# Extraction of Emblica Officinalis and Evaluation of their Antioxidant and $H^+K^+$ ATPase inhibition activity

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***Abstract: Emblica Officinalis is a common plant mostly used in ayurvedic formulations due to its therapeutic activity. Alcoholic, Ethanolic, and Hydro-alcoholic extract of amla fruits having antioxidant as well as H+K+ ATPase inhibition activity. In the present study extractive value of amla fruit was determined by using various solvents. The aqueous, ethanolic, and hydro-alcoholic extracts were prepared and determined Antioxidant activity and H+K+ ATPase inhibition activity. ethanolic extract showing greater H+K+ ATPase Inhibition activity $IC_{50}$ 52.25±2.45 µg/ml and Antioxidant activity $IC_{50}$23.45 ± 4.26 µg/ml as compared to other types of extract.***

***Key-Word: Amla, Antioxidant, Polyphenol, Extraction.***

## I.INTRODUCTION

Amla (Emblica Officinalis Gaertn. Belonging to the family Euphorbiaceae, It is also known as the Indian gooseberry. In the traditional indigenous system, amla has more value in the preparation of medicines, including folklore Ayurveda, it has more importance in formulations of nutritional and medicinal formulations to gain lost vigor as well as vitality. (Variya et al., 2016) Geographically amla is found throughout in India. Amla plant grows 8-18 meters in height. It has light grey colored bark exfoliating in small thin irregular flakes. Leaves are subsessile, simple, and closely set along with the branchlets, amla having greenish to yellow colored flowers, in auxiliary fascicles, unisexual, males numerous on short slender pedicels, females few, subsessile, ovary 3- celled; fruits globose, fleshy, pale yellow with six obscure vertical furrows enclosing six trigonous seeds in 2-seeded 3 crustaceous cocci. (R. Jain et al., n.d.). Medicinally each and Every part of amla possesses high medicinal value but fruits. Fruits of amla are the most valuable part of folklore and therapeutic uses as compared to other parts of plant. Amla are the source of polyphenol, tannins and flavonoids. These active elements are key responsible elements for major bioactivities. (Yadav et al., 2017) Amla consist of highly nutritious value and is one of the richest source of vitamin-C, amino acids and minerals. Amla contains various chemical constituents such as tannins, alkaloids and phenols. Among all hydrolysable tannins, Emblicanin A and B; gallic acid, ellagic acid are reported to possess biological activity.(Yadav et al., 2017)

## II.MATERIAL AND METHOD

Amla dried Fruits, Methanol, Folin-Chiocalteu reagent, Sodium carbonate, Gallic acid, g 1, 1-diphenyl-2-picrylhydrazyl (DPPH), tris-HCl, magnesium chloride, potassium chloride, adenosine-5'-triphosphate (ATP), omeprazole. Etc.

1. Extract Preparation:

1.1 Preparation of Hydro-alcoholic Extract:

Hydro-alcoholic extracts from dried fruits of amla were prepared by slight modifying the method of (Agarwal Madhu et al., 2012). All the dried fruit flakes of amla was initially rinsed with distilled water to remove the foreign matter and soil particles and dried in to tray drier in between 65-70$^0$C. After drying the fruit flakes were grinded and sieved through #16 mesh. The retained material above sieve further grinded and completely pass through #16 mesh.

50 gm of coarse powder were dispersed into 250 ml of solvent (50:50 % v/v). Stirred all material up 30 min. at 900 rpm on stirrer. Filtered all material using 100 mesh muslin cloth and further again extracted obtained mark in another 250 ml of solvent (50:50%v/v) with 30 min stirring. Filter all material collect all decoction and centrifuge at 3000 rpm for 5 min and collect supernatnt liquid. Mixed weighed quantity of microcrystalline cellulose in to above collected supernatant liquid and dry completely on tray dryer at 60$^0$C to obtained Loss on drying NMT 5%w/w. collected all dried extract milled and passed through 60 mesh and packed in closed container.

1.2 Preparation of Ethanolic Extract:

Ethanolic extract were prepared by slightly modification of (Ahmad et al., 1998). 50 g of well cleaned and dried coarse powdered plant material in 500 ml of ethanol for 72 h. with frequently shaking e every 24 h using a sterile glass rod. At the end of extraction, all solution filtered through Whatman filter paper no. 1. The filtrate obtained were concentrated and dried as per process given in hydro-alcoholic extraction method.

1.3 Preparation of Aqueous Extract:

Aqueous extract were prepared by slightly modification of (Ahmad et al., 1998)50 g of cleaned and dried coarse powdered plant material in 500 ml of distilled waters for 72 h. with frequently shaking e every 24 h using a sterile glass rod. At the end of extraction, all solution filtered through Whatman filter paper no. 1. The filtrate obtained were concentrated and dried as per process given in in hydro-alcoholic extraction method.

1.4 Determination of Extractive Value:

The dry powdered of amla fruit were extracted with water, methanol, and Combination of water and methanol (50:50%v/v) using a maceration process. 2 gm. of coarsely powdered plant material was weighed and transferred into a dry 250 ml conical flask. Flask was filled with solvents (50 ml) separately. The flasks were shake for 24 hrs at room temperature, frequently. The mixtures were centrifuge at 3000 rpm and collect supernatent and dried in tray dryer to evaporate moisture completely. The extractive value in percentage was calculated by using following formula and recorded.

