# COMPARATIVE IN-VITRO ANTIOXIDANT POTENTIAL OF SOME HERBAL PLANTS

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## ABSTRACT
The use of plants in treatment of burns, dermatophytes and infectious diseases is common in traditional medicine. Based on ethno pharmacological and taxonomic information, antibacterial activities of aqueous and ethanolic extracts of some medicinal plants were determined by in vitro by agar diffusion-method against some human pathogenic bacteria. The leaves of five different plants, belonging to the different family and which have some ethnomedicinal applications were studied for antibacterial activity. Powdered leaf materials of all selected plants were extracted with aqueous and methanol. The solvent extracts were evaporated to dryness using rotary flash evaporator. Dry residue was dissolved in ethanol (1:10 w/v) and tested for antibacterial activity. The antibacterial screening of aqueous and methanol extract carried out in vitro on the following bacteria viz., Bacillus cereus, Bacillus megaterium, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus, Streptococcus faecalis and Yersinia enterocolitica. It has been showed that the methanol extracts had wider range of activity on these organisms than the aqueous extracts, which indicates that the methanol extracts of all selected plants may contain the active components. This study supports, the traditional medicines (herbal extracts) to cure many diseases like diarrhea, intestinal tract, throat, ear infections, fever and skin diseases.

**Key words: Antioxidant Medicinal plants Human pathogens**

## 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**)

### 1.1 Host- parasite; definitions & nomenclature

The majority of species rely on scarce natural resources like nutrient-rich water, adequate mating grounds, or other elements for their survival. It costs time, effort, and involves hazards like predation to gain access to these resources. The success of the organism's reproduction is significantly influenced by the expense of resource exploitation and the benefit from it. Organisms associate with different life forms while utilizing the natural resources, resulting in a variety of interactions. A partner in an association may gain (+), lose (-), or have no effect at all (0). Increased reproductive success in association as compared to living alone is the definition of benefit (+) from an affiliation. Loss (-) represents a decline in reproductive success. Theoretically, interactions between populations of two species can take six different forms depending on how the three possible outcomes are combined. Particularly intriguing and varied is the (+, -) interaction. A (+,-) link exists between predatory behavior, parasitism, and herbivory (or grazing). When the predator is the primary consumer (usually an animal) and the host or prey is the primary producer (plant), the interaction is 'herbivory'. **(Aho JM *et al.*2016)**

### 1.2 Human parasites and parasitic diseases

Important human and animal diseases are frequently brought on by parasites. The parasite, also known as its host, is a living entity that lives inside (endoparasite) or on (ectoparasite) any other organism. It receives defense and sustenance without giving anything back. As a result, the host experiences a variety of infections, illnesses, and discomforts. In other instances, the host might not exhibit any symptoms of parasite infection. The majority of tropical disorders, including trypanosomiasis, malaria, leishmaniasis, schistosomiasis, lymphatic filariasis, onchocerciasis, and helminthiases, are caused by parasitic infections. More than 1-2 billion infections, which have resulted in millions of deaths annually, are primarily caused by parasites. Through furthermore to such economically beneficial parasitic infections, a percentage of ectoparasites have negatively impacted public health, including bed bugs, lice (Pediculus capitis, Phthirus pubis), flea (Pulex irritans, Tunga penetrans)..(**Biren N, *et al* 2012)**

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|---|---|
| **Synonym**<br>Gahvara , Laksmana, Amogha, Patala, Vidanga, Tandula, , Krmighna,<br>**Vernacular names**<br>**Sanskrit**; Amodha,<br>**Hindi:** Baba-rang, Baberang,<br>**Bengali:** Bhaibirrung, Biranga,<br>**Kannada:** Amogha, |  |

***Zizyphus xylopyrus* (Retz.) Willd**

North-western China, Pakistan, and India are all home to Zizyphus xylopyrus (Retz.) Willd. A big, sprawling shrub , three-legged plant up to 4 meters tall that is equipped with spines.

| | |
|---|---|
| **Common name:**<br>**Sanskrit:** Gotika,<br>**Bengali:** Kulphal,<br>**English:** Jujab,<br>**Gujrati:** Gatbadar,<br>**Hindi:** Ghunta, Kakora,<br>**Kannada:** Yeranu,<br>**Marathi:** Bhorghoti,<br>**Tamil:** Kottai,<br>**Telugu** : Gotti, Gotiki |  |

## 1.3 PHYTO-CHEMICAL SCREENING

The concentrates of root was subjected to different subjective substance tests to decide the nearness of different phytoconstituents like alkaloids, glycosides, sugars, phenolic etc. Utilizing detailed techniques in (Khandelwal et al (2007).

**1.3.1 Alkaloids:** The weak HCL corrosive was combined with the dried concentrate, mixed firmly, and then separated. The unique test was also conducted using the filtrate.

