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Effect of bael bark extracts against *Escherichia coli* - A food borne pathogen

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ABSTRACT

Food borne illness has developed serious threat to children and elderly people and the causative organism is gaining resistance over usage of synthetic formulations and drugs. The usage of medicinal plants could cover up the health aspects and offer newer source of antibacterial, antifungal agents which can be effective against pathogenic organism. The increase in resistant of pathogenic food borne illness causing organism has to be declined by developing newer type of drug which is consists of significant effect over it. The *Aegle marmelos* [bael] of family Rutaceae is considered as sacred tree among Indians and possess good medicinal properties. The food borne pathogen *Escherichia coli* were tested against bark extracts of *Aegle marmelos* by standard well diffusion method at varying concentration from 50mg/ml-250mg/ml. The extracts were prepared by standard procedure of 1:5 ratio of bark and solvent in soxhlet apparatus. The test results were compared with commercial antibiotic ampicillin. The sensitive of *E.coli* against hexane extract of bael bark is 30mm at concentration of 250mg/ml and 24mm of inhibition zone for aqueous extract of bael bark. The commercial antibiotic sensitive against test species is recorded about to be 30mm at 250mg/ml concentration. The bark extracts against *E.coli* were showing sensitive inhibition zones as that of the commercial antibiotic which is of maximum at 250mg/ml concentration and it could easily lead to the development of newer synthetic, chemical and pharmaceutical compound for the treatment or control of food borne illness which is making serious impact on both developing and developed countries. Effective studies against the definite active biological compound could be significantly studied to develop further more chemical compounds for treating other pathogens.

KEYWORDS

Food borne pathogen;
Medicinal plant;
Pathogenic organism;
Well diffusion;
Sensitive zones.

INTRODUCTION

Food borne illness has developed to be a serious threat to public health and incidence of appearance is increasing from developed countries to developing countries. The development of modern and new chemically synthesized drugs is used to treat the pathogenic organism, but some strains develop resistance against these commercial antibiotics. The food borne pathogens has been caused by various microorganisms in which less effective treatment over lack of potential drugs to aid it.

Escherichia coli

Escherichia coli, is a Gram negative typical opportunistic pathogen, which is facultative anaerobic rod shaped bacterium. It is commonly found in the lower intestine of warm blooded animals. *E.coli* is mostly used as model organism in the fields of Biotechnology and Microbiology. In humans, the *E.coli* colonizes in the gastrointestinal tract and coexists in good health conditions. When the animal host is immunocompromised the normal gastrointestinal layer gets weaken and breached for causing illness.

Pathogenesis

The *E.coli* has various pathogenic strains which causes discomfort or illness over the period of time. The clinical syndrome of the pathotypes infections are enteric or diarrhoeal disease, urinary tract infections and sepsis or meningitis. In those intestinal infections are caused by Enteropathogenic *E.coli* [EPEC], Enterohaemorrhagic *E. coli* [EHEC], Enterotoxigenic *E.coli* [ETEC], Enteroinvasive *E.coli* [EIEC], Enteroaggrative *E.coli* [EAEC] and Diffusively adherent *E.coli* [DAEC][1]. The *E.coli* pathotypes which causes extraintestinal infections have been named as ExPEC[2]. The EPEC, EHEC and ETEC can also cause disease in animals using the same virulence factors that are present in human strains.

EPEC

Enteropathogenic *E.coli* [EPEC] was the first outcome of the pathotype of *E.coli* in which the infant diarrhea caused in United Kingdom were the children are not with proper health condition which occurred in 1945. This EPEC *E.coli* remains an important cause for fatal infant diarrhea in developing countries[1]. The bacteria intimately attach to the intestinal epithelial cells and induce pathogenicity. In industrialized countries a typical EPEC *E.coli* are more frequently isolated from diarrhoeal cases that contain the EAF plasmid which is a main causative agent[3]. It has caused large cases of diarrhoeal disease involving both children and adults in developed countries. The model of EPEC pathogenesis is more complex than simple binding to epithelial cells and secretion of an enterotoxin that induces diarrhoea[4,5].

EHEC

Enterohaemorrhagic *E.coli* [EHEC] was first recognized in 1982 which caused bloody diarrhoea, non bloody diarrhoea and hemolytic uremic syndrome. The EHEC *E.coli* settles at bovine intestinal tract by the consumption of under cooked food, unpasteurized milk and juice contents. The transmission of EHEC *E.coli* by airborne were also reported[6]. The EHEC strains of E.coli O157:H7 serotype is mostly pathogenic in food borne illness conditions and prominent in the countries like North America, United Kingdom and Some developing countries. The potential adhesion is released by EHEC E.coli O157:H7 which causes the significant gastrointestinal tract infections[7].

ETEC

Enterotoxigenic *E.coli* [ETEC] causes watery diarrhoea which can be mild raging to severe purging disease. The organism of ETEC *E.coli* in developing countries is the main cause of childhood diarrhoea and causing travelers diarrhoea[1]. The ETEC *E.coli* colonizes in the bowel mucosa and elaborates enterotoxins which give rise to intestinal secretion. ETEC enterotoxins belong to one of two groups of heat labile enterotoxins and heat stable enterotoxins. In that heat labile toxins are class of enterotoxins which closely related and expressed by *Vibrio cholera*[8].

EAEC

Enteroaggrative *E.coli* [EAEC] is recognized at children and adults with persistent diarrhoea in developing countries and developed countries. EAEC *E.coli* infection comprised by colonization of the intestinal mucosa followed by secretion of enterotoxins and cyto-

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toxins[9]. EAEC studies on the human intestinal tract indicates and significant mucosal damage and inflammatory changes[10].

EIEC

Enteroinasive *E.coli* [EIEC] which is genetically closely related to shigella SPP. EIEC *E.coli* causes an invasive inflammatory colitis and occasionally dysentery or watery diarrhoea[1]. EIEC *E.coli* infection is through to represent an inflammatory colitis and comprises of epithelial cell penetration and followed by lysis of endocytic vacuole and invasion into epithelial cells which induce apoptosis in infected macrophases[11].

DAEC

Diffusively adherent *E.coli* [DAEC] which has been the causative agent for diarrhoea for the children of less than 12 months of age[1,12]. DAEC *E.coli* strains induce cytopathic effect that is characterized by the development of long cellular extensions[13]. DAEC strain might induce expression of MICA [MHC Class I Chain Related Gene A] by intestinal epithelial cells indicating that the infection could be proinflammatory or bowel inflammatory disease[14].

The ability of producing toxins by E.coli reinforces that to convert commensal E.coli to Pathogenic E.coli[15].

BAEL [Aegle marmelos]

Bael is the only member of monotypic genus Aegle. Its botanical name is *Aegle marmelos*; it grows in dry forest on hills and plains of northern, central and southern India. It is commonly found in dry hilly areas, gardens, road sides and Lord Shiva temples. It is a medium sized deciduous tree grows upto 18m tall, armed with straight sharp auxiliary throns. Leaves are alternate, foliate occasionally with five foliate and leaflets are ovate, terminal long petioled. Flowers are large, greenish white, sweets scented in short auxiliary panicles. Fruits are globas, yellowish or gray, woody, numerous seeds and colored sweet pulp[16].

Traditional uses

Parts used in the bael tree are fruits, seeds, roots, leaves and bark. The fresh leaf juice is mixed with honey and given as a mild laxative for fever, cattrah and asthma. The leaf juice is mixed with black pepper and given for constipation and jaundice. A decotion of leaves is used as expectorant and asthmatic complaints. The ripe fruit tonic is used as laxative and given for heat, chronic cases for diarrhoea, dysentery and irritation of alimentary canal. It is a useful adorant in the after treatment of dysentery and intestinal disorders. The root and bark are used in the form of decotion as a laxative in intermitted fever and poisoning.

MATERIALS AND METHODS

Plant collection

Bark sample was collected from nearby lord Shiva temple and bark is cut into small pieces. Then the bark sample is kept for shadow drying for 3 weeks and powdered with electric blender.

Extraction

The powdered sample was weighed 25g and packed in the sox-let apparatus with 250 ml of solvent which is 1:5 ratio. The solvent system is used of high polar gradient from hexane-chloroform and distilled water. Then extraction was carried out and the residues were air dried and then the residues were collected and stored in 4ÚC for future use.

Concentration of the extract

The residue of extracted sample was taken and weighed 1g and dissolved in 100 ml of mother solvent and then the varying concentration was taken for analyzing antibacterial activity.

