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Study Of Invitro Antioxidant Activity Of Aqueous And Honey Extract Of Emblica Officinalis -Wild And Hybrid Indian Gooseberry

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ABSTRACT:

This study is about two different strains of Emblica officinalis (Amla) which is Wild Indian gooseberry and Hybrid Indian gooseberry. Both the strains of Emblica officinalis(Amla) possess antioxidant activity. As the comparative study of both strains of Emblica officinalis (Amla) is not proven on antioxidant activity. In this a comparative study is performed between two strains of Emblica officinalis (Amla), to find out which one of the strains of Emblica officinalis have better antioxidant activity.

Keywords: Antioxidant activity, Emblica officinalis, Hybrid indian gooseberry,Wild Indian gooseberry.

Introduction

Amla (Emblica *officinalis*) has a hallowed position in Ayurveda - an Indian indigenous system of medicine, which belongs to the family of Euphorbiaceae and is also known as Phyllanthus Emblica or Indian gooseberry[1]. Emblica *officinalis* fruit extract has various therapeutic benefits such as radioprotective, antidiabetic, antiaging, gastroprotective[4]. Amla is native to India and grows in tropical and subtropical regions of Pakistan, Uzbekistan, Sri Lanka, Southeast Asia, China, and Malaysia[1]. Amla is highly nutritious and is one of the richest sources of vitamin C, amino acids, and minerals. It contains several chemical constituents like tannins, alkaloids, and phenols. Among all hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid are reported to possess biological activity[1]. Pharmacological research reports on Amla reveals its analgesic, anti-tussive, anti-atherogenic, apoptogenic, cardio, gastro, nephron, neuro protective and anticancer properties. Amla also possess chemo preventive, radio, chemo and immunomodulatory, free radical scavenging, antioxidant, anti-inflammatory, anti-mutagenic activities[2]. Almost all parts possess medicinal properties, particularly fruit. Emblica *officinalis* extracts contains various antioxidant such as emblicanin A, emblicanin B, Gallic acid, ascorbic acid, ellagic acid.[3] Amla fruit is widely used in the Indian system of medicine as alone or in combination with other plants and is used to treat common cold and fever, as diuretic, laxative, liver tonic and dyspepsia.[1]

Antioxidant means "against oxidation". Any substance at low concentrations compared to that of an oxidizable substrate that significantly delays or prevents oxidation of that substrate is called as antioxidant. Antioxidants play vital role in preserving the quality of food and maintaining health of human being.[9]

Oxidation reaction depending upon site of occurrences presents specific repercussions. If the site of occurrence is food system, then food deteriorates. When oxidation occurs in biological cell system, it causes damage or death to the cell. Most tests are performed by shortening the induction period of chain reaction, by using high temperature[10].

2.1 PLANT PROFILE

2.1.1 WILD INDIAN GOOSEBERRY[3,7]

BOTANICAL NAME	: Phyllanthus Emblica Linn.
FAMILY	: Euphorbiaceae
KINGDOM	: Plantae
CLASS	:Dicotyledonae
ORDER	:Geraniales
GENUS	:Emblica

SPECIES :Phyllanthus Emblica

2.1.1.1 Vernacular Names:

English : Emblic, myrobalan-tree, Indian gooseberry

Tami : Nelli

Malayalam: Nelli

Hindi : Amla, aonla, onilika

2.1.1.2 Morphology

Leaves, 10 to 13 mm long, 3 mm wide, closely set in pinnate fashion, making the branches feathery in general appearance. The leaves develop after the fruit set. Flowers, unisexual, pale green, 4 to 5 mm in length, borne in leaf-axils in clusters of 6 to 10; staminate flowers, tubular at the base, having a small stalk, gamosepalous, having 6 lobes at the top; stamens 1 to 3, polyandrous, filaments 2 mm long; pistillate flowers, fewer, having a gamopetalous corolla arid a two-branched style; both staminate and pistillate flowers are borne on the same branch, but the staminate flowers occur towards the apices of small branches. Fruits, fleshy, almost depressed too globose. 2.14 cm in diameter, 5.68 g in weight, 4.92 ml in volume, primrose yellow.

Fig 2.1: Fruit of Wild Indian gooseberry

2.1.1.3 Chemical Constituents

CHEMICAL CONSTITUENT	PERCENTAGE
Moisture content	70.5%
Proteins	0.75%
Mineral	2.922%
Tannins	2.73%
Pectin	0.59%
Protein	0.75%
Ascorbic acid	1094 mg/100 ml of juice

Table 2.1: Major Chemical Constituents and Its Percentage

2.1.2 HYBRID INDIAN GOOSEBERRY[1]

BOTANICAL NAME : Emblica *officinalis* Geartn

FAMILY : Euphorbiaceae

KINGDOM : Plantae

CLASS : Dicotyledonae

ORDER : Geraniales

GENUS : Emblica

SPECIES : officinalis Geartn.

2.1.2.1 Vernacular names

Hindi : Amla

English : Gooseberry, Embolic myrobalan

Tamil : Nelli

Malayalam: Nellimaram

2.1.2.2 Morphology

Amla tree is a small to medium sized deciduous tree with an average height of 8-18 m, with thin light grey bark exfoliating in s mall thin irregular flakes, exposing the fresh surface. The average girth of the main stem is 70 cm. In most cases, the main trunk is divided into 2 to 7 scaffolds very near to the base. Leaves are 10 -13 mm long, 3 mm wide, closely set in pinnate fashion which makes the branches feathery in general appearance. After setting of the fruit's leaves develop. Flowers are unisexual, 4 to 5 mm in length, pale green in colour, borne in leaf axils in clusters of 6 to 10.

Fig 2.3: Fruit of Hybrid Indian Gooseberry

CHEMICAL CONSTITUENTS	PERCENTAGE YEILD
Carbohydrate	14.1
Fat	0.5
Fibres	0.1
Calcium	0.05
Vitamin C	600mg/100g
Nicotinic acid	0.2mg/100g
Moisture	81.2

Table2.2MajorChemical Constituent and Its Percent

RESULT

Summary of Preliminary Phytochemical Screening of Crude Fruit Extract of

Hybrid and Wild Indian Gooseberry

Sl No	Tests	Phytochemical Constituent	Hybrid Indian Gooseberry-Aqueous Extraction	Hybrid Indian Gooseberry-Honey Extraction	Wild Indian Gooseberry-Aqueous Extraction	Wild Indian Gooseberry-Honey Extraction
1.	Bromine water test	Phenol	++	++	++	++
	Litmus test	Phenol	++	++	++	++
2.	Mayer's test	Alkaloid	+	+	+	+
3.	Molish test	Carbohydrate	++	++	++	++
	Fehling's test	Carbohydrate	++	++	++	++
4.	Alkaline reagent test	Flavonoid	+	+	+	+

(+) Positive – Reaction intensity is normal

(++) Positive – Reaction intensity is high

EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY OF HYBRID AND WILD INDIAN GOOSEBERRY

The fruit extract of hybrid and wild Indian gooseberry was subjected to antioxidant evaluation by using Ferric reducing antioxidant power (FRAP) Assay, DPPH radical scavenging assay and ABTS Radical Scavenging Assay.

5.4.1 5.4.1 Ferric Reducing Antioxidant Power (FRAP) Assay

When comparing ferric reducing activity of AHG and AWG at the concentration 20, 40, 60, 80 and 100 mcg/ml, the AHG shows more ferric reducing antioxidant power activity ($P<0.05$). When comparing HWG and HHG at concentrations 20, 40, 60, 80and 100 mcg/ml, there is no significant difference in the antioxidant activity of both extracts. Almost identical antioxidant activity was shown by HWG and HHG at all concentrations. When comparing all the test sample with the standard, the standard (ferrous sulphate) shows higher ferric reducing antioxidant activity. when comparing all the four samples, at all tested concentration there is no significant difference in ferric reducing activity and Concentration dependent increase in absorbance value at 593 nm.The absorbance value at different concentration are given in Table No-5.4

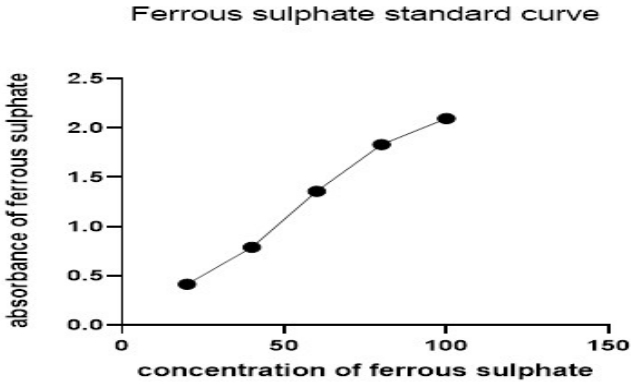

Fig 5.26 –Standard Curve of Ferrous Sulphate

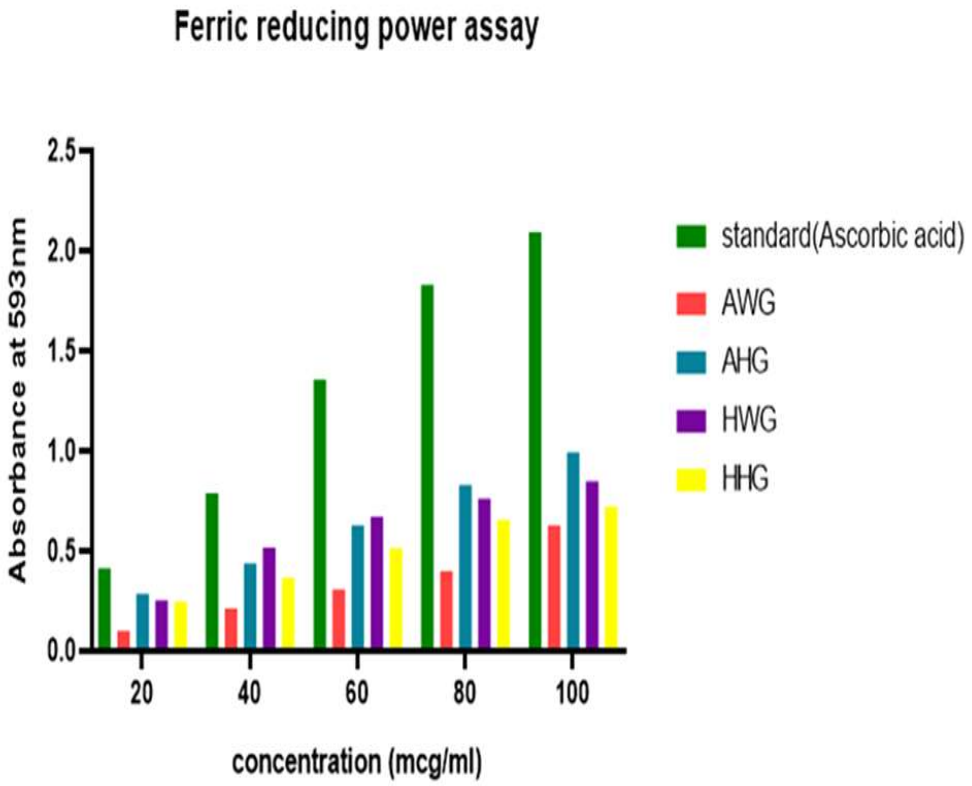

Fig 5.25 – Effect of various fruit extract of Wild and Hybrid Indian Gooseberry in FRAP assay

5.4.2 1,1, Dipheny-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

When comparing the DPPH activity of AHG and AWG at the concentrations 50, 100, 200, 400 and 800 mcg/ml the AHG shows more DPPH radical scavenging activity ($P<0.05$). When comparing HHG and HWG at all the tested concentrations there is no significant difference in DPPH radical scavenging activity, both shows almost identical DPPH radical scavenging activity at all concentrations. When comparing all the test sample with the Standard, the standard(ascorbic acid) shows higher DPPH radical scavenging activity. Overall, when comparing all the four samples, AHG shows significant DPPH radical scavenging activity ($P <0.01$).The percentage inhibition value at different concentration are given in Table No-5.5