- Mayer's test: Mayer's reagent was used to prepare the filtrates (potassium mercuric iodide). The placement of a yellow cream hasten shows how close alkaloids are..
- Wagner's test: Wagner's reagent was used to treat the filtrates. Alkaloids are present because no brown and reddish-brown precipitate occur.
- Dragendorff's test: Dragendorff's reagent was used to prepare the filtrates. Red hasten's arrangement shows how close the alkaloids are..

- Hager's test: Hager's reagent was used to treat the filtrates. The placement of yellow hasten shows how close alkaloids are..

**1.3.2 Carbohydrates**

- Molisch's test: Concentrated sulphuric corrosive was added precisely at the test tube's edges after extracts were treated with a dipsomaniac setup in the test tube. Starches are close by as evidenced by the growth of the violet ring at the crossing.
- Fehling's test: The filtrate was heated for 5–10 seconds over a water shower after adding the Fehling A and B arrangement. Next, it was killed with soluble base and fermented with moderate hydrochloric acid. Initially yellow, a block red acceleration then signaled the imminence of decreasing sugars.

**1.3.3 Glycosides**

Selection of concentrate's residual sugar content, and then hydrolyze the extract with a mineral corrosive. Decide once more what the hydrolyzed remove's total sugar content is. Glycosides are revealed by an increase in sugar concentration..

- Legal's test: The concentration was treated using $C_5H_4FeN_6Na_2O_3$ in pyridine and methanolic base. The pattern of pink and red colors indicated the proximity of cardiac glycosides.
- Killer Killani test: A small amount of dry concentrate was dissolved in two milliliters of icy acidic solution that contained one drop of ferric chloride solution. After the expansion of 1 ml of concentrated H2SO4, these remained stable for quite some time. A darker ring acquires at the nearness of cardenolides.

**1.3.4 Saponin glycosides**

- Froth test: The focus was weakened by shaking for 15 minutes in a graded barrel with 20ml of refined water on its own. Saponins can be detected by a layer of foam that is about 1 centimeter thick.

**1.3.5 Flavonoids**

- Shinoda test : Since being purged with 10ml of ethanol for 15 minutes in a water shower, specific dry concentrations were filtered .The filtrates were mixed with a tiny amount of magnesium bind and concentrated HCl. The increase of pink shading demonstrates how related flavonoids are.
- FeCl3 test: FeCl3 arrangement is placed in the sample arrangement. The change in shade from green to dark indicates the presence of flavonoids..

**1.3.6 Amino acids & Proteins**

- Million's test: Million's reagent was used to handle the concentrations. The configuration of a white particle, which turned red upon warming, demonstrates the proximity of proteins and amino acids.
- Ninhydrin test: 0.25 percent Ninhydrin reagent was added to the concentrates and bubbled for a short while. The placement of blue shading indicates the proximity of amino acids.

**1.3.7 Tannins**

- FeCl3 test: 2-3 drops of neutral $FeCl_3$ were taken to the concentration as a treatment. a remedy (5 percent ). The arrangement of dark shades of pale blue indicates the presence of tannins.
- Lead acetic acid derivation test: The 10% concentration was treated with a few drops of neutral Lead acetic acid synthesis. The quickness with which yellow appears is evidence of tannins.

### 1.3.8 Triterpenoids and Steroids

- Liebermann Burchard response; A mixture of ethanol, ethanol-chloroform-ethyl acetic acid derivation, and ethanol will be applied to the dried amounts. To the aforementioned mixture, 1 to 2 drops of a strong sulfuric corrosive must be added. The presence of steroids is indicated by the Solution's subtle green tint, whilst triterpenoids are indicated by the arrangement's faint pink color.
- Salkowaski response: After a few drops of concentrated sulfuric acid corrosive were added, chloroform was added to the dry concentrations, thoroughly agitated, and permitted to sit for a while. The presence of triterpenoids was indicated by the appearance of a yellow tint in the lower layer, whereas the presence of steroids was indicated by the appearance of red shading in the lower layer.

## 2. Quantitative Evaluation of Phyto¬Constituents

### 2.1 Determination of Total Phenolic Content

The total phenolic content of all medication concentrates was evaluated using the procedure outlined in the Ayurvedic Pharmacopeia of India. The total phenolic content per gram of concentrate is represented as a reciprocal of gallic corrosive milligrams. A methanol (1 mg/ml) stock of the concentrate will be prepared. The 25 ml volumetric flagon will be filled with 10 ml of water, 1.5 ml of the folin ciocalteau reagent, and the correct amount of concentrate from the stock arrangement. A 20 percent sodium carbonate solution and up to 25 milliliters of twice-refined water were added after the first five minutes. The blend's absorbance at 765 nm was assessed after 30 minutes. The amount of total phenolics was calculated from the adjustment bend of gallic acid using the aforementioned method and reported in conformity with the Ayurvedic Pharmacopeia of India (2008).