Bacterial species

The food borne pathogen *Escherichia coli* MTCC No: 443 have been procured from Microbial type culture collection centre, IMTECH, Chandigarh, India. The bacterial culture of *Escherichia coli* was sub cultured in Trypticase soy agar slants at 37ÚC and stored at 4ÚC for future use. For the antibacterial screening of the organism, it was sub cultured in the nutrient broth and tested in nutrient agar at 37ÚC.

Media components

The media compounds were purchased from Micro fine chemicals, India.

Composition of Trypticase soy broth:

Ingredients	Gm/lit
Trypticase Soy Broth	0.5
Agar	15.0

Composition of Nutrient agar:

Ingredients	Gm/lit
Beef extract	1.0
Yeast extract	2.0
Peptone	5.0
NaCl	5.0
Agar	15.0

Composition of Nutrient broth:

Ingredients	Gm/lit
Beef extract	1.0
Yeast extract	2.0
Peptone	5.0
NaCl	5.0

Antibacterial assay

Agar well diffusion method was followed for the antibacterial study against varying concentration of the bark extracts ranging from 50mg/ml, 100 mg/ml, 150 mg/ml, 200mg/ml and 250 mg/ml. and compared with commercial antibiotic of ampicillin with varying concentration. The Petri plates were swabbed with *Escherichia coli* MTCC NO: 443 from the broth culture and the wells were made by 6mm of sterile cork borer at minimum distance to the each well. Then the plant extracts has been added to it for the screening of antibacterial effectiveness.

RESULTS

Antibacterial activity

The well diffusion method has shown the extracts effectiveness against bacterial pathogen with varying concentration with respective inhibiting zones [TABLE 1]. The extracts actively diffused for the growth microorganism which has inhibited their growth and resulted in positive note.

The effect of Bael bark chloroform extracts does not have any of inhibition zone ranges for the tested concentration. For the hexane extracts of bael bark has relatively given results comparing with commercial antibiotic of 30mm inhibition zone at the test concentration of 250mg/ml. The *Aegle marmelos* bark of aqueous extract shown sensitivity range of 24mm at the concentration of 250mg/ml. the commercial antibiotic Ampicillin has been recorded for test concentration at 250mg/ml showing 30mm of inhibition range.

DISCUSSION

Recently much attention for the development of plant and their biologically active compounds has directed newer formulations of synthetic compound. The usage of medicinal plants would cover the health aspects for the children to the adult and offer the new source of antibacterial, antifungal and antiviral agents with significant and effective against microorganism. In reference to the earlier studies of *Agele marmelos* parts tested against *E.coli* has been resulted and recorded. The methanol extract of bark and chloroform extract of the bark has shown sensitivity of 4mm each respectively and the leaves extract of methanol has shown 10mm, chloroform extract shown 8mm and 4mm of aqueous extracts of *Aegle marmelos* leaves. The fruit extracts of methanol and chloroform has shown sensitivity of 8mm and 2mm respectively of the inhibition zone ranges[17]. On 10-20mg concentration of *Aegle marmelos* leaves extract against *E.coli* has shown re-

TABLE 1: Effect of bark extract against bacterial pathogen and their inhibition zones.

S.NO:	Species	Con. of the well mg/ml	Zone of inhibition (in mm)			
			Hexane extract	Chloroform extract	Aqueous extract	Standard Antibiotic (Ampicillin)
1.	Escherichia coli	50	11	-	-	12
		100	15	-	7	20
		150	20	-	15	25
		200	25	-	20	27
		250	30	-	24	30

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sistance[18] and does not offer any inhibition zones. The hexane extract of bael bark has shown resistant at concentration of 250mg/ml which has compared with commercial antibiotic has relatively lesser effect on the bark extract[19]. *Aegle marmelos* leaves extract has been tested against *E.coli*, ethanol extract has shown 10.5mm at 400mg/ml concentration, methanol extract recorded has 22.5mm of 400mg/ml concentration and acetone extract revealed 9.0mm at the 400mg/ml concentration[20]. The bark extracts has been relatively to certain extent tested for antibacterial sensitivity against *E.coli* and the results recorded earlier were testing of sensitivity against leaves and fruits. The subject to the tested extracts of *Aegle marmelos* bark has given maximum of 30mm for hexane extracts and 24 mm for aqueous extracts, on comparing to the commercial antibiotic ampicillin has shown relative sensitive of 30mm at 250mg/ml concentration.

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