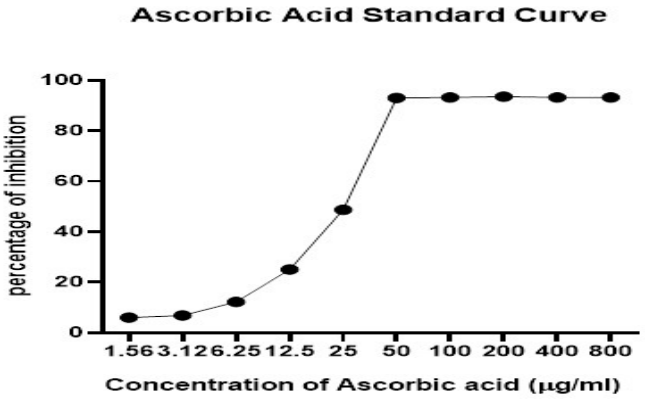

Fig 5.28 –Standard Curve of Ascorbic Acid

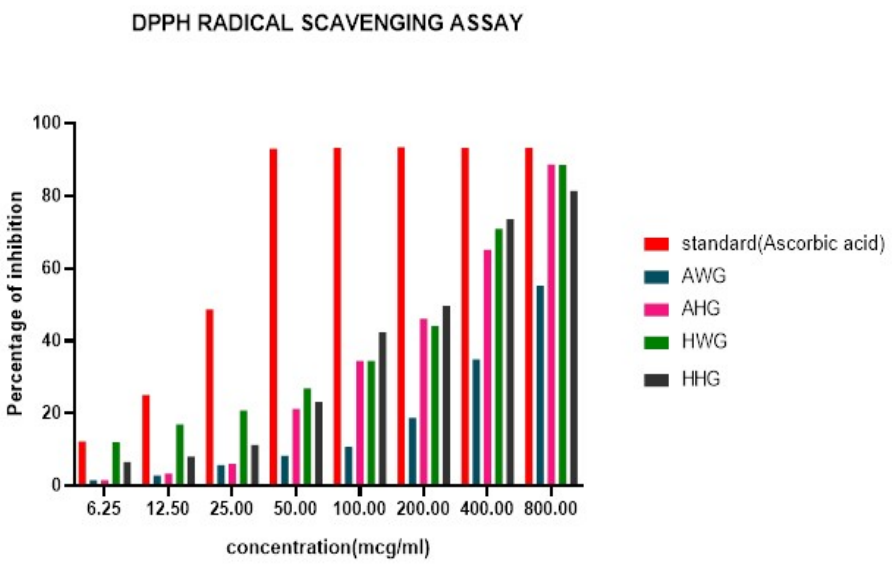

Fig 5.27 – Effect of various fruit extract of Wild and Hybrid Indian Gooseberry in DPPH

5.4.3 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic Acid) (ABTS) Radical Scavenging Assay

When comparing ABTS 0f AHG and AWG at the concentrations 100, 200, 400 and 800 mcg/ml ,the AHG shows more ABTS radical scavenging activity ($P<0.05$). When comparing HWG and HHG at concentration of 100 mcg/ml, both the extract shows same ABTS radical scavenging activity. When comparing ABTS activity of HWG and HHG at the concentrations 12.5, 25, and 50 mcg/ml, HWG shows more ABTS radical scavenging activity ($P<0.05$). When all the test sample is compared with the standard, the standard (Ascorbic acid) shows higher ABTS radical scavenging activity. When comparing all the test sample AHG & HWG shows significant ABTS radical scavenging activity than others ($P<0.01$). The percentage inhibition value at different concentration are given in Table No-5.6