Extractive value (%) = Weight of dried extract
Weight of plant material X 100

1.5 Determination of percentage of amla Extract:

Dried percentage of extract was calculated by subtracting added filler during drying from the total weight of dried extract powder. Percentage of Aqueous, Hydro-alcoholic and Ethanolic extracts were calculated using below formula:

Percentage of Extract= $\frac{weight\ of\ total\ dried\ extract - weigh\ of\ filler\ added}{weight\ of\ total\ dried\ Extract} \times 100$

1.6 Determination of H+K+ ATPase:

The H+K+ ATPase activity was determined in the presence of different concentrations of test extracts. The enzyme source was pre-incubated with various concentration of the test extract material (10-70µg) for 30min. The reaction was started with the addition of 2mM adenosine-5'-triphosphate (ATP) and incubated for 30 mins at 30ºC and terminated by the addition of 10% trichloroacetic acid. Reaction mixtures contained 300 µg of gastric vesicular protein, 1 mM ATP, 20 mM MgCl2 and 50 mM Tris–HCl (pH 7.4) The amount of inorganic phosphorous released from adenosine-5'-triphosphate (ATP) was determined colorimetrically by measuring the release of inorganic phosphate (Pi) from ATP (1 mM) as described previously (Fiske and Subbarow, 1925). The enzyme source was also treated similarly with the standard drug omeprazole and the enzyme activity was measured(Ajay Shukla et al., 2016)

1.7 DPPH radical scavenging assay:

The free radical scavenging of prepared amla extracts were determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay. All extracts were prepared in various concentrations from 5-200 µg/mL in 50% methanol solution. The reaction assay contained 3 ml of 0.060 mM DPPH in methanol and 200 µL of extract solution.In dark room all extraction mixture were incubated and absorbance was measured by spectrophotometer at wavelength 517 nm. Gallic acid was used as references and 50% methanol solution was used as vehicle control(Charoenteeraboon et al., 2010). The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical scavenging activity (%) = $\frac{\text{Abs.control} - \text{Abs.sample}}{\text{Abs.control}} \times 100$

## III. RESULT AND DISCUSSION

Table 1: Results of aqueous, ethanolic and Hydro- alcoholic extract

| Type of Extract | Extractive Value (%w/w) | percentage of amla Extract (%w/w) | H+K+ ATPase Inhibition ($IC_{50}$ µg/ml) | DPPH radical scavenging ($IC_{50}$ µg/ml) |
|---|---|---|---|---|
| Aqueous Extract | 15.32±2.35 | 70.24 | 60.65±5.23 | 25.32±5.65 |
| Ethanolic Extract | 13.32±3.74 | 50.65 | 52.25±2.45 | 23.45±4.26 |
| Hydro-alcoholic Extract | 16.65±2.85 | 68.27 | 68.89±4.35 | 26.12±5.32 |

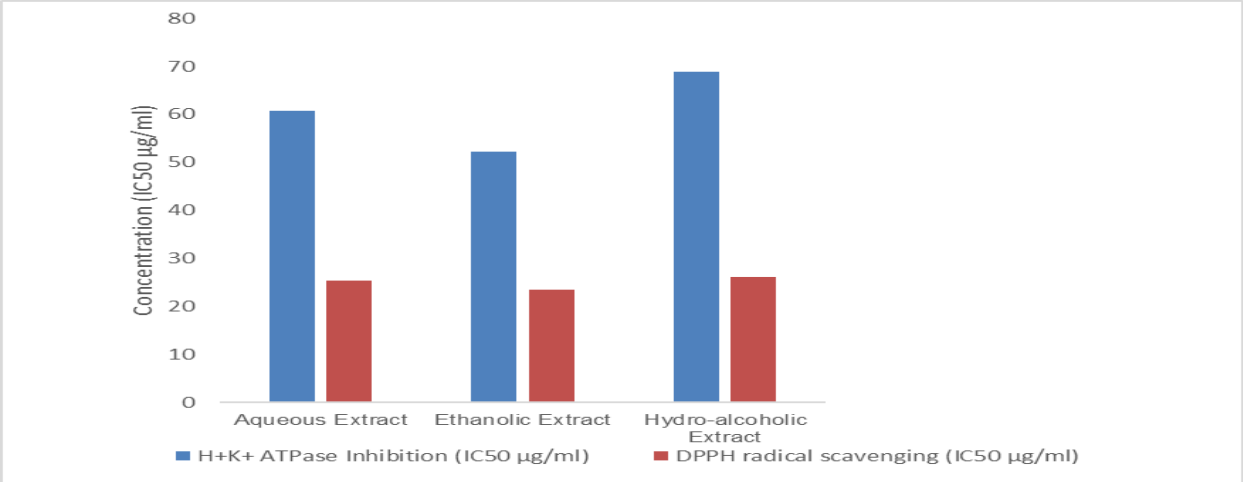


Figure1: Graphical presentation of types of extracts and their H+K+ ATPase inhibition and antioxidant activity

In current study different types of amal extract were prepared like aqueous, ethanolic and hydroalcoholic extracts and determine extractive value, percentage of dried extract, antioxidant activity and H+K+ ATPase inhibition activity. Extractive value in ethanol, water and hydroalcohol was found 15.32±2.35, 15.32±2.35 and 16.65±2.85 %w/w respectively. Percentage of prepared aqueous, ethanolic and hydroalcoholic extract were found 70.24, 50.65, 68.27 %w/w respectively. All extracts showing H+K+ ATPase Inhibition activity and antioxidant activity which is shown in the form of $IC_{50}$ Value. It was observed ethanolic extract of amla showing greater H+K+ ATPase Inhibition activity $IC_{50}$ 52.25±2.45 µg/ml and Antioxidant activity $IC_{50}$23.45 ± 4.26 as compared to aqueous and hydro-alcoholic extract.

## III.CONCLUSION

Aqueous, Ethanolic and Hydro-alcoholic extracts of amla having potent antioxidant and H+K+ ATPase Inhibition activity.

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