Total phenolic content = GAE×V×D×10–6×100/W GAE-Gallic acid equivalent (µg/ml),

V-Total volume of sample (ml),

D-Dilution factor,

W-Sample weight (gm)

**Reagents**

Sodium carbonate reagent (20%): After being broken up into 20 g of sodium carbonate and 100 ml of water, it was separated after being given a medium-term Gallic corrosive stock solution (1 mg/ml): Disintegration of 10 mg of gallic corrosive in 10 ml of purified water.

### 2.2 Determination of Flavonoids Content

For calculating the total amount of flavonoids in the concentrated plant root, the aluminum chloride method was used. Concentrate arrangements have been taken in aliquots, each of which contains 3 ml of ethanol. 2.8 ml of pure water, 0.1 ml of a potassium acetic acid derivative, and 0.1 ml of aluminum chloride (10%) were then gradually added. The test setup

was continuously shaken. After brooding for 30 minutes, absorbance at 415 nm is measured. A std. adjustment plot created at 415 nm utilizing known groupings of rutin According to Mervat and Hanan, the convergences of flavonoids in the test samples were calculated from the alignment plot and reported as mg rutin identical/g of the example. (2009).

**Reagents**

1. Aluminum chloride arrangement 10 %: 100 ml of methanol was used to dissolve 10g of aluminum chloride.
2. 1M Potassium acetic acid derivation arrangement: 9.815g $C_2H_3KO_2$ derivation was broken up in 100 ml of refined water.

Standard rutin arrangement (1mg/ml): 10 mg rutin was broken up in 10 ml methanol.

## 2.3 PHARMACOLOGICAL ACTION

### 2.3.1 Antioxidant activity

#### *2.3.1 In-vitro* free radical scavenging activity:

Due to the spare electron being delocalized across the entire molecule, the free radical is stable and does not dimerize. When a DPPH solution is combined with one of a chemical that can donate an atom of hydrogen, the reduced form is produced, and this violet color is lost.

**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) Dilution of test solution: The test samples' 100, 200, 300, 400, and 500 g/ml solutions were made from stock solution..

(b) Preparation of DPPH solution: 10 ml of methanol were used to dissolve 15 mg of DPPH. To shield it from light, the finished design was wrapped in aluminum foil.

**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

### 3.1 EXTRACTION OF PLANT MATERIAL

To determine the percentage yield, color, and uniformity of successive solvent extracts of plant roots, the results are shown in the table. 6.1

**Table 3.1 Percentage Extractive Value and Characterization of *Embelia ribes***

| S.No. | Extracts | Colour | Odour | Consistency | Yield |
|---|---|---|---|---|---|
| 1. | petroleum ether extract | Pale yellow | Characteristic | Semi-solid | 0.46±0.5 |
| 2. | chloroform extract | Pale yellow | Characteristic | Semi-solid | 0.53±0.42 |
| 3 | ethyl acetate extract | Reddish brown | Characteristic | Semi-solid | 3.43±0.50 |
| 4. | ethanolic extract. | Reddish brown | Characteristic | Semi-solid | 2.16±0.56 |

To determine the percentage yield, color, and uniformity of successive solvent extracts of plant roots, the results are shown in the table. 6.9

**Table 3.2 % Extractive Value and Characterization of *Zizyphus xylopyrus***

| Extracts | Colour | Odour | Consistency | Yield (%w/w) |
|---|---|---|---|---|
| petroleum ether extract | Pale yellow | Characteristic | Semi-solid | 0.46±0.5 |
| chloroform extract | Pale yellow | Characteristic | Semi-solid | 0.53±0.42 |
| ethyl acetate extract | Reddish brown | Characteristic | Semi-solid | 3.43±0.50COm |
| ethanolic extract | Reddish brown | Characteristic | Semi-solid | 2.16±0.56 |

### 3.2 PRELIMINARY PHYTOCHEMICAL SCREENING

Qualitative preliminary phytochemical screening of *both plant* is mentioned in Table 6.10

**Table 3.3 Preliminary phytochemical screening of *Embelia ribes***

| Class of constituents | PEE | CE | EAE | EE |
|---|---|---|---|---|
| Amino acids | - | - | - | - |
| Proteins | - | - | - | - |
| Carbohydrates | - | - | - | - |
| Steroids/Triterpenoids | + | + | - | - |
| Alkaloids | - | + | - | + |
| Saponin | - | - | + | + |

| | | | | |
|---|---|---|---|---|
| Flavonoids | - | - | + | - |
| Tannins | - | - | + | + |
| Phenolics | - | - | + | + |