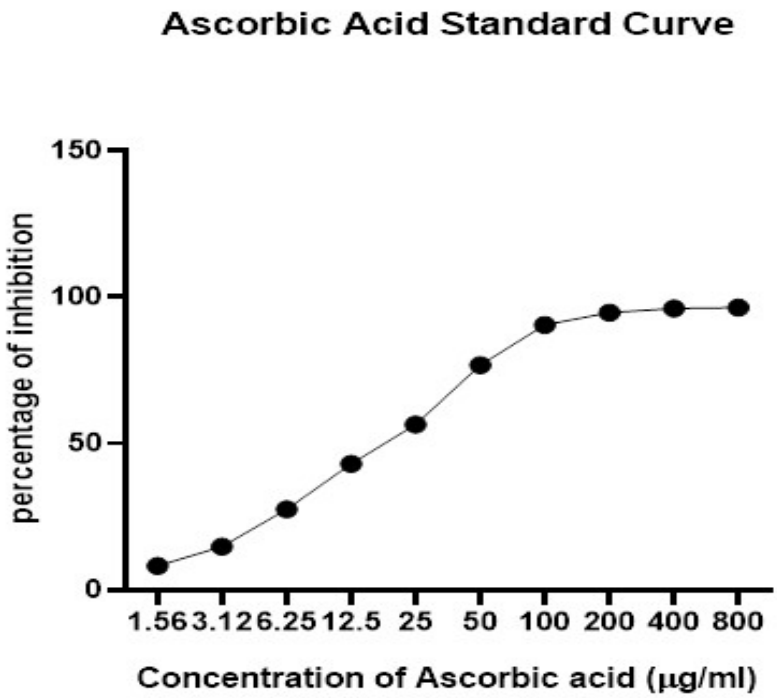

Fig 5.30 – Standard Curve of Ascorbic acid

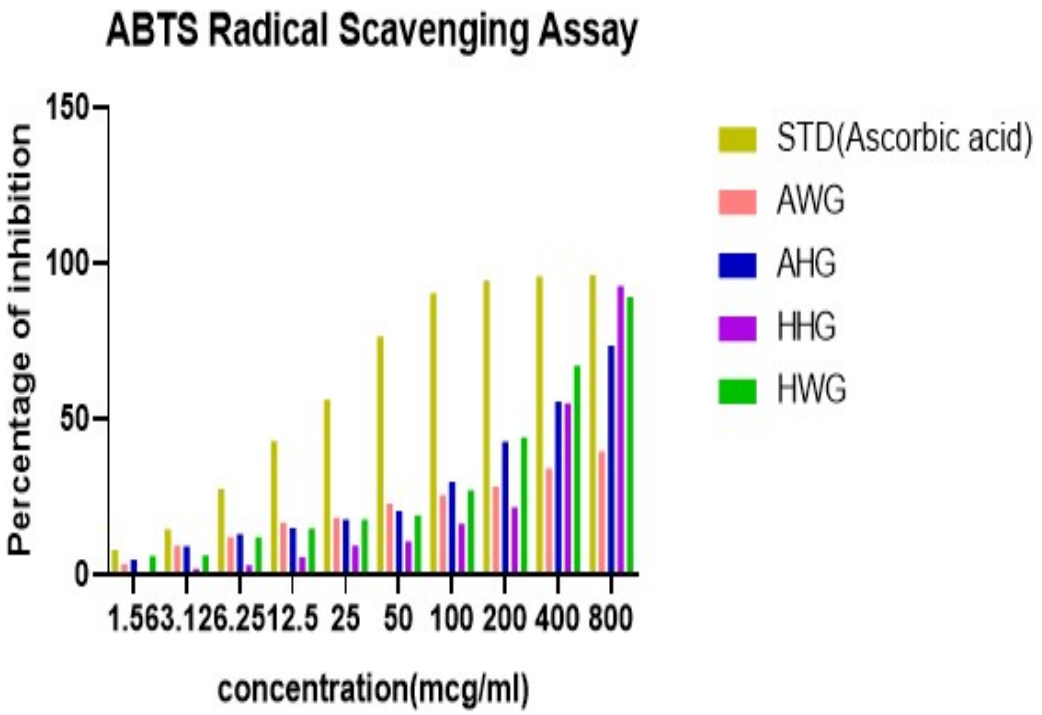

Fig 5.29 – Effect of various fruit extract of Wild and Hybrid Indian gooseberry

CONCLUSION

Amla belongs to the family of Euphorbiaceae and is also known as Phyllanthus Emblica or Indian gooseberry. Amla is native to India and grows in tropical and subtropical regions .[1] It has a beneficial role in degenerative diseases like cancer, diabetes, liver treatment, ulcer, anaemia, heart trouble1 and also is an important constituent in hepatoprotective formulas available. Amla is highly nutritious and is one of the richest sources of vitamin-C, amino acids, and minerals. It contains several chemical constituents like tannins, alkaloids and phenols. Among all hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid are reported to possess biological activity.

Antioxidant means "against oxidation". Any substance at low concentrations compared to that of an oxidizable substrate that significantly delays or prevents oxidation of that substrate is called as antioxidant.[26]

The freshly prepared fruit extract of wild and hybrid Indian gooseberry was subjected to phytochemical screening tests for the detection of various active constituents. The phytochemical analysis of fruit extract of wild and hybrid Indian gooseberry showed the Prescence of phenol, alkaloid, flavonoid, and carbohydrates.

In the present study the antioxidant activities of aqueous and honey extract of wild and hybrid Indian gooseberry were evaluated by using different antioxidant models that is Ferric Reducing Antioxidant Power(FRAP) Assay, DPPH radical scavenging assay, ABTS radical scavenging assay.

The result obtained in the study shows that the aqueous and honey extract of wild and hybrid Indian gooseberry has antioxidant activity in different antioxidant models.The aqueous hybrid Indian gooseberry (AHG) shows higher antioxidant activity in FRAP, DPPH, ABTS assay models. When the standard sample ($FeSo_4$ and Ascorbic acid) of FRAP, DPPH and ABTS assay is compared with Aqueous Hybrid Indian gooseberry (AHG),the standard samples ($FeSo_4$ and Ascorbic acid) show higher antioxidant activity.

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