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Antimicrobial activity of serial extracts from of *Aeglemarmelos*(linn.) Against dysenteric causing gram negative organisms

Manojkumar.K[1] ,seema[2]
1(Department of Microbiology , Baba Haridass College of pharmacy and technology / Board of Technical Education New delhi / India.)
2 (Department of Microbiology, Baba Haridass College of pharmacy and technology / Board of Technical Education New delhi / India.)

Abstract:*The in vitro antimicrobial activity of methanol extracts from Leaves , stream , bark , fruit of Aeglemarmeloswere investigated against bacterial and fungal species. All the extracts exhibitedbroad spectrum antimicrobial activity with zones of inhibition ranging from 10 to 22 mm against bacteriashigelladysenteriae, shigellaflexneri, vibrio cholerae, vibrio parahaemolyticus, Escherichia coli, salmonella typhi, The minimal inhibitory concentrations (MIC) and the minimal microbicidal concentrations(MMC) of the extracts ranged from 1.25 to 10 mg/mL and 2.5 to 20 mg/mL respectively. Assessment of antibacterial efficacy of different extract revealed that The ability of the leaf extracts of Aeglemarmelosto inhibit growth of bacteria and fungi isan indication of its broad spectrum antimicrobial activity which could be a potential source for development ofnovel bioactive antimicrobial agents.*

Key words :*Aeglemarmelos, antimicrobial activity, phytochemicals , serial extracts*

I. Introduction

The continued emergence or persistence of drug resistant organisms and the increasing evolutionaryadaptations by pathogenic organisms to commonly used antimicrobials have reduced theefficacy of antimicrobial agents currently in use. In addition to this, antibