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# Study of Antibacterial activity of Mulethi and Amla against gram negative bacteria Escherichia coli

**H A Padwal, N B Kamble, C P Thomas, A J Rathore, V S Narayane**
Department of Zoology, Birla College, Kalyan, Maharashtra, India

## ABSTRACT

Present investigation is performed on comparative study of antibacterial potential of two plants viz., Glycyrrhiza glabra and Emblica officinalis commonly known as Mulethi and Amla respectively, against the gram negative bacteria Escherechia coli. E.coli is a well-known and a commonly used bacterium which is a useful tool for genetic research because of its relatively small size, easy availability and ability to grow rapidly under conducive situations. Glycyrrhiza glabra is commonly used as condiment in sweets, teas, soft drinks and tobacco products. It not only acts as a good flavouring agent but is also widely used home remedy because of its medicinal properties. This medicinal herb has wide reaching health benefits in naturally treating sore throat, chest congestion, rheumatoid arthritis, strengthening of bones and muscles, kidney problems, mouth ulcers, etc. According to Ayurveda, Emblica officinalis balances all the three fundamental bio-elements of the body (viz., pitta, vatta and kapha). Apart from its ability to soothe digestion, Amla may be used as a rejuvenating agent, anti-inflammatory agent, and anti-diabetic agent, helps to reduce fever, stimulate hair growth and enhance intellect. Aqueous and crude extracts of these plants were prepared to find their antibacterial efficacy. The crude extracts of Mulethi and Amla -exhibited greater antibacterial properties against the bacteria, as confirmed with the help of antibacterial assays. The growth inhibition of bacteria was studied using growth curve assay at 660 nm using Elico CL63 photometer. Well diffusion assay was performed for 1%, 10% and 100% crude extracts for either plant materials and it was found that 100% extract had maximum antibacterial effect.

**Keywords:** E.coli, Glycyrrhiza glabra, Emblica officinalis, antibacterial assay, growth curve, well diffusion, crude extract, Photometer.

## I. INTRODUCTION

Indian subcontinent and India in particular is known for its rich wealth of medicinal plants, spices, ornamental plants as well as the plants of agricultural importance. In the vastness of dense Indian forests many tropical medicinal herbs are hidden some with known ethnic uses and some, yet to be explored.

'Green medicines' obtained from these plants has high curative ability and gaining the importance around the globe as they are considered as very good alternatives with lesser known side effects over the synthetic drugs.

Glycyrrhiza glabra also called liquorice is known for many medicinal uses. It is also known for its other uses as confectionary and in preparation of tobacco. Studies over the past 50 years have yielded information about pharmacological and physiological effects of liquorice on the intact adrenal gland. Earlier studies suggested that G.glabra is useful in treatment of Addison's disease due to the presence of chemicals which mimic corticosteroids secreted by healthy adrenal gland;

however, wide information is available about its role in treating diabetes as its inherent sweetness is thought to reflect its medicinal use to treat high sugar levels. G.glabra is sweet in taste due to the presence of a sugar glycyrrhizine (a demulcent starch) in its roots[1]. It has also been reported to have various pharmacological applications like Anti-tussive & expectorant, Antioxidant, Skin lightening and skin tightening, Anti-bacterial, Anti-malarial, Anti-inflammatory, Anti hyperglycaemic, Immunostimulatory effects[2]

Emblica officinalis also known as Amla or Indian gooseberry is native to India and is believed to have the capacity to increase the defence against diseases. It has its curable and beneficial role in treating cancer, diabetes, heart trouble, ulcer, anaemia and various other diseases. It shows a variety of applications as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective. Additionally, it is useful in enhancing memory, curative on ophthalmic disorders and effective in lowering cholesterol level[3]. Previous studies have shown that these plants had been of great medicinal use. Stems and roots of GG are used in the treatment inflammation, ulcers and allergies as it contains certain active components which are said to be responsible for anti-ulcer, anti-inflammatory, anti-diuretic, anti-allergic and antioxidant properties[4]. EO plays a major role in the treatment of Diabetes mellitus, hepatomegaly, leucorrhoea, inflammations, skin diseases, greying of hair and also has an antisenescent activity[5,6]. Studies have shown different properties of GG and EO. Both of these herbs are well known for its anti-microbial and anti-bacterial property. Present investigation is based on the antibacterial property of both G.glabra and E.officinalis.

## II. METHODS AND MATERIAL

**Collection of Sample**:
Fruits of E.officinalis and stems of G.glabra were collected from the local market.

**Preparation of Extracts**:
The cleaned and cut samples were oven dried over 37°C for 2 days. The dried samples were powdered using clean grinder and were stored in sterile containers at room temperature before extraction. Soxhlet Apparatus was used for extraction of Mulethi and Amla. Extract was stored at room temperature in sterile containers. For crude aqueous extraction, decoction process was done by boiling dried powder of the plant with sterile water in 1:1 proportion.

**Preparation of bacterial suspension**:
Pure isolated colonies of E.coli were obtained from the Department of Microbiology, Birla College, Kalyan. E.coli was sub-cultured by growing the colonies on nutrient agar slants and preserved in refrigerator.

**Preparation of nutrient broth:**
25ml of sterile nutrient broth was prepared using distilled water, and stored at 4°C

**Preparation of nutrient agar plates:**
20ml of sterile nutrient agar was poured on sterile plates.

**ANTIMICROBIAL ASSAY: GROWTH CURVE**:
24ml of sterile autoclaved nutrient broth was inoculated with 1ml of soxhelated mulethi stem extract and 1ml of E.coli culture in triplicate. It was then inoculated in Remi Orbital shaking inoculator at 37°C for 24h, growth was determined at regular intervals of 1hr using Elico CL 63 Photometer at 660 nm. Similar methods were followed for dry and wet Amla extract. Experimental control group was also studied for comparison.

**ANTIMICROBIAL ASSAY: WELL DIFFUSION METHOD:**
Sterile Agar plates were used as medium for screening antibacterial activity. About 15 to 20 ml of agar was poured in the sterile petridishes which were allowed to solidify. Wells of 1cm were dug out using a sterile cork borer in solidified agar medium. 1ml of 24hr old cultures of E.coli was inoculated evenly using a sterile spreader. Dilutions were made from the crude extract that was used for inoculation. Dilutions of 1%, 10% and crude extract that is 100% of solutions were used. The plates were incubated at

37°C for 24hrs and observed for inhibition zone of growth around the wells[7].

## III. RESULTS AND DISCUSSION

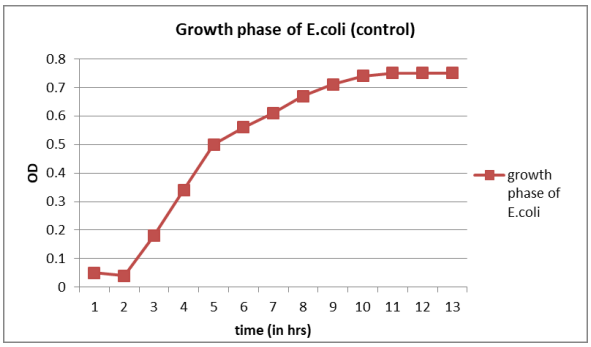


**Figure 1.** Growth curves of E.coli for Control.

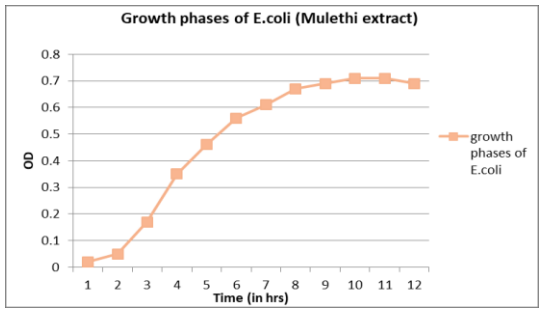


**Figure 2.** Growth curves of E.coli for Mulethi aqueous extract.

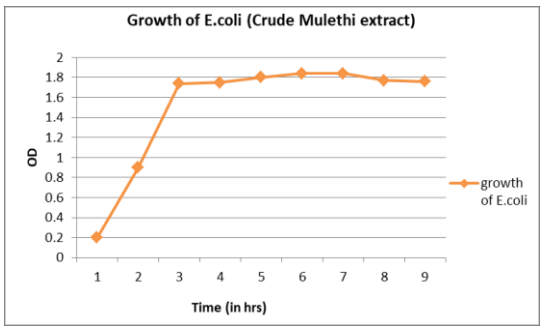


**Figure 3.** Growth curves of E.coli for Mulethi crude extract.

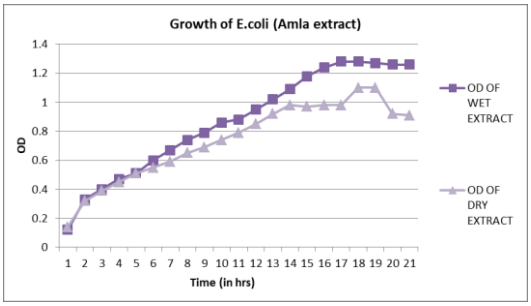


**Figure 4.** Growth curves of E.coli for Amla aqueous extract.

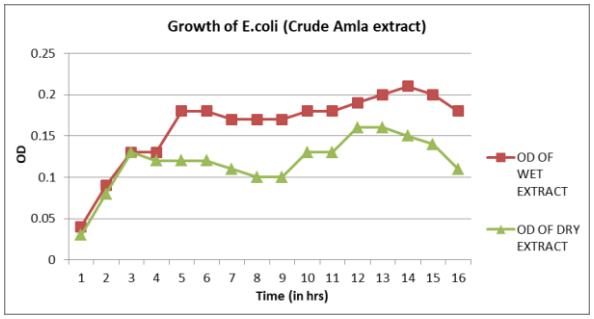


**Figure 5.** Growth curves of E.coli for Amla crude extract.

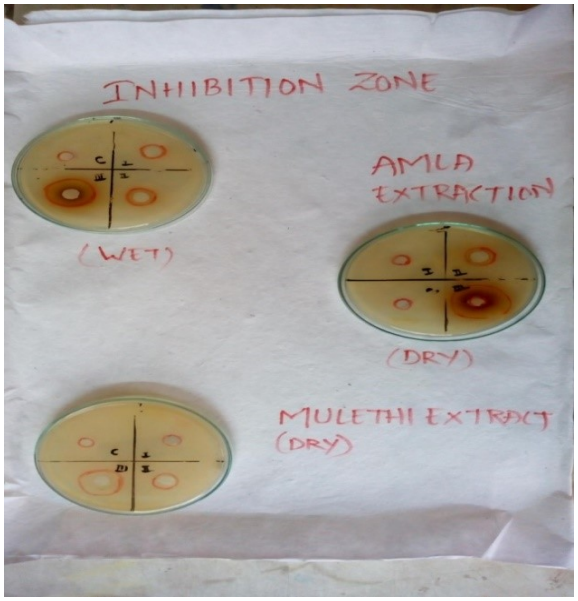


**Figure 6.** Inhibition zones (mm) for the wet extract of Amla, dry extract of Amla and dry extract of Mulethi obtained by a well diffusion method

**Table. 1.** Inhibition Zones (Mm) For The Wet Extract Of Amla Obtained By A Well Diffusion Method

| Extract used | Control | Dry extract of Mulethi | | | Dry extract of Amla | | | Wet extract of Amla | | |
|---|---|---|---|---|---|---|---|---|---|---|
| % Dilution | 100% | 1% | 10% | 100% | 1% | 10% | 100% | 1% | 10% | 100% |
| Average Diameter of Zone of Inhibition in mm | 0 | 1 | 6 | 14 | 2 | 8 | 17 | 7 | 9 | 15 |

Comparison of control bacterial culture with that of Soxhlet aqueous extract (Pure extract) yielded expected outcome. Although very little difference was seen in the growth curve, it is noticeable that the stationary phase arrived slightly earlier in case of pure extract "Fig.2" than that of the control "fig1" on the other hand it can be commented from the growth curve prepared after using crude extract "Fig.3" that the stationary phase arrived much earlier than control as well as pure samples. Time taken by the culture to arrive at stationary phase in case of crude sample is nearly half to its other counter parts.

