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ORIGINAL ARTICLE

Improved Reproductive Efficacy of *Phyllanthus emblica* L. on Testis of Male Swiss Mice and a Pilot Study of its Potential Values

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ABSTRACT **Introduction:** The extract of 'Amlaki' [*Phyllanthus emblic*a L.] of Phyllanthaceae family is high antioxidant and protective properties in damaged tissue. In this study, we investigate the histo- physiological development of testis of adult Swiss mice by introducing its fruit extract. An effort has been made in combination with the experiment and review process to raise awareness for the medicinal significance and utilization of the Amlaki as natural rich sources of novel bioactive components.

Aims: This article examines to assess the efficacy of the male gonadal development of adult Swiss mice of aqueous extract (20 mg/100 gm body weight/30 days) of fruit of 'Amlaki' [*Phyllanthus emblica* L.]. Besides the experimental works, the article also aims to review its remarkable significance on pharmacology and in-vitro antimicrobial activities.

Materials and Methods: Histological parameters and major nutritional components in *P. emblica* fruit extracts (20 mg/100 gm body weight for 30 days) were noticed. Besides, briefly summarizes other medicinal and therapeutic usefulness of this valuable medicinal plant.

Results and Discussions: Treatment with low amount of aqueous extract of an Amlaki fruit, the increased seminiferous diameter, percentage of spermatid and spermatozoa of the testis than an untreated animal. A pilot study proves that every component of Amlaki is beneficial for the treatment of various disease ailments. The fruits of Amlaki in alone or in combination with other plant parts used to treat various diseases. The summarized information might be helpful for future generations who are interested in developing natural drugs also.

Keywords: Amlaki, *Phyllanthus emblica* L. , Reproductive activity, germ population, Testis

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INTRODUCTION

P. emblica L. is an indigenous medicinal plant, found all over India [1, 2]. The entire plant parts including fruits represent a rich source of organic compounds which are used for human health [3]. It contains a high amount of vitamin-C, tannin, polyphenol, carbohydrate, 70 organic bioactive compounds by GC-MS [4]. Treatment with 20mg *E. officinalis* /kg bw/day to infertile male rats exhibited recovery of its fertility[5]. The aim of the study was to understand its male gonadal improvement with an introduction with *P. emblica* if faced any toxicological or smoking effect on its testis and also awareness of its medicinal significance.

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METHODS

Adult male Swiss mice were collected from local suppliers. The mice were maintained in individual metallic cages and kept under ambient temperature conditions (12 L : 12 D, hours of light, humidity 75 ± 2%) in the laboratory. Mice were fed a specially prepared diet (40 g/d/mice) and given water *ad libitum* before treatment. 12 mice of almost equal body weight (60 ± 5 g) were included. Mice were allocated into two separate groups, control and aqueous

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extract (20 mg/100 gm body weight for 30 days) of an Amlaki fruit.

The Amla fruits were freshly collected during February and March from the North Bengal hill slope of West Bengal, India. Fruits parts were dried under Sunlight, then grounded in an electric grinder and submerged in 50% methanol for 48 hrs. The extract was filtered and concentrated to dryness in a flash evaporator under reduced pressure and controlled temperature (50°-55°C) to obtain a Crude extract and was stored at 0°C. The crude extract was diluted with distilled water (20 mg/ml) before use. The aqueous extract was administered orally (20 mg/100 gm body weight for 30 days) by a glass syringe fitted with a specially designed blunt needle. The controls were similarly treated with distilled water.

After 30 days, all male mice were weighed and killed by cervical dislocation between 9:30 AM and 10:30 AM. The right testes were weighed, fixed in Bouin's solution, and embedded in paraffin. These tissues were cut into 6 µm thick sections and stained with hematoxylin- eosin. The germ cell populations were counted at a magnification of 1000× (oil-immersion objective 100× and ocular 10×) from 100 different Seminiferous tubules that had been randomly selected and counted from each mice. With the help of an all Britt disk planimeter, the Seminiferous tubular areas of the testis were noted and the area was magnified with a light microscope of low power (10×10) magnification.