**Table 3.4 Preliminary phytochemical screening of *Zizyphus xylopyrus***

| Class of constituents | PEE | CE | EAE | EE |
|---|---|---|---|---|
| Amino acids | - | - | - | - |
| Proteins | - | - | - | - |
| Carbohydrates | - | - | - | - |
| Steroids/Triterpenoids | + | + | - | - |
| Alkaloids | - | + | - | + |
| Saponin | - | - | + | + |
| Flavonoids | - | - | + | - |
| Tannins | - | - | + | + |
| Phenolics | - | - | + | + |

## ANTIOXIDANT ACTIVITY

### 3.5 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.

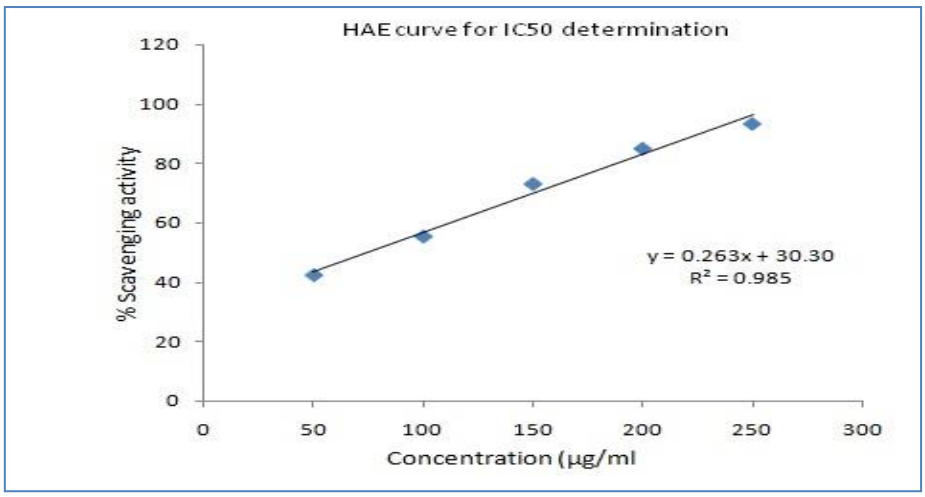


**Fig. 3.1 Standard Curve of Ascorbic Acid**

**Table 3.6 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 3.7 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 |

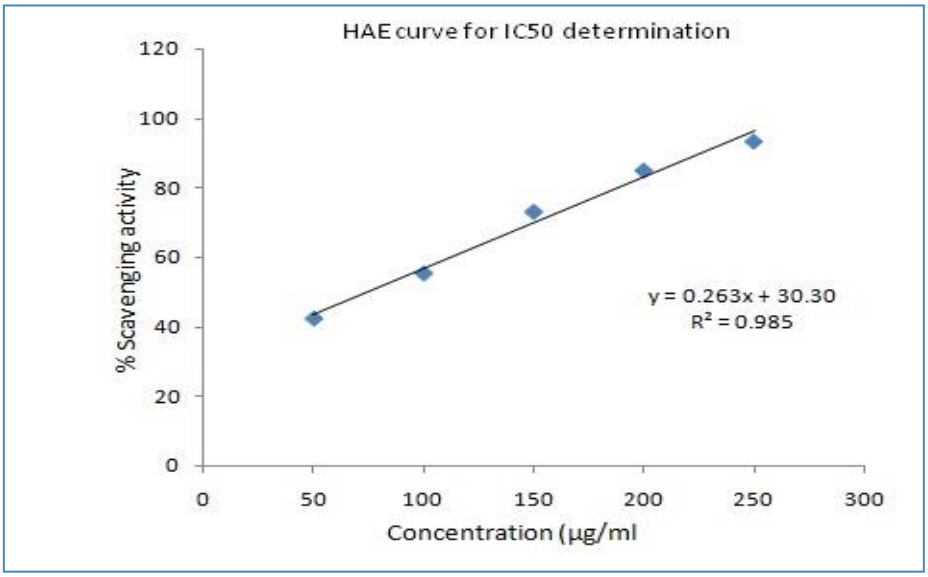


**Fig. 3.2 Absorbance of *Embelia ribes* (Hydro- Alcoholic Extract)**

**Table 3.7 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 |

The present study was aimed at evaluating *Embelia ribes and Zizyphus xylopyrus* extract formulated as antioxidant and antimicrobial. This chapter provides a methodical summary of the entire project.

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.
This study thus demonstrates the easy and effective formulation of *Embelia ribes and Zizyphus xylopyrus* extract and its antimicrobial property. Considering the various properties attributed to *Embelia ribes and Zizyphus xylopyrus*, we conducted a complete and detailed analysis of the research required to assess the formulation's therapeutic utility using several in-vitro and in-vivo models.

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