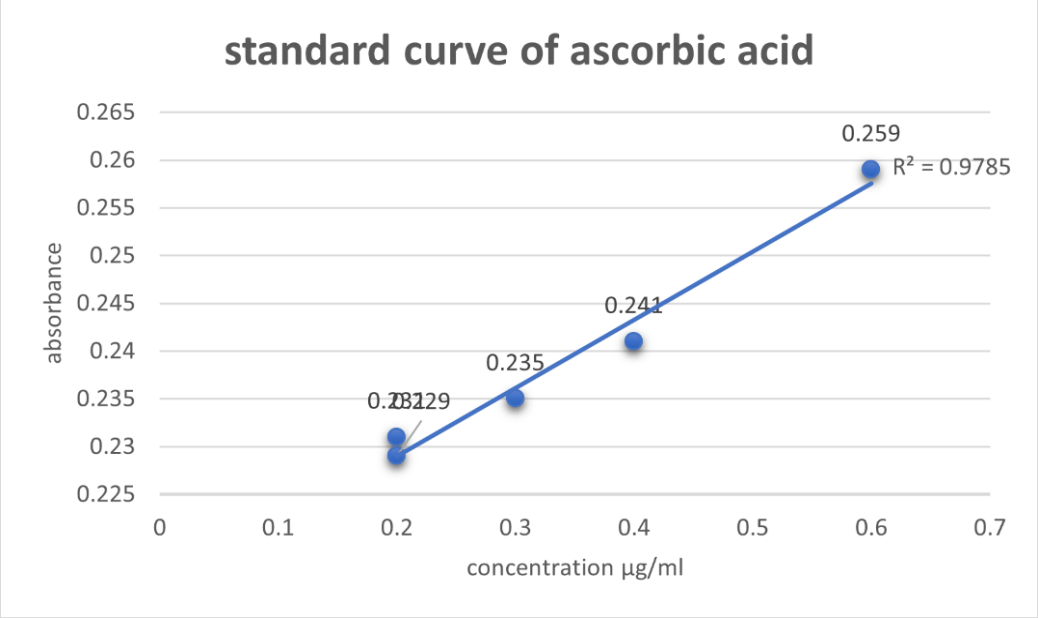

Figure:3 standard curve of scorbic acid

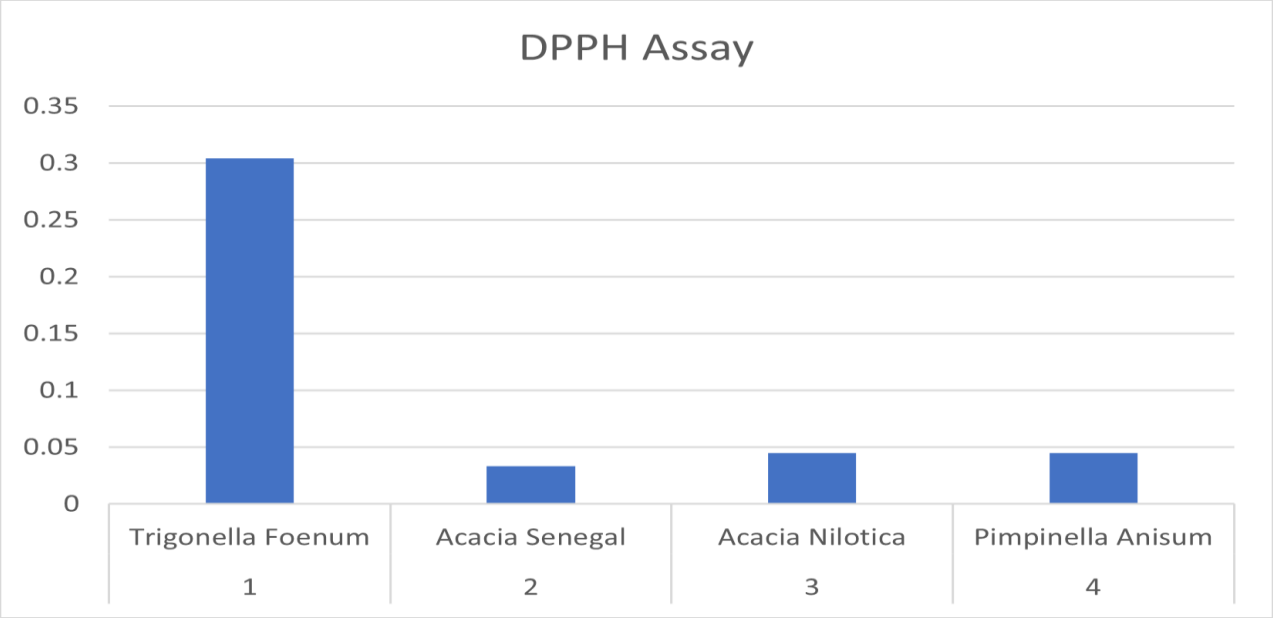