Bivariate correlation analyses were performed on the different study parameters using IBM SPSS version 25. Pearson correlation coefficients were calculated based on the Z-score values to maintain a normalized distribution. P-values less than equal to 0.05 were considered to be statistically significant. Initially, the values of the different parameters were on a different scale. Therefore, to compare them with one another and estimate their relative changes, the values were first scaled on a common range (1-10). Then the values were plotted on a box plot using SPSS software version 25.0 and were grouped based on their category (i.e., Control and treatment). In other words, each parameter is comprised of values from the Control and the treatment group. The variables were standardized by calculating their z-scores. Then the z-scores were subjected to PCA analyses with varimax rotation using SPSS version 25.

RESULTS AND DISCUSSION

The Proximate analysis of the fruit sample showed that Amla contains total ash content (0.22 g/100 g), acid insoluble ash (0.02 g/100 g), moisture content (82.59 g/100 g), total polyphenol (14.99 g/100 g), total Carbohydrate (16.54 g/100 g), tannin Content (23.68 g/100 g) and vitamin C/ascorbic acid (177.67 mg/100 g).

The size of the testis of control and treated mice were normal in shape. Numerous seminiferous tubules, spermatids, and spermatozoa were present in both mice. A very thin basement membrane also is noticed. But in post-treated mice of the 30 days, the percentage of spermatids and spermatozoa were increased significantly. On the other hand, the percentage of spermatogonia was decreased significantly. Seminiferous tubular area and diameter of Ledig cell also increased compared with control (Table 1).

Table 1: Effect of Germ Cell Population, Seminiferous Tubular Area and the Nuclear Diameters of Sertoli and Leydig Cells of Treated with Fruit Extract and Control Mice

Parameters	Control (12 L : 12 D) #	12 L : 12 D + Treatment
Spermatogonia (%) (Mean ± SE)	21.07 ±0.54	14.42±0.89*
Primary spermatocyte (%) (Mean ± SE)	18.85±0.55	17.76±0.58ns
Secondary spermatocyte (%) (Mean ± SE)	17.68±0.47	19.86±0.75**
Spermatid (%) (Mean ± SE)	18.73±0.63	21.0±0.60 **
Spermatozoa (%) (Mean ± SE)	18.59±0.77	21.79±0.63***
Sertoli cell (%) (Mean ± SE)	4.67±0.62	5.33±0.42ns

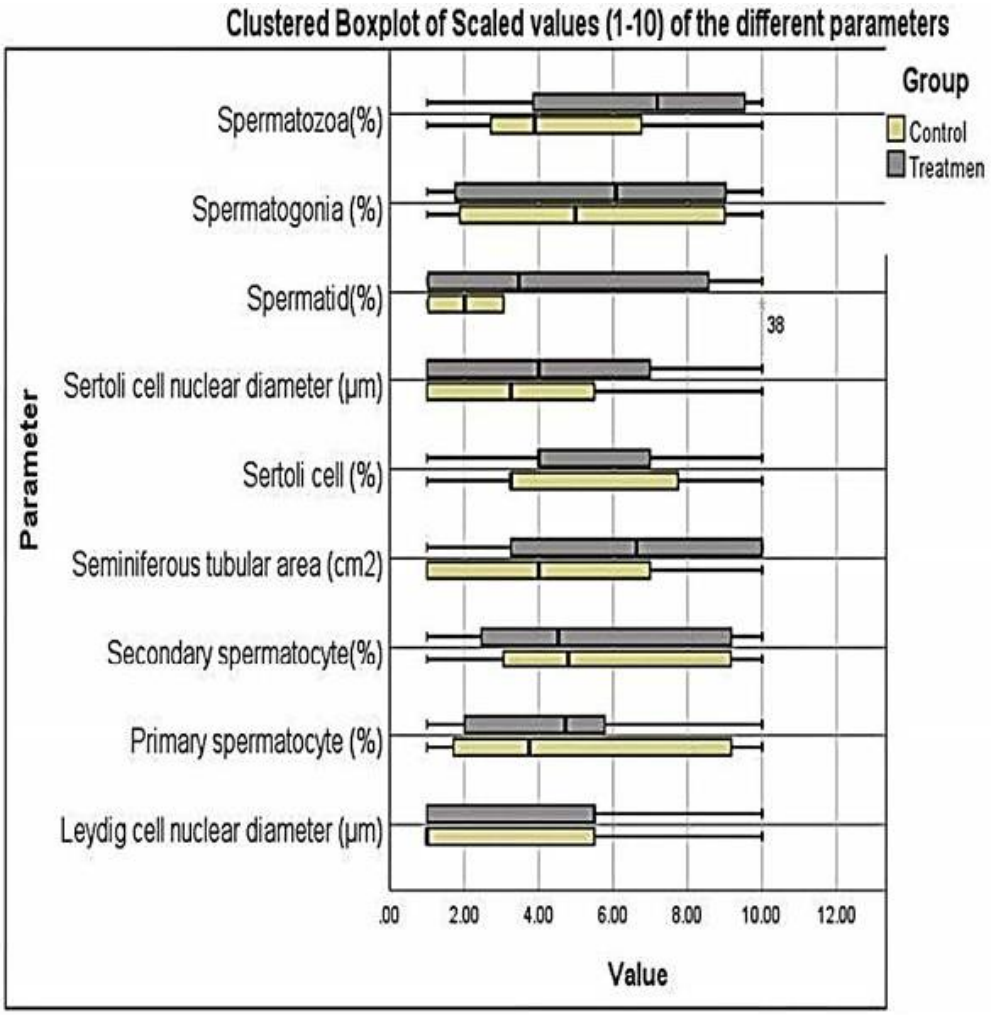
Table 1 (Cont.)

