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Therapeutic Effect and Characterisation of Ethanol and Acetone Extracts of *Aegle marmelo*

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ABSTRACT: Indian historical medicine has a significant property to heal various human diseases. Bael (*Aegle marmelo*) leaves contains natural antioxidant source helps to promote several health benefits and has many health promoting effects. This plant is well known to possess hypoglycaemic, antibacterial, antifungal, anti-inflammatory, radio protective, anti-oxidative and anti-cancer activities. The present study is to investigate *Aegle marmelo* leaves using ethanol and acetone extract by cold extraction method. Phytochemical qualitative and quantitative analysis were studied. The amount of total anti-oxidant capacity (TAC), ABTS assay, DPPH assay also studied. This antioxidant efficacy revealed the activity of ethanol and acetone extracts of *Aegle marmelo*. The Percentage of Protection of anti-inflammation for samples and standard (Diclofenac) were found. The anti-diabetic capacity by Alpha amylase and glucosidase inhibition assay showed the anti-diabetic capacity for both ethanolic and acetone extracts. The results infer that ethanolic extract shows nearly best efficacy compared to standard (Acarbose). The Antibacterial activity was analysed against *Staphylococcus aureus* and *E. coli* by resazurin method. Hence we confirmed that the *Aegle marmelo* ethanolic extract shown the best efficacy to that of acetone extract, when comparing all the study results. Both the extracts are potential source of phytoconstituents, along with natural anti-oxidant, anti-inflammatory, anti-diabetic and anti-bacterial efficacy, which helps to understand the traditional knowledge of the medicinal plants and can be suggested as therapeutic agent's usage.

KEYWORDS: *Aegle marmelo*, anti-bacterial, anti-diabetic, anti-inflammatory, anti-oxidant and phytochemicals

I. INTRODUCTION

Aegle marmelo L. (L.) is an herbal plant which usually used in Ayurveda treatment, has come under the genus *Sapindales* and *Rutaceae* family, origin to Southeast Asia and India and it is alone and only member of monotypic genus *Aegle*. The common name of *Aegle marmelo* L. is commonly called as golden apple, bael, bengal quince and Japanese bitter orange. For the integration and normalization of this herb systematically in nutraceuticals and Ayurvedic medicines, the identification and standardisation of bioactive ingredients of this plant is essential [1]. Extracts from its leaves, roots, fruit and bark revealed the effectiveness on various infections causing bacterial strains [2]. In India, on account of cost effective, health care and toxicity, when compared to synthetic medicine *A. marmelo* is one among most important medicinal plant and has tremendous medicinal importance. It was recognised that plant extracted elements are used against several infections, mainly used in the rural areas, who mainly affect from malnutrition and various health complications [3].

Medicinally, as a whole part of the tree has significant importance and also used in Indian medicinal system. Each and every parts of the plant proves its effect of anti-viral, anti-diarrheal, anti-bacterial, anti-fungal, radio protective, anticancer, chemo preventive, antiulcer, antioxidant, antigenotoxic, antimalarial and anti-inflammatory capacity, which helps for the prevention and helps to heal several acute and some chronic infections [4]. Secondary metabolites are implemented continuously in an extensive range of approaches such as modern pharmacology, in plant science, geomedicine and agrochemical and also in food industry [5]. Tannins, saponins, flavonoid, proteins, steroids, alkaloid, terpenoids, coumarin derivatives, amino acid and carbohydrates are the secondary metabolites present in leaf extract as reported [4]. Antioxidants suppress oxidation by several methods like free radical scavenging, pair with electrons and chelate the free catalytic metals. Natural anti-oxidant compounds lack in different conditions. Therefore, intake of natural antioxidant as antioxidant compounds is highly important. The therapeutic advantages of certain significant

plants are consistently recognized to their antioxidant phytochemicals. It could be recommended that there is an opposite correlation between rich in anti-oxidant foods and have highly prevailing for pathogenic diseases. Plant form anti-oxidants can be suggested better effect than artificial ones based on its multiple implement of actions and also it is harmless in nature. These facts have inspired universal screening of plants for medicinal and antioxidant activity [6].

Based on the negative side effects of artificial anti-oxidant, nutritionally and healing capacity of antioxidant has highly insistent for future expectation. Moreover, parasites, which can causes major health issues by producing communicable diseases, obtained capacity to resist the available antibiotics. Currently, there has gained a more interest on to discover new drugs utilizing from medicinal plants with an overview to minimizing notable reactions associated with artificial antimicrobials [7]. Therefore, the current study aimed to investigate on the leaves extract of *Aegle marmelo* L using ethanol and acetone. To study its phytochemical qualitative and quantitative screening, anti-oxidant, anti-inflammatory, anti-diabetic and anti-bacterial potential of both the extract. The research findings are expected to give scientific and baseline information of *Aegle marmelo* L.

