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COMPARATIVE ANTIOXIDANT ACTIVITY OF *AEGLE MARMELOS* AND *TERMINALIA BELLERICA*

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1. ABSTRACT

The antioxidant activity of different concentration (5,10,15 mg/ml) ethanol, methanol of *Aegle marmelos* was determined by DPPH method. In the present study the methanolic extracts of *Aegle marmelos* is showing higher antioxidant activity comparative to others. The scavenging effect on the ethanol extract from *Aegle marmelos* were 58.62 in 5 mg /ml, 64.34 in 10mg/ml and 67.46 in 15mg/ml respectively . The scavenging effect on the methanol extract from *Aegle marmelos* were 76.73% in 5mg/ml, 83.64% in 10mg/ml and 84.38% in 15mg/ml respectively. In the present study the methanolic extract of *Terminalia bellerica* showing higher antioxidant activity. The scavenging effect on the ethanol extract from *Terminalia bellerica* was 55.32 in 5 mg /ml, 68.34 in 10mg/ml and 62.46 in 15mg/ml respectively . The scavenging effect on the methanol extract from *Terminalia bellerica* were 46.73% in 5mg/ml,78.64% in 10mg/ml and 85.38% in 15mg/ml respectively.

Key Words- *Terminalia bellerica,* Antioxidant, *Aegle marmelos,* DDPH, etc.

2. INTRODUCTION

Today higher plants are acting an important role in the management of immeasurable diseases counting cancer, lymph sarcomas, AIDS, senile dementia and auto-immune diseases. Classically superior plants are occupying a main position in the construction of new therapeutic agents. Thus, the plant containing drugs are capable to dwell in an important place in contemporary medicine. The World Health Organization (WHO) have been assumed that 4 billion person, 80% of the total world population, currently use herbal remedy for some aspect of primary health care. Herbal medicine is a major component in all indigenous traditional medicine and a common element in Ayurvedic, Homeopathic, Naturopathic, traditional oriented, medicine. *A. marmelos,* in general said as Bael and belonging to the family Rutaceae is an important medicinal plant in the traditional Indian system of medicine.

The extract equipped by boiling the bark, leaves or roots in water is useful as laxative, febrifuge, and expectorant. The extract is also constructive in ophthalmia, deafness, inflammations, catarrh, diabetes, and asthmatic complaints. The fruits are used in treating diarrhea, dysentery, stomachache and cardiac aliments. *Terminalia bellerica* Roxb (combretaceae) known as **bahera** or **beleric** is found widely throughout the Indian subcontinent , Sri Lanka, Bangladesh, Indonesia, Nepal, Bhutan, China, Cambodia, South- East Asia, as a medicinal plant.

3.1 Methodology Involved In Antioxidant Activity of *Aegle Marmelos Leaves*

3.1.1 Plant Materials:

The leaves of *Aegle marmelos* were collected from G.B. Pant University Pantnagar. The identified plant parts were washed and air dried at room temperature and was powdered with the help of mortar and pestle. The fine particles were separated and stored in clean containers until used.

3.1.1.1 Chemicals:

1, 1- Diphenyl-2-picrylhydrazyl (DPPH), (Hi-Media Lab. Pvt. Ltd., India), Ethanol (CDH chemicals. (India.), Methanol (Merk chemicals Ltd., India.)

3.1.1.2 Instruments:

The instruments used for different analyses during the study. UV Spectrophotometer, Atomic absorption spectrophotometer, Centrifuge,Cooling centrifuge, Rotary shaker, Digital Ultrasonic Cleaner, Water bath, Rotary Shaker, Glass Bead Sterilizer, Electronic Balance, Laminar air flow, Autoclave, Quick freezer, Hot air oven, Microwave (LG) etc.