Seminiferous tubular area (cm^2) (Mean ± SE)	15.17±0.48	19.33±0.67*
Sertoli cell nuclear diameter (µm) (Mean ± SE)	3.67±0.48	4.17±0.33ns
Leydig cell nuclear diameter (µm) (Mean ± SE)	3.50±0.34	4.83±0.31**

Note: (# = mice number= 6; SE = Standard Error; ns = Not significant, '*' = > Significant at P = 0.001; '**' = > Significant at P = 0.05, '***' = > Significant at P = 0.01).

From the above table, six significant correlations were observed which are as follows as Sertoli Cells(C) x Primary Spermatocyte (C) = 0.013, Leydig Cell Nuclear Diameter (C) x Primary Spermatocyte (C) = 0.032, Spermatozoa (C) x Sertoli Cell Nuclear Diameter (C) = 0.048 and Sertoli Cell (C) x Leydig Cell Nuclear Diameter (C) = 0.003. It is evident from the Box plot that in most of the cases the median value in the treatment group is higher than the Control group for each parameter except in the case of secondary spermatocyte. In the case of spermatid (%) in the control samples, it has been found that only one case has an extremely high value compared to the others (Figure 1).

Figure 1: Clustered Box Plot to Compare the Distribution Pattern of the Different Parameters Between the Control and the Treatment Samples