Figure:4 DPPH Assay

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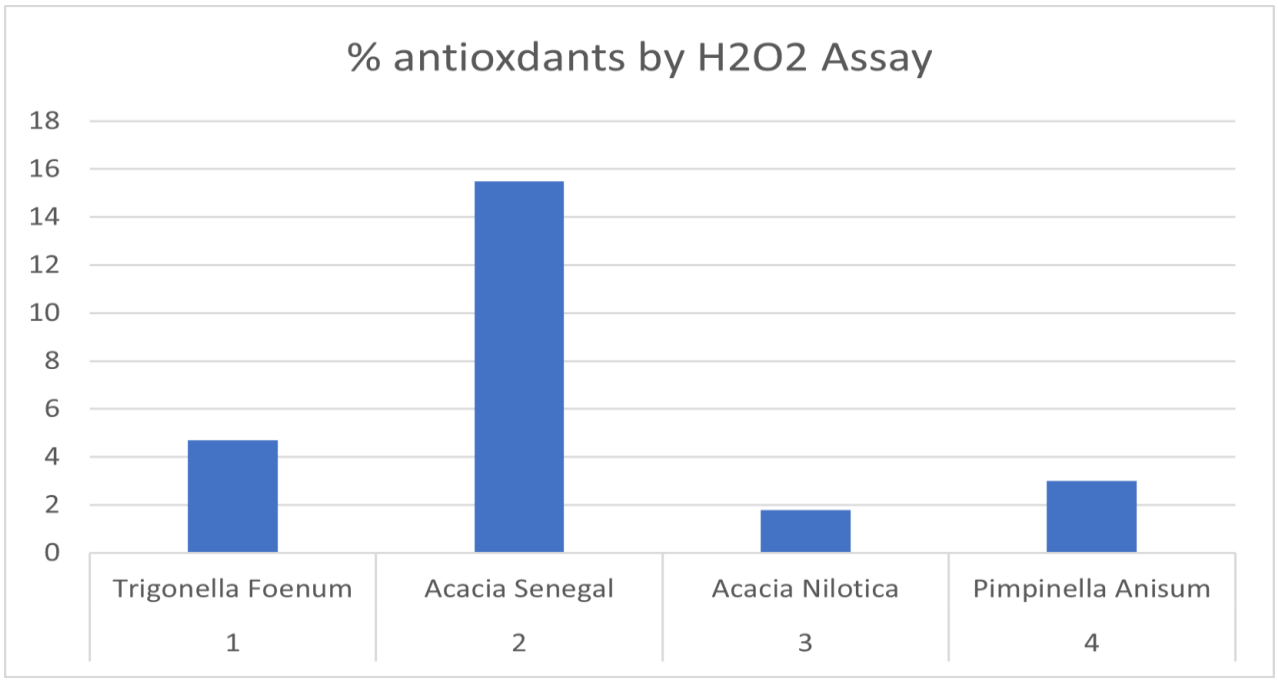