3.1.1.3 Glass wares: Borosil flasks, culture tubes, pipettes, beakers etc.

3.1.1.4 Sterilization of leaves

The disease free and fresh plant were selected priorly for this investigation. About 4 gm of fresh and healthy *Aegle Marmelos* leaves were taken for each solvent extraction. These are washed with tap and distilled water for four times. Then, surface sterilized with 0.3% alcohol for few seconds. Then it washed with distilled water and then the leaves were dried under shade. This dried material was mechanically powdered and stored at a dry place. This powdered material was used for further antioxidant analysis.

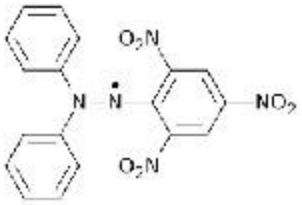
3.2.2 Extract Preparation of Leaves for Determining Antioxidant Activity

Collected leaves of *Aegle marmelos* were weighed prior to drying. 15 gm of accurately weighed powdered leaves of *Aegle marmelos* was extracted with 150 ml solvent (methanol, ethanol, separately) in a conical flask, plugged with cotton wool and put the sample in a mechanical shaker (100rpm) in room temperature for 8 hours. Take the sample after 8 hrs and centrifuge for 15 min (6000-8000rpm). The extracts were filtered

through whatmann no. 1 filter paper and filtrates were evaporated at 42C° under reduced pressure to dryness in a rotary evaporator. The extract obtained was weighed and stored in airtight container in refrigerator until further antioxidant screening purpose.

3.2.2.1 DPPH Solution

Take 4g DPPH powder in a flask and added 100ml of methanol, ethanol seperately and then flask covered with a foil paper and kept in a cool condition. **Scavenging assay (DPPH)**



$C_{18}H_{12}N_5O_6$

The DPPH assay method is based on the reduction of DPPH a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). The antioxidant activity of different *Aegle marmelos* extract solution were measured in term of hydrogen donating or radical scavenging ability using the stable DPPH method. The ability of extracts to scavenge DPPH radical is determined according to the method of Blois (1958). 1 ml of 0.1mM DPPH solution was mixed with 3ml of extract in methanol. The mixture was shaken vigorously and left for 35 minutes in the dark at room temperature. The absorbance was measured at 517 nm spectrophotometrically. Methanol/Ethanol were used to set auto zero. All determinations were performed accordingly. The radical scavenging activities of the tasted samples expressed as percentage of inhibition were calculated according to the following equation.

Radical scavenging activity (% Inhibition) = [(A0-A1)/A0] × 100

Where A0 is the absorbance of the control; A1 is the absorbance of test samples.

3.2 Methodology Involved In Antioxidant Activity of *Terminalia Bellerica Leaves*

3.2.1 Plant Materials:

The leaves of *Terminalia bellerica* were collected from G.B. Pant university Pantnagar. The identified plant parts were washed and air dried at room temperature and was powdered with the help of mortar and pestle. The fine particles were separated and stored in clean containers until used.

3.2.1.1 Chemicals:

1, 1- Diphenyl-2-picrylhydrazyl (DPPH), (Hi-Media Lab. Pvt. Ltd., India), Ethanol (CDH chemicals**,** India.), Methanol ((Merk chemicals Ltd., India.)

3.2.1.2 Instruments:

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3.2.1.3 Glass wares: Borosil flasks, culture tubes, pipettes, beakers etc.

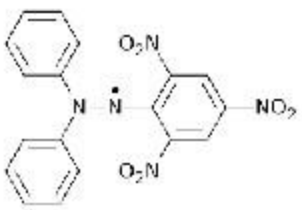
3.2.1.4 .Sterilization of leaves

The disease free and fresh plant were selected priorly for this investigation. About 4 gm of fresh and healthy *Terminalia bellerica* leaves were taken for each solvent extraction. These are washed with tap and distilled water for four times. Then, surface sterilized with 0.3% alcohol for few seconds. Then it washed with distilled water and then the leaves were dried under shade. This dried material was mechanically powdered and stored at a dry place. This powdered material was used for further antioxidant analysis.

3.2.2 Extract Preparation of Leaves for Determining Antioxidant Activity collected leaves of *Terminalia bellerica* were weighed prior to drying. 15 gm of accurately weighed powdered leaves of *Terminalia bellerica* was extracted with 150 ml solvent (methanol, ethanol, separately) in a conical flask, plugged with cotton wool and put the sample in a mechanical shaker (100 rpm) in room temperature for 8 hours. Take the sample after 8 hrs and centrifuge for 15 min (6000-8000rpm). The extracts were filtered through whatmann no. 1 filter paper and filtrates were evaporated at 42C° under reduced pressure to dryness in a rotary evaporator. The extract obtained was weighed and stored in airtight container in refrigerator until further antioxidant screening purpose.

3.2.2.1 DPPH Solution

Take 4g DPPH powder in a flask and added 100ml of methanol, ethanol seperately and then flask covered with a foil paper and kept in a cool condition. **Scavenging assay (DPPH)**



$$C_{18}H_{12}N_5O_6$$

The DPPH assay method is based on the reduction of DPPH a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purplecolour). The antioxidant activity of different *Terminalia Bellerica* extract solution were measured in term of hydrogen donating or radical scavenging ability using the stable DPPH method. The ability of extracts to scavenge DPPH radical is determined according to the method of Blois (1958). 1 ml of 0.1mM DPPH solution was mixed with 3ml of extract in methanol. The mixture was shaken vigorously and incubated for 35 minutes in the dark at room temperature. The absorbance was measured at 517 nm spectrophotometrically. Methanol/Ethanol containing DPPH were used to set auto zero. All determinations were performed accordingly. The radical scavenging activities of the tasted samples expressed as percentage of inhibition were calculated according to the following equation.

Radical scavenging activity (% Inhibition) = [(A0-A1)/A0] × 100

Where A0 is the absorbance of the control; A1 is the absorbance of test samples.

4. RESULTS AND DISCUSSION

4.1.Various Finding of the Antioxidant Activity *Aegle Marmelos Leaves*

Aegle marmelos showed a good antioxidant activity in ethanol and methanol extracts. The model of scavenging the DPPH radical is widely used method to evaluate the free radical scavenging ability of different solvent extracts (ethanol, methanol) on the DPPH radical which increase with increasing concentration. The scavenging effects on DPPH radical were determined. Measuring the decrease in absorbance at 517 nm due to the DPPH radical reduction, indicating the antioxidant activity of the *Aegle marmelos* in a short time. The antioxidant activity of different concentration (5,10,15 mg/ml) ethanol, methanol of *Aegle marmelos* was determined by DPPH method. In the present study the methanolic extracts of *Aegle marmelos* is showing higher antioxidant activity comparative to others. The scavenging effect on the ethanol extract from *Aegle marmelos* were 58.62 in 5 mg /ml, 64.34 in 10mg/ml and 67.46 in 15mg/ml (Fig 5) respectively (Table 8). The scavenging effect on the methanol extract from *Aegle marmelos* were 76.73% in 5mg/ml, 83.64% in 10mg/ml and 84.38% in 15mg/ml respectively (Fig 6) (Table 9)

Table 8: The scavenging effect on the ethanol extract from *Aegle marmelos*

Concentration of Leaves Extract (mg/ml)	Percentage of inhibition Ethanol
5	58.62
10	64.34
15	67.46

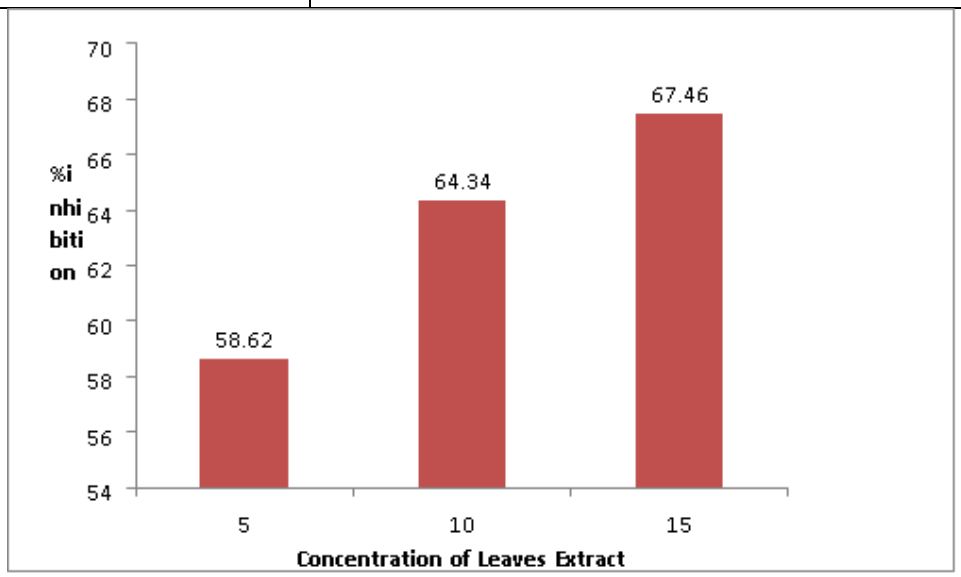


Fig. 5: Antioxidant activity of *Aegle marmelos*

***Table 9: The scavenging effect on the methanol extract from* Aegle marmelos**

Concentration of Leaves Extract (mg/ml)	Percentage of inhibition Methanol
5	76.73
10	83.64
15	84.38