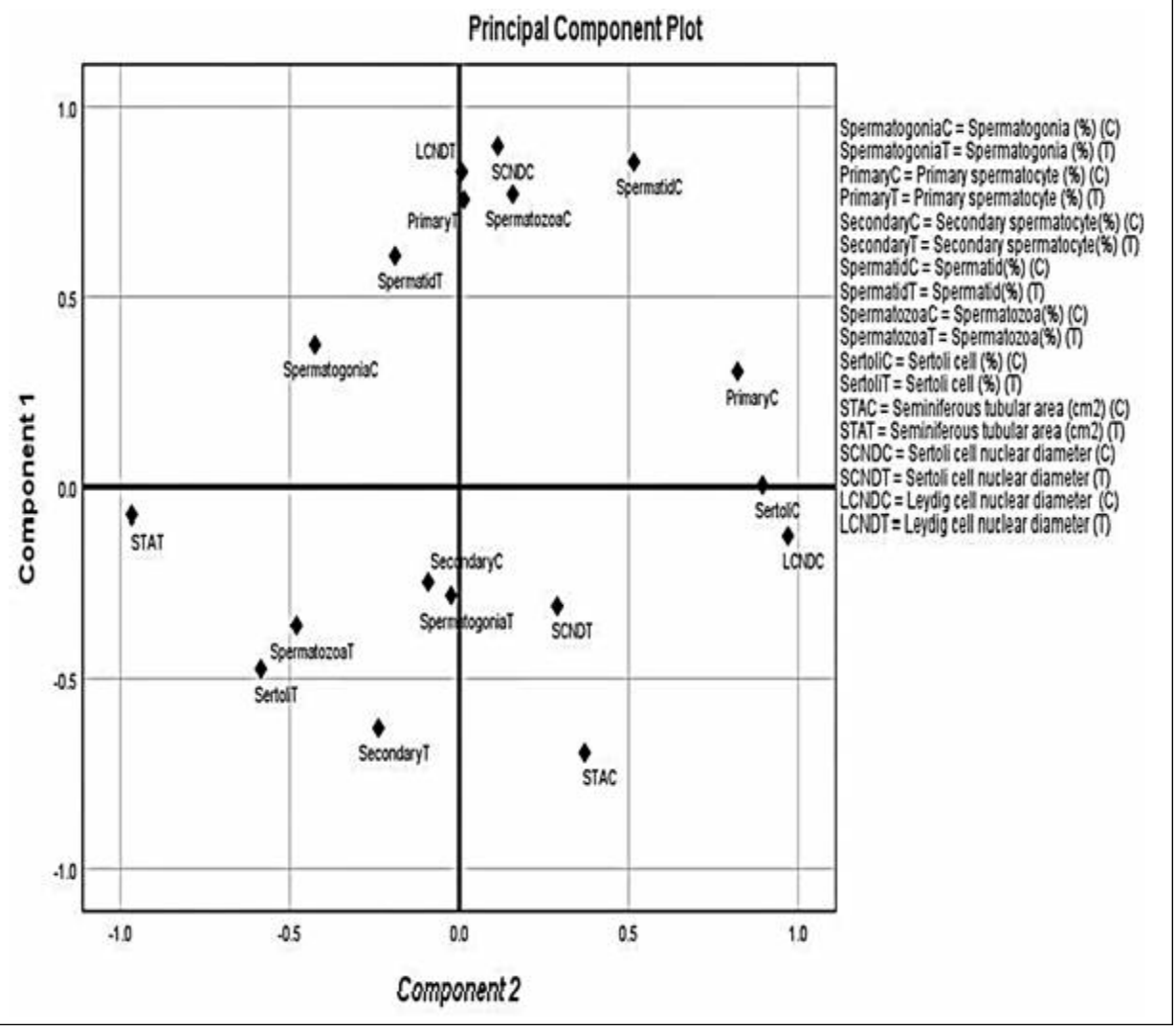


The variables that share the same quadrant in the PCA score plot are likely to have a strong correlation between them. It is observed from the table that 5 out of 18 variables contributed significantly to Component 1 which include Leydig Cell Nuclear Diameter (C), Sertoli cell (%) (C), Primary spermatocyte (%) (C), Seminiferous tubular area (cm^2) (T) and Sertoli cell (%) (T). Similarly, 6 out of these 18 variables contributed to the second component as evident from Supplementary followed by 5 variables each in the case of Component 3 and Component 4, and finally 4 out of 18 variables in case of the 5th component (Figure 2).

From the present pilot study, we observed that Indian gooseberry fruit 'Amlaki' is a traditionally multi-component phytochemicals having clinically proven pharmacological and antimicrobial activities [(Table 2 (a and b)]. Large numbers of biologically active components are present such as alkaloids, flavonoids, tannins, terpenoids, etc which indicate varieties of pharmacological properties such as antitumor, antigenotoxic, antioxidant, anticancer, and ant carcinogenic effects.

In the present study from fruits, we found ash (soluble and insoluble), moisture content, polyphenol, Carbohydrate, tannin, and vitamin C/ascorbic acid. Experimentally in the mice model, large numbers of active fertilizing germ cells viz., spermatid and spermatozoa were noticed in the fruit extract-treated mice than untreated/control. It is well established that spermatogenesis is accelerated on treated mice than untreated which were controlled by various sets of genes. A high amount of vitamin C (Ascorbic acid) was found in the fruit. The role of water-soluble ascorbic acid, important antioxidant properties, in the process of spermatogenesis is well-known. Ascorbic acid was vital for the functional and structural integrity of androgen-dependent reproductive organs. However, some authors claimed that excessive intake of ascorbic acid causes reproductive failure in the male. Under this study, it is noticed that the increased diameter of the

Figure 2: PCA Score Plot to Compare the Distribution Pattern of the Different Parameters Between the Control and the Treatment Samples



seminiferous tubular area indicates the high level of testosterone and nutrients were formed in the testis due to ingestion of a low amount of aqueous fruit extract of amlaki. Ascorbic acid is known as a catalyst for both lipid peroxidation and alteration of unsaturated fatty acid composition. On the other hand, by reviewing the process, the authors are tried to validate clinically their medicinal potentialities. Amlaki is the richest natural source of vitamin C. Due to the presence of strong antioxidants and other biological properties to prevent innumerable health disorders it can be used as a possible food additive in biopharmaceutical industries. In this review, we tried to summarize the medicinal based on therapeutic

Table 2a: Experimental Pharmacological Studies on Various Parts of *Phyllanthus emblica* L. Plant. (w = Week, d = Day, g = gram, mg = milligram, ml = mililitre, µl = microlitre, kg = kilogram, h = hour)

S. No.	Compounds/ Extrac ts	Pharmacological Activity	Experimental M odel/ Study Design	Findings of Effectiveness	Ref.
1	Fruit: Dried aqueous Extract.	Anti-cancer Activity	Human HPV16-(+Ve)(SiHa) and HPV18-(+Ve) (HeLa)cervical cancer cells.	Exhibits its anticancer activities through inhibition of AP-1 and targets transcription of viral oncogenes.	[6]
2	Fruit: Methanoli c Extract.	Anti- diabetic Activity	Streptozotocin (STZ)-induced diabetic rats.	Quercetin changes in the levels of glucose, cholesterol, and triglycerides.	[7]
3	Fruit: Aquous Extract.	Anti- inflammator y and Analgesic Activity	Male Sprague-Dawley rats, weighing 40-60, 100-120, and 200-250 g as well as male ICR mice weighing 30-40 g.	Inhibitory action on the synthesis and/or release of inflammatory or pain mediators may be the main mechanisms of action of *P. emblica* .	[8]
4	Fruit: Ethanolic Extract.	Anti- oxidant and antitumor activity	HT-29 cancer cells MTT colorimetric assay.	*Phyllanthus emblica* possessed strong antioxidant and anticancer activity.	[9]
5	Bark: Hydro- alcoholic Extract.	Anti- oxidant Activity	Male Wistar rats (150-200 g)	The IC 50 value was 188.80 g/mL while that of ascorbic acid was177.7 g/mL.	[10]
6	Fruit: n-hexane, carbon- tetra-chloride, chlorofor m, and aqueous Extract.	Anti-viral Activity	Human Peripheral Blood Mononuclear Cells (PBMCs).	Fruit extract has anti- HIV activity via inhibition of HIV reverse transcriptase activity.	[11]
7	Fruit and leaf: Ethanol Extract.	Anticonvuls ant activity	Strychnine Induced Convulsions in Mice Model.	Fruit extract have more anticonvulsant activity than leaf extract.	[12]
8	Fruit:Aquous Extract.	Anti-apoptosis effect	Male Sprague Dawley rats on contrast-induced acute kidney injury (CI-AKI) model.	Anti-apoptotic activities of PE extract could attenuate renal injury in the CI-AKI model.	[13]
9	Fruit powder.	Anti- hyperlipide mic, hypolipide mic, and anti- atherogenic activity	30 healthy albino rats of Wister strain (*Rattus norvegicus*) weighing 150-200 g either sex.	Amla has shown to possess significant hypolipidemic and anti- atherogenic activity slightly lesser as compared to Atorvastatin.	[14]
10	Fruit: Ethanolic Extract.	Biphasic effect on NSAID (Non steroidal anti-inflammator y drug)-induced ulcer	Male Swiss albino mice (6-8 w, 25 ± 2 g) were kept in 12-h light/dark cycles and housed at 25°C ± 1 °C.	The biphasic effect is due to switching from anti-oxidant to pro-oxidant shift and immunomodulatory property.	[15]

Table 2b: *In-Vitro* **Experimental Studies on Antimicrobial Activities of Various parts of** *Phyllanthus emblica* L. **Plant**

S. No.	Used Extract/ Plant Parts	Microbes Type	Name of Microbes	Remarks/ Experimental Outcome	Ref.
1	Aqueous extracts (Fruits, seed, stem, leaves and root)	Bacteria	*E. coli, Salmonella typhi,S. paratyphi, Staphylococcus aureus, Bacillus sp., Proteus sp., Pseudomonas sp. and Klebsiella sp.*	*Staphylococcus aureus* exhibited the maximal antibacterial activity against the fruit extract. Minimum activity of the extracts was observed against *Salmonella paratyphi.*	[16]
2	Ethanolic Branch Extract (EBE) and Methanolic Branch extracts (MBE).	Bacteria	*Staphylococcus aureus, S. epidermidis, Escherichia coli, Salmonella sp* and *Pseudomonas aeruginosa*	Both EBE and MBE similarly inhibited *S. epidermidis, E. coli, Salmonella sp* and *P. aeruginosa.* However, EBE inhibited *S. aureus* slightly more than MBE.	[17]
3	Petroleum ether (Leaf extract)	Bacteria and *Fungi*	Bacteria–E *nterobacter feacalis, Staphylococcus aureus, Salmonella typhi, Escherichia coli Bacillus subtillus. Fungi- Aspergillus niger, Candida albicans* and *Penicillium notatum.*	Activity–Maximum–*S. aureus* and *E. Coli.* Moderate– *S.typhi* . Comparatively less–*B.subtilis.* Zero– *Enterobacter feacalis*	[18]
4	Hexane, Ethyl acetate, Methanol, Aqueous (Fruit extract)	Bacteria	*Serratia marcescens, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli. Escherichia coli, Staphylococcus aureus, Vibrio cholerae, Salmonella paratyphi A, Salmonella paratyphi B, Shigella spp.,* and *Bacillus cereus.*	All the extracts exhibited significant antibacterial activity, more against *S. aureus* than *E. coli.*	[19]
5	Aqueous (Fruit extract)	Bacteria	*Salmonella typhi,*	Effective in killing *E. coli* , *Salmonella paratyphi A* , *Salmonella paratyphi B* , *Vibrio* spp, *Shigella* spp, and *Bacillus cereus* ; but was most effective against *Staphylococcus aureus* . APE showed a reduced antibacterial activity against *Salmonella typhi.*	[20]
6	Petroleum ether, Chloroform, Alcohol, Amphicillin (40 g/ml) (Leaf and fruit Extract)	Bacteria	*Escherichia coli Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis.*	Alcohol leaf extracts of *P. emblica* exhibited good activity against *S. aureus* The fruit extracts exhibited superior activity against *S. aureus.*	[21]
7	Methanolic, aquous (Leaf and fruits extracts)	Bacteria and *Fungi*	*E. coli, Klebsiella . pneumonie, K. ozaenae, Pseudomonas aeruginosa, S.typhi, S. paratyphi* A2 and B, *S. marcescens. Candida albicans.*	Maximum activity on– Aqueous infusion and decoction against *B. subtilis* and *S. haemolyticus* respectively. Minimum activity on– *Candida albicans.*	[22]
8	Methanol, water, ethyle acetate, chloroform, hexane. (Fruit extract)	Bacteria	*Proteus mirabilis, Klebsiella . pneumoniae, A. baylyi* and *Pseudomonas aeruginosa.*	The ethyl acetate extract (and the methanolic extract to a lesser extent) good inhibitor of the growth of the autoimmune bacterial triggers. Potent against *P. aeruginosa* (prevention and treatment of multiple sclerosis).	[23]
9	Methanol and aqueous. (Fruit extract).	Bacteria	*E. coli* and *Salmonella typhi* (gram –ve), *Staphylococcus aureus* (gram +ve)	Highest antibacterial activity was against gram positive bacteria (*S.aureus*) then the gram negative bacteria (*S.typhi* and *E.coli).*	[24]

Table 2b (Cont.)					
10	Aqueous and methanol (Leaf extract).	Bacteria	*E. coli* and *Bacillus subtilis* .	Combination of amla extract and ofloxacin at 5% concentration is most effective against the *B. subtilis*	[25]

usefulness and clinically proven pharmacological activities of Amla. Amla with its multi-faceted properties may occupy a prominent position in herbal medicinal systems in the next few decades because of its tremendous pharmacological applications and its high nutritious value. The authors are also tried to summarize the conventional use of sun-dried Amlaki for household treatment by indigenous people. In this context, it is essential to generate awareness among general people regarding the beneficial resources of amlaki for a healthy life. Herein, an effort has been made in this review to raise awareness for the medicinal significance and utilization of the amlaki as natural rich sources of novel bioactive components for the welfare of mankind.

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