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Comparative In-Vitro Antioxidant and Antimicrobial Potential of Some Medicinal Plants

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ABSTRACT:

In traditional medicine, herbal remedies are often used for treating burns, dermatophytes, and other infectious illnesses. Based on ethno pharmacological and taxonomic data, we tested the antibacterial activities of aqueous and ethanolic extracts of selected medicinal plants in vitro using the agar diffusion-method against selected human pathogenic bacteria. The antimicrobial properties of the leaves of five different plants were tested. Alternative medical practises have long made use of several plant species from all over the globe. There was an extraction process including water and methanol for the powdered leaves of each plant. To dry out the solvent extracts and concentrate them, a rotary flash evaporator was utilised. The dried residue's bactericidal effectiveness was measured by dissolving it in ethanol (1:10 w/v). Various bacteria, including Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis, and Yersinia enterocolitica, were employed for in vitro antibacterial screening. Methanol extracts demonstrated more diverse effects on these species than water extracts, indicating that the active components may be present in methanol extracts of all the plants we studied. Many different types of ailments, including as those affecting the immune system, nervous system, digestive tract, respiratory system, skin, and even a high temperature, have been proven to respond well to traditional herbal treatment.

Keywords: Antimicrobial, Antioxidant, Medicinal plants, Human pathogens

1. Introduction

It is thought that almost all wild animals contract parasites, which are frequently many and diverse, at some point in their lives. Without giving the host any direct benefits, parasites obtain their resources from another living thing. However, they frequently have no noticeable impact on wild hosts. Early pioneers in the study of evolutionary ecology ignored parasitism as a factor that can influence an animal's ecology and life-history decisions due to this fact and the prevalence of parasites in ecosystems. In fact, David Lack believed that parasite infection had little bearing on the host. (**Afzal M *et al* 2012)**

Numerous parasites have developed during the course of human evolution and use humans as a host. In most cases, a parasite wouldn't kill its host (at least not right away) because doing so would cause that particular parasite to reach an evolutionary dead end. However, the majority of parasites are uncomfortable for us or harm our health. The illnesses caused by parasites, like trypanosomiasis, malaria and Chagas, however, might be fatal if patients are not given enough therapeutic care. Due to the unfavorable hygienic conditions that humans are prone to, parasites are able to spread among people. (**Aho JM *et al.*2016**)

Antioxidant Agents

Concept of Oxidative Stress

Oxidative stress the term used to describe the condition of oxidative damage resulting when the critical balance between free radical generation and antioxidant defenses is unfavorable (Rock et al., 1996). Oxidative stress, arising as a result of an imbalance between free radical production and antioxidant defenses, is associated with damage to a wide range of molecular species including lipids, proteins and nucleic acids (Me Cord, 2000). Short-term oxidative stress may occur in tissues injured by trauma, infection, heat injury, hypertoxia, toxins and excessive exercise. These injured tissues produce increased radical generating enzymes. (e.g. xanthine oxidase, lipogenase, cyclooxygenase) activation of phagocytes, release of free iron, copper ions, or a disruption of the electron transport chains of oxidative phosphorylation, producing excess reactive oxygen species. The initiation, promotion and progression of cancer, as well as the side effects of radiation and chemotherapy, have been linked to the imbalance between reactive oxygen species and the antioxidant defense system. Reactive oxygen species have been implicated in the induction and complications of diabetes mellitus, age related eye disease, and neuro degenerative diseases such as Parkinson’s disease.

Mechanism of Action of Antioxidant: Two principal mechanisms of action have been proposed for antioxidants (Rice-Evans and Diplock, 1993). The first is a chain - breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of reactive oxygen species/reactive nitrogen species initiators by quenching chain - initiating catalyst (secondary antioxidants). Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, coantioxidants or by gene expression regulation.

Plants as Source of Antioxidants

Synthetic and natural food antioxidants are used routinely in foods and medicine especially those containing oils and fats to protect the food against oxidation. There are a number of synthetic phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) being prominent examples. These compounds have been widely uses as antioxidants in food industry, cosmetics and therapeutic industry. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated

temperature, strict legislation on the use of synthetic food additives and carcinogenic nature of some synthetic antioxidants e.g. BHT, BHA and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants (Papas, 1999). In view of increasing risk factors of human to various deadly diseases, there has been a global trend towards the use of natural substance present in medicinal plants and dietaiy plants as therapeutic antioxidants. It has been reported that there is an inverse relationship between the dietary intake of antioxidants rich food and medicinal plants and incidence of human diseases. The use of natural antioxidants in food, cosmetic and therapeutic industry would be promising alternative for synthetic antioxidants in respect of low cost, high compatibility with dietary intake and absence harmful effects inside the human body. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers (Brown and RiceEvans, 1998) Attempts have been made to study the antioxidant potential o f a wide variety of vegetables like potato, spinach, tomatoes and legumes (Furuta et a/., 1997). There are several reports showing antioxidant potential of fruits (Wang et a l, 1996). Strong antioxidants activities have been found in berries, cherries, citrus prunes and olives. Green and black teas have been extensively studied in the recent past for antioxidant properties since they contain up to 30% of the dry weight as phenolic compounds.