II. RELATED WORK

Some scientists have reported that flavonoids and terpenoids content were present in extract of *A. marmelo*, *C. roseus* and *A. conyzoids* leaves which was relevant when compared with the present study. [7]. Arumugam Perumal et al 2018 have discussed properties of *A. marmelo* leaf powder using polar and non-polar solvent like Hexane, Ethyl acetate, Aqueous, Methanol and Ethanol, In which ethanol extract of *A. marmelo* revealed that antioxidant DPPH scavenging activity of IC_{50} was 78.36 $\mu\text{g/mL}$, Phosphomolybdenum reduction assay shown IC_{50} of 41.35 $\mu\text{g/mL}$, ferric reducing power assay shown 20.58 $\mu\text{g/mL}$ respectively, and also study conveyed that the flavonoid content 92.43 $\mu\text{g/mg}$ were found to be higher when compared to total phenolic content 9.510 $\mu\text{g/mg}$ [22]. The result from relevant research was significant in anti-diabetic capacity ranges from 41.4% - 12.42% for various concentrations of aqueous extract and also performed 50% pancreatic lipase inhibition for fresh leaves and roots of *Aegle marmelo* [23]. *Aegle marmelo* leaves extracts of ethanol and acetone shown good antimicrobial efficacy by various concentrations against different bacterial strains such as *S. aureus* and *E. coli* [24]. Hence present study aimed to prove the effectiveness of ethanol extract compared to the acetone extract with the baseline methods.

III. MATERIAL AND METHODOLOGY

Collection of the drug *Aegle marmelo*: The fresh leaves powder of *Aegle marmelo* were collected from local market and used for this study.

Cold extraction: 10 g of sample was measured and soaked in 100 ml of ethanol and acetone respectively. The extracts were allowed to stand overnight and filtered using sterile filter paper. The filtrate was collected in sterile container and incubated at room temperature for evaporation. Then measure the weight and find the yield by calculating initial weight - final weight.

Phytochemical analysis: Qualitative screening of ethanol and acetone extracts were tested for the presence of bioactive compounds by using standard methods respectively [8-12]. 1ml of crude extract is taken in each test tube for the qualitative analysis. Quantitative screening of total tannin content was estimated using Sulaiman et al. [13] method. Where Gallic acid is used as a standard. Total flavonoids are quantitatively analysed as per Sulaiman et al. [13]. Quercetin was used as a standard. Quantitative protein analysis were followed from Sarkar et al. [14]. Different volumes of standard (Bovine serum Albumin-BSA) and samples were tested.

Anti-oxidant activity: The total antioxidant capacity (TAC) was evaluated with customized Aliyu et al. [15] method. Where ascorbic acid used as a standard. Evaluation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays for the extracts were performed according to Shalaby et al. [16] and Tailor et al. [17]. The ABTS scavenging capacity of the plant extracts were compared with butylated hydroxyl toluene (BHT- Standard), the free radical scavenging capacity of the extracts was compared with BHT (standard), and the scavenging activity percentage was calculated using the following formula,

ABTS radical scavenging activity (%) or % DPPH Radical Scavenging Activity,

$$= \{(\text{O.D}_{\text{control}} - \text{O.D}_{\text{sample}}) / (\text{O.D}_{\text{control}})\} \times 100$$

Where, $\text{OD}_{\text{sample}}$ – Absorbance of the tested compound; $\text{OD}_{\text{control}}$ – Absorbance of the control.

Anti-inflammatory Assay (Human red blood cell (HRBC) membrane stabilization assay): The percentage of anti-inflammatory activity was measured using Bag, Anwesa, et al. [18]. The blood was collected from one normal volunteer (1ml from 31 years old healthy female). Diclofenac (1mg/ml) was used as standard and a blank was prepared without sample. The percentage (%) of HRBC protection (Erythrocyte membrane stability) was calculated by,

$$\text{Percentage of Protection (\%)} = 100 - (\text{OD}_{\text{sample}} / \text{OD}_{\text{Blank}}) \times 100$$

Where, $\text{OD}_{\text{sample}}$ – Absorbance of the tested compound; OD_{Blank} – Absorbance of the blank.

Anti-diabetic activity: The inhibition of α - amylase of the extracts was evaluated by DNS method according to the procedure described by Suhashini et al. [19]. The inhibition of α - glucosidase tests were carried out as described with customized Abdel-Hady, Heba et al. [20]. Ethanol and acetone extract of different concentrations of samples and standard as acarbose were taken. The percentage of alpha amylase and glucosidase inhibition were calculated by,

$$\% \alpha\text{- amylase inhibition} = ((\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / \text{OD}_{\text{Sample}}) \times 100$$

$$\% \alpha\text{- glucosidase inhibition} = ((\text{OD}_{\text{Blank}} - \text{OD}_{\text{Sample}}) / \text{OD}_{\text{Blank}}) \times 100$$

Where, $\text{OD}_{\text{sample}}$ – Absorbance of the tested compound; OD_{Blank} – Absorbance of the blank.