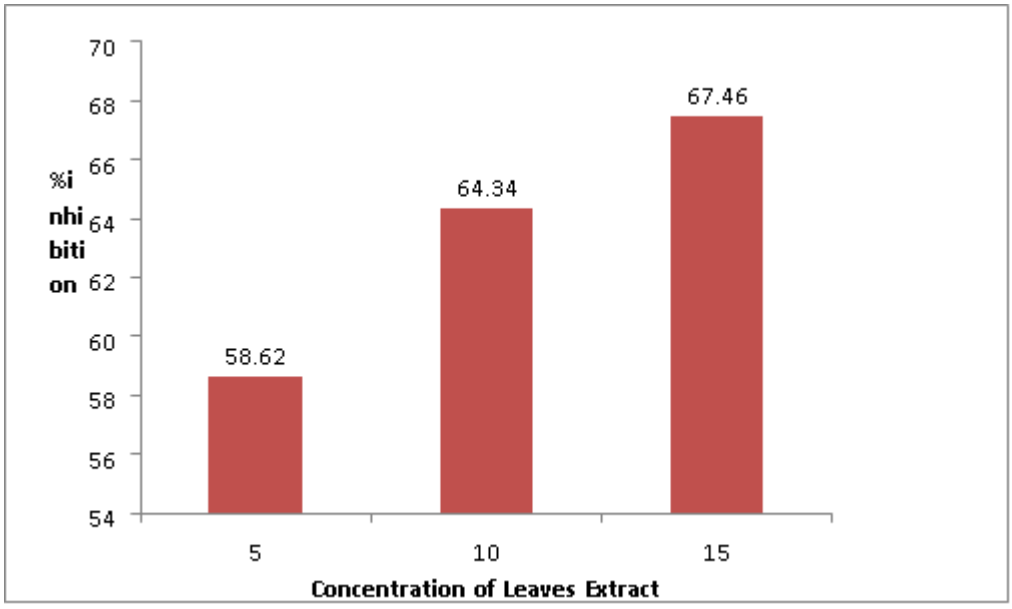


Fig. 6: Antioxidant activity of *Aegle marmelos*

4.2 Antioxidant Activity of *Terminalia Bllerica* Leaves

*Terminalia bellerica*showed a good antioxidant activity in ethanol and methanol extracts. The model of scavenging the DPPH radical is widely used method to evaluate the free radical scavenging ability of different solvent extracts (ethanol, methanol) on the DPPH radical which increase with increasing concentration. The scavenging effects on DPPH radical were determined. Measuring the decrease in absorbance at 517 nm due to the DPPH radical reduction, indicating the antioxidant activity of the *Terminalia bellerica* in a short time.The antioxidant activity of different concentration (5,10,15 mg/ml) ethanol, methanol of *Terminalia bellerica* was determined by DPPH method. In the present study the methanolic extract of *Terminalia bellerica* showing higher antioxidant activity. The scavenging effect on the ethanol extract from *Terminalia bellerica* were 55.32 in 5 mg /ml, 68.34 in 10mg/ml and 62.46 in 15mg/ml (Fig 7) respectively (Table 10). The scavenging effect on the methanol extract from *Terminalia bellerica* were 46.73% in 5mg/ml,78.64% in 10mg/ml and 85.38% in 15mg/ml respectively (Fig 8) (Table 11).

Table 10: The scavenging effect on the ethanol extract from *Terminalia bellerica*

Concentration of Leaves Extract (mg/ml)	Percentage of inhibition Ethanol
5	55.32
10	68.34
15	62.46

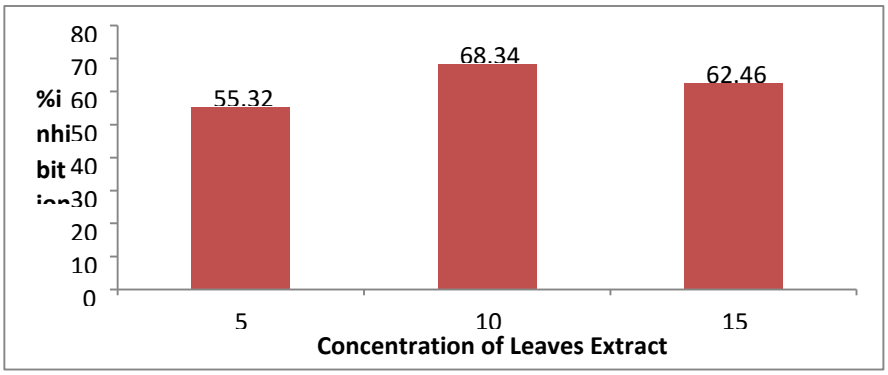


Fig. 7: Antioxidant activity of *Terminalia bellerica in ethanol*

Table 11 The scavenging effect on the methanol extract from *Terminalia bellerica*

Concentration of Leaves Extract (mg/ml)	Percentage of inhibition Methanol
5	46.73
10	78.64
15	85.38

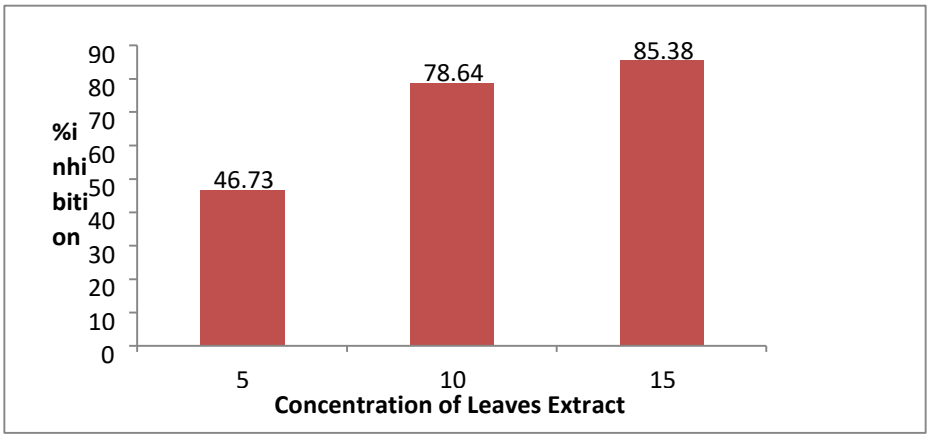


Fig. 8: Antioxidant activity of *Terminalia bellerica in methanol*

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