Collection and Authentication

The plant was harvested in August and September in the Tirupati (Hyderabad) area of India and was certified by the department of botany.

Antioxidant Activity

5.3.1 In-Vitro Free Radical Scavenging Activity:

Due to the delocalized nature of the spare electron over the whole molecule, the free radical is stable and does not dimerize. The violet hue is gone from a DPPH solution when it is reacting with a molecule that may give hydrogen.

Procedure

The spectrophotometer measured the DPPH scavenging activity. 75 l of the stock solution were produced to give an initial absorbance when combined with 3 ml of methanol. After 15 minutes, a decrease in absorbance was seen when sample extract at various concentrations (100-500 g/ml) was present.

Protocol for DPPH Free Radical Scavenging Activity

The test sample's stock solution was made by dissolving 100 mg of the extract in 100 ml of methanol to create a 1000 g/ml solution.

(a) The stock solution was diluted to provide test solutions at concentrations of 100, 200, 300, 400, and 500 g/ml.

(b) Solution of DPPH was made by dissolving 15 mg of DPPH in 10 ml of methanol. Aluminum foil was used to block the light from reaching the completed product.

Evaluation of DPPH Radical Scavenging Activity:

1. After diluting 75 µl of the DPPH solution with methanol to make 3 ml, the absorbance was measured right away at 517 nm for the control reading..
2. A series of volumetric flasks were filled with 50 µl of the test material at various concentrations and 75 µl of DPPH. With the help of methanol, the final volume of each was changed to 3 ml. Three test samples were collected, and they were all handled similarly. The mean was ultimately chosen.
3. For each concentration, absorbance at zero time was measured.

4. After 15 minutes, at 517 nm, DPPH absorbance finally decreased with the sample at a varied concentration.

Calculation of % Reduction = Control Absorbance − Test absorbance/ Control Absorbance × 100

2. Results

Antioxidant Activity

6.11.1 DPPH Radical Scavenging Activity

The HAE of *Embelia ribes and Zizyphus xylopyrus* indicated promising free radical searching impact DPPH in a focus subordinate way. Ascorbic acid was utilized as the reference is appeared in the figure 6.9. The diminishment of DPPH by the HAE was high and the rummaging capacity expanded with expanding focus when contrasted with the standard. The outcomes were communicated as the dosage required for half restraint by HAE (IC50) and the outcomes are portrayed in Table. 6.14

The IC_{50} value for the Hydroalcoholic extract (HAE) of *Embelia ribes and Zizyphus xylopyrus* is 43.45±1.05 µg/ml and ascorbic acid was utilized as the reference standard for antiinflammation prevention action, and the IC_{50} value is 26.08±1.90µg/ml. Table 6.12 IC_{50} values of standard and HAE in DPPH radical scavenging activity.

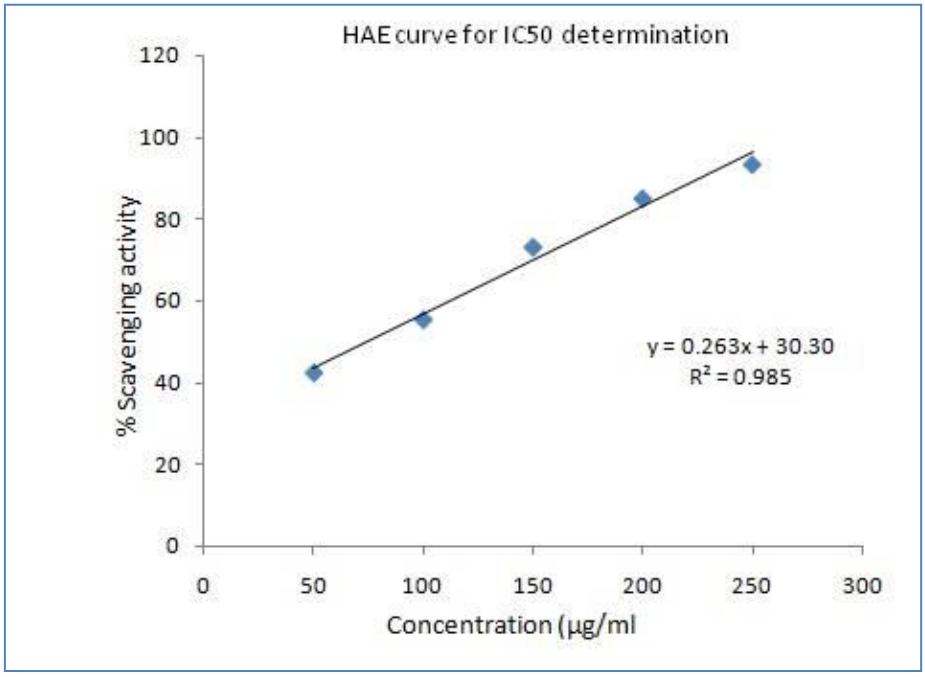

Fig. 6.10 Standard Curve of Ascorbic Acid

Table 6.12 DPPH Radical Scavenging Activity

S. No.	Standard/Extract	IC_{50} Value*
1	**Ascorbic acid**	26.08±1.90
2	**HAE**	43.45± 1.05

*** n=3, Mean ± SD**

Table 6.13 Percentage Inhibition of Standard in DPPH Radical Scavenging Activity

S. No.	Concentration (Ascorbic acid)	% Inhibition
1	1 µg/ml	0.43
2	2 µg/ml	2.57
3	3 µg/ml	4.76
4	4 µg/ml	6.8
5	5 µg/ml	8.16

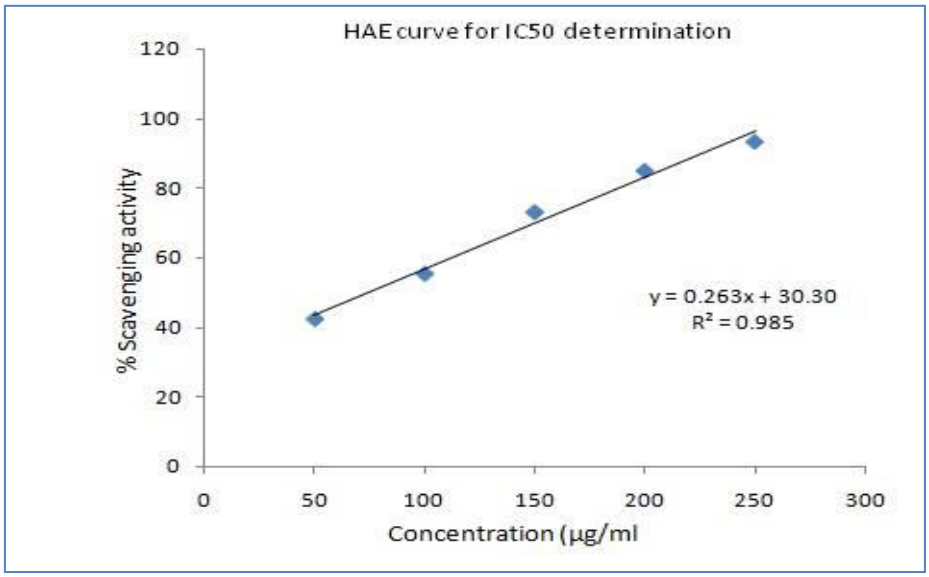

Fig. 6.12 Absorbance of *Embelia ribes* (Hydro- Alcoholic Extract)

Table 6.13 Percentage Inhibition of HAE of *Embelia ribes* in DPPH Radical Scavenging Activity

S. No.	Concentration (HAE)	% Inhibition
1	50 µg/ml	42.34
2	100µg/ml	55.39
3	150 µg/ml	73.17
4	200 µg/ml	85.08
5	250 µg/ml	93.47

3. Summary and Conclusion

Various secondary and main phytoconstituents were found in the extract during early phytochemical studies. Tannins, polyphenols, phytosterols, and flavonoids are common in most plants and are gaining popularity for their antioxidant properties. They are alsefficient hydrogen donors. Numerous studies have shown that flavonoids have strong antioxidant properties that can scavenge superoxide anions and lipid peroxy radicals. Flavonoids' strong antioxidant potential has been linked to a number of their pharmacological qualities, including their anti-inflammatory, antibacterial, hepatoprotective, anti-ulcer, and anti-allergic effects.

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