Antibacterial activity: Determination MIC value (minimum inhibitory concentration): The antibacterial activity for the plant extract were performed using Resazurin Microtitre Assay described by Satyajit D. Sarker et al. [21]. Streptomycin (1mg/ml) used as positive control and DMSO (10 μ L) was used as negative control.

IV. EXPERIMENTAL RESULTS

The yield of ethanol and acetone leaves extract of Bael (*Aegle marmelos*) obtained and found to be 1.05 and 1.23g respectively. The phytochemical qualitative assay showed the presence of bioactive compounds like tannins, flavonoid, Alkaloids, protein, terpenoids, cardiac glycosides and phenol in ethanol extract whereas tannin, saponins, flavonoid, Alkaloids, protein and phenol in acetone extract of *Aegle marmelos*. The amount of tannins present in the samples were found to be 117 μ g and 112 μ g in 500 μ g of samples (refer table 1 in Annexure I). The amount of flavonoid content in the samples were found to be 270 μ g and 249 μ g in 500 μ g of sample (refer table 2 in Annexure I). The amount of protein present in the samples was found to be 638 μ g and 588 μ g in 500 μ g (refer table 3 in Annexure I). The amount of phytochemical estimation was shown in the Fig.1 (a-c). Secondary metabolisms such as terpenoids, flavonoids, alkaloids, phenols, cardiac glycosides, tannins, saponins and quinones neither present nor absent in herbs [7]. The enzymatic and non-enzymatic antioxidant assays revealed the activity of the ethanol and acetone extracts of *Aegle marmelos*. The total anti-oxidant in the samples were found to be 320 μ g and 301 μ g at 500 μ g based on TAC respectively (refer table 4 in Annexure I). ABTS assay infers that standard (Ascorbic acid) has 75.3% inhibition at 100 μ g whereas the samples exhibit 50% and 44.2% of inhibition at 500 μ g (refer table 5 in Annexure I). The DPPH assay result (refer table 6 in Annexure I) reveals that, the samples were highly active as the inhibition percentage of the ethanol and acetone extracts of *Aegle marmelos* for 500 μ g were found to be 52.5%, 32.2% and butylatedhydroxytoluene (standard) 99.8%. Fig 2 (a-c) refers the significance of effective anti-oxidant activity of the extracts. The Percentage Protection of anti-inflammation for standard (Diclofenac) was found to be 60.4 % whereas for sample it was found to be 45.9% and 29.2% at 500 μ g (refer table 7 in Annexure I). Anti-diabetic assays such as alpha amylase and alpha glucosidase were done (refer table 8 & 9 in Annexure I). From figure 4 (a) we can infer that the amylase assay, 50% inhibition of standard (Acarbose) attain at 330 μ g whereas the ethanol and acetone extract exhibits at 372 μ g and 489 μ g. Also from figure 4 (b) glucosidase inhibition assay infers that standard (Acarbose) has 50% inhibition at 200 μ g whereas the sample exhibited 33.3 % and 38.8 % activity at 500 μ g. Both assays showed that both the extracts has the anti-diabetic capacity. The Antibacterial activity was analysed against *E.coli* and *Staphylococcus aureus* by resazurin method and sorted the values in table 1. The MIC value for ethanol and acetone extract was found to be 62.5 μ g, 31.2 μ g and 250 μ g, 125 μ g respectively. Compared with acetone, ethanolic extract revealed the best antibacterial activity [24].

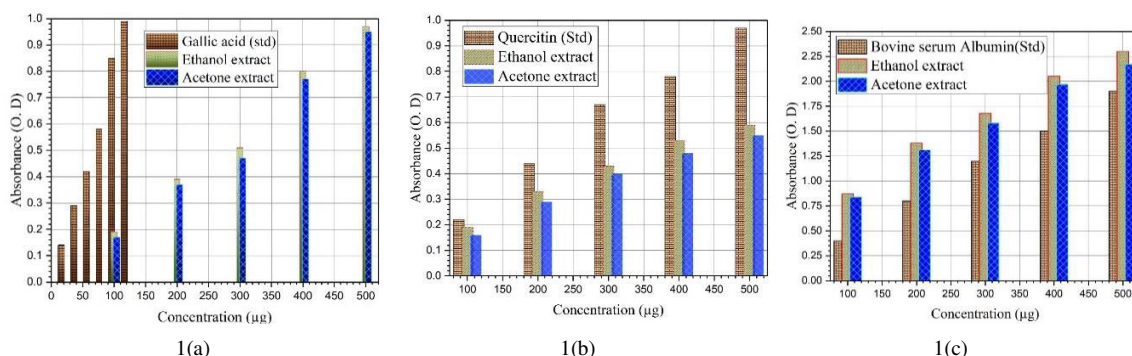


Fig: 1. ethanol and acetone leaves extracts of Bael (*Aegle marmelos*) (a) estimation of tannins with standard (Gallic acid), (b) estimation of flavonoid with standard (Quercetin) and (c) Estimation of protein with standard (Bovine serum Albumin- BSA).