Results produced by well diffusion method are in corroboration with those shown by growth curve as it can be seen from the "Fig.6" and table.1 that the zone of inhibition is much larger when crude extract is incorporated in nutrient agar.

Two separate samples of Amla were prepared as given in the methodology where one sample was prepared using wet Amla and the other using dry Amla. In both cases a general comment can be made that the stationary phase arrived much latter than their control counter parts. When compared among them it can be commented that the extract made out of dry Amla was less effective than the wet amla as again the arrival of growth curve was slower in former than the latter "Fig.4 and 5".

Growth curve obtained using crude extract showed contradictory results as it was found that extracts made using wet sample were more effective than the one made out of dry sample confirmed as the stationary phase appeared late in the former than the latter.

Results obtained from well diffusion "Fig.6" assay showed nearly similar results and hence on that basis it is difficult to comment which sample is more effective.

Plants have formed the basis for traditional medicine system and natural product make an excellent and wide way out for any new drug development[8]. Approximately 80% of the world's population rely on the natural remedies as their primary health care system[9]. The World Health Organization (WHO) is encouraging, promoting and facilitating programs based on herbal medicines and their uses[10]. Bacteria have the genetic ability to transmit and acquire resistance against the drugs which are utilised as the therapeutic agents.[11]

Glycyrrhiza glabra (GG) (licorice, Fabaceae/Papilionaceae) is a plant with a rich ethnobotanical history[12].The active components are triterpene saponins, glycyrrhizin and glycyrrhetic acid, which makes this plant more effective in treating many diseases and thus becoming one the best home remedy due to its antioxidant property which also helps to fight against low blood pressure[13.] Studies on GG extracts have been shown to possess antidepressant-like, memory-enhancing activities and produce antithrombotic effects. On the other hand, the root extracts are reported to exhibit anti androgenic and antitumor activities and radio-protective effects. Besides that, the extracts from GG roots viz. glabridin (an isoflavan) and isoliquiritigenin (a flavonoid), are known to be

pharmacologically active compounds[2]. Glabridin is reported to be a potent antioxidant towards LDL oxidation, whereas isoliquiritigenin is known to exert anti-platelet, anti-viral, estrogenic activities and has the protective potential against cerebral injury.[14]

Inhibition zone for Methanolic extract of GG was found to be 9mm, 11mm and 25mm[15] when compared with the results after investigation it can be clearly seen that the zone of inhibition with crude aqueous extract arrived much earlier (Table.1). Therefore, crude aqueous extract of GG shows more potency against E.coli than the methanolic extract.

Emblica officinalis (EO) is a good source of polyphenols, flavones, and tannins and other bioactive compounds[16]. It contains many alkaloids like phyllantine, phyllembein, phyllantidine, flavonoids like kaempferol, quercetin, citric acid, and phenolic compounds like gallic acid, methyl gallate, ellagic acid & trigallayl glucose as its active components and Chebulagic acid, Emblicanin-A, Gallic acid, Emblicanin-B, Punigluconin, Pedunculagin as its few chemical compounds[3].(3). The major components like tanins, citric acid, phyllembein; pectin smoothens the digestion and also helps in other digestion related problems. EO is one of the important ingredients of Chyavanprash and a constituent of Triphala powder[17]. It is reported to be one of the important constituent in many of the ayurvedic tonics, as the active compounds helps in reducing nausea, excessive salivation, giddiness, regurgitation, and spermatorrhea. EO also helps in maintaining the body homeostasis and menstrual cycle in women.[18, 19].

Earlier studies showed that the inhibition zone for both methanolic extract and aqueous extract were found to be more or less similar[20]. However, when compared with results it was found to be similar, but, significant differences can be seen for the crude extract of Dry and Wet Amla“Fig.6”.

## IV. CONCLUSION

The present study concludes that the crude extract is more effective than that of pure aqueous extract prepared using Soxhlet in both cases such as Mulethi as well as Amla. When compared among them it can be said that Mulethi is more potent as antimicrobial agent than Amla.

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