Figure:5 H_2O_2 Assay

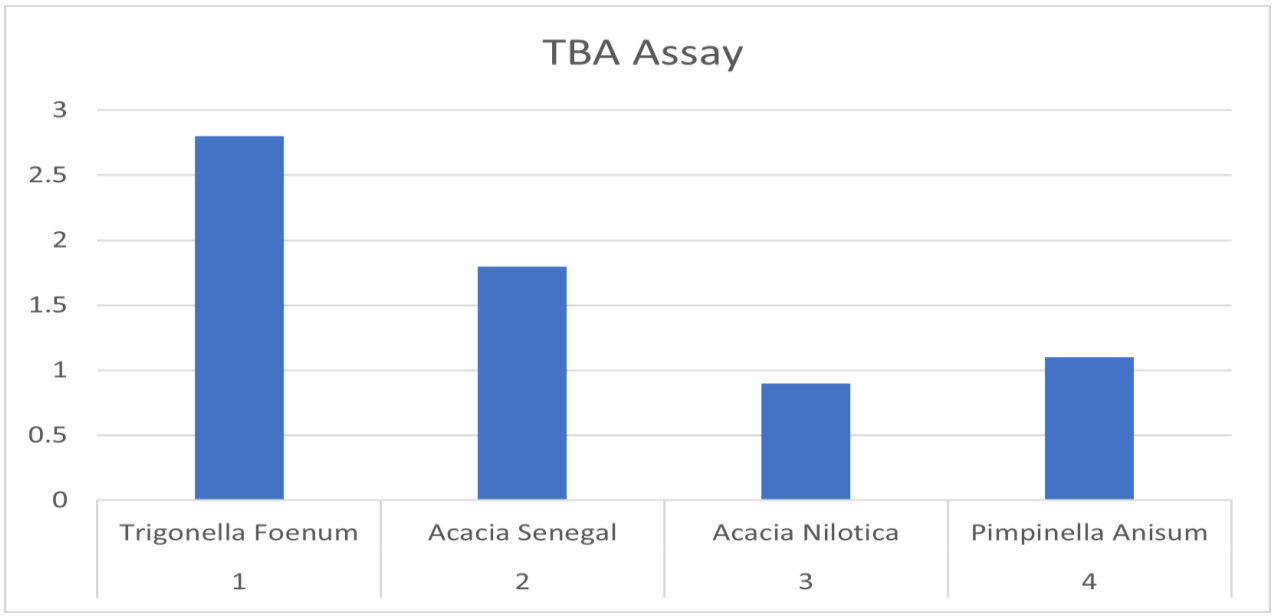

Figure:6 TBA Assay

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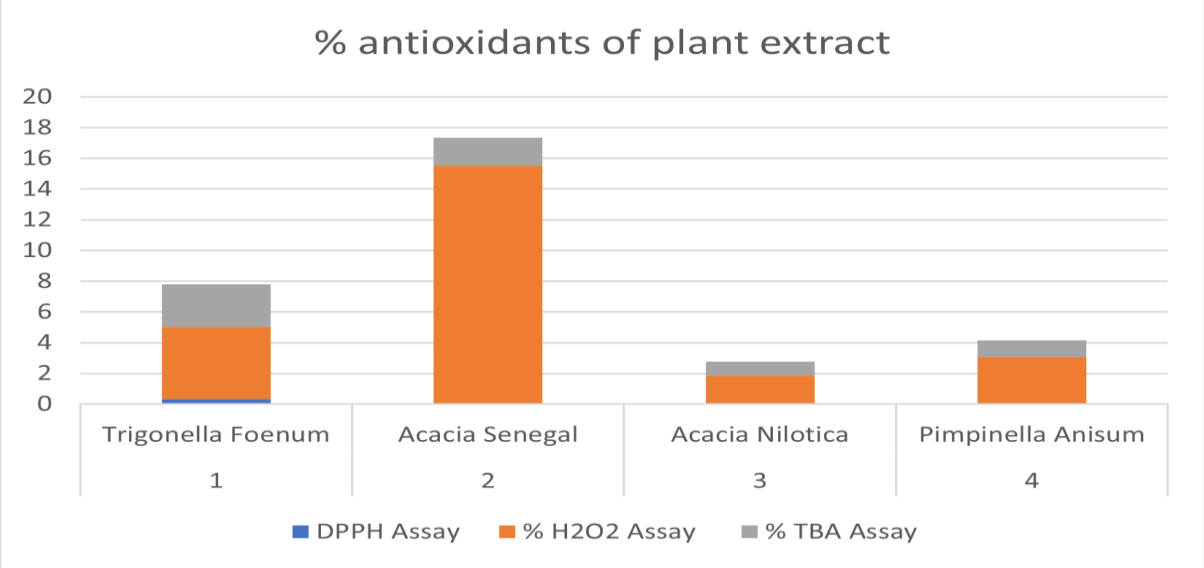

Figure:7 Percentage of antioxidants activity *among Trigonella foenum,acacia Senegal, acacia nilotica,* and *Pimpinella anisum*.

IV. DISCUSSION

Evaluation of antioxidants activity of various plant extracts *Trigonella Foenum, Acacia Senegal*, *acacia Nilotica*, and *Pimpinella anisum* using in vitro methods DPPH, H_2O_2, and TBA show percentage difference among extracts thus this difference related to antioxidants activity of plant extract in vitro method significance antioxidants could be various due method used to assist antioxidants.

V. CONCLUSION

Comparing the impacts of four plant extracts tested for antioxidant activity show the efficacy of *acacia Senegal* extract for antioxidant activity towards the Hydrogen peroxide assay method (Fig.7) so a quietly useful antioxidant for various disease curing as more antioxidant value is reported with the extract. *Trigonella foenum* and *Pimpinella anisum* show significantly less antioxidant when compared with *acacia Senegal* extract. Overall plant extract remains the best natural source of antioxidants.

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