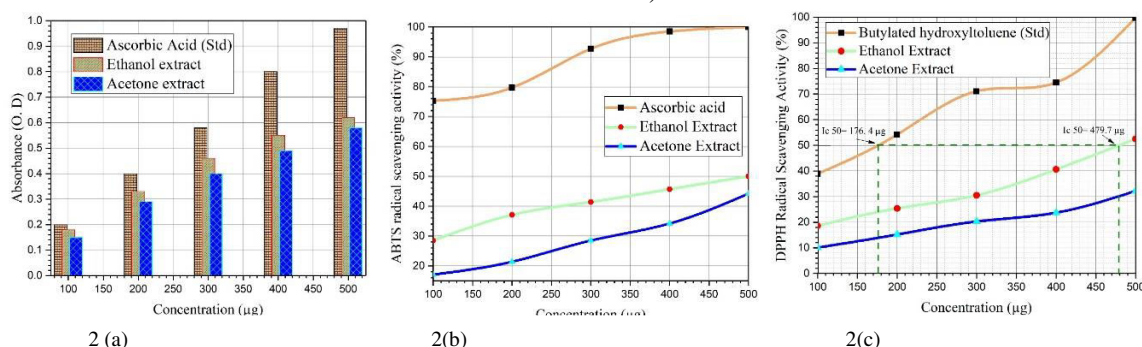


Fig: 2. Antioxidant activity of ethanol and acetone leaves extracts of Bael (*Aegle marmelos*) (a) Total antioxidant capacity with standard (Ascorbic acid), (b) ABTS radical scavenging assay with standard (Ascorbic acid) and (c) DPPH scavenging assay with standard (BHT).

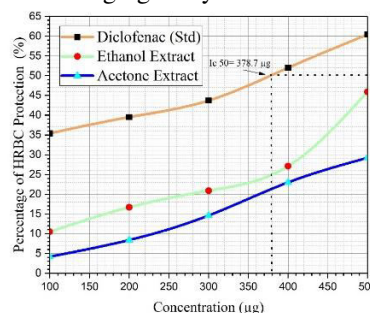


Fig: 3. Anti-inflammatory activity of membrane stability assay for ethanol and acetone leaves extracts of Bael (*Aegle marmelos*) and standard (Diclofenac).

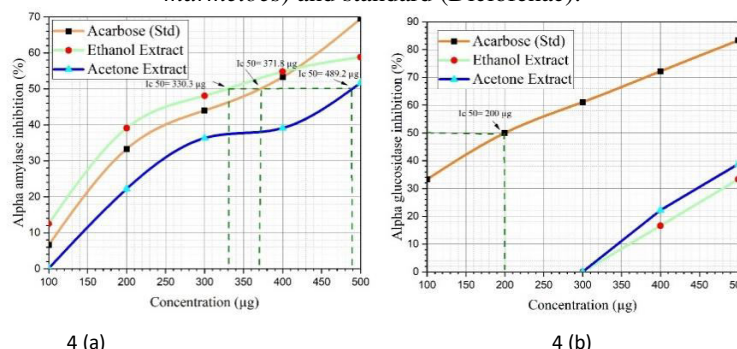


Fig: 4 Antidiabetic activity of ethanol and acetone leaves extracts of Bael (*Aegle marmelos*), inhibition of (a) alpha amylase with standard (Acarbose) and (b) alpha glucosidase with standard (Acarbose).

Table1. Antibacterial assay: MIC Value of ethanol and acetone leaves extracts of Bael (*Aegle marmeloes*) against *Staphylococcus aureus* and *E.coli* by resazurin method.

Microorganisms/sample	MIC Value (μ g)
Ethanol extract	
<i>E.coli</i>	62.5
<i>Staphylococcus aureus</i>	31.2
Acetone extract	
<i>E.coli</i>	250
<i>Staphylococcus aureus</i>	125

V. CONCLUSION

In conclusion, the present study ethanolic and acetone extract of *A. marmeloes* leaves revealed biologically active phytochemical qualitative and quantitative activity. *Aegle marmeloes* can be considered as promising resources for antioxidants, anti-diabetic, anti-inflammatory and potent antibacterial activity. Further study is needed to isolate chemical compounds responsible for such natural secondary metabolites and may lead to their use as safe alternatives to synthetic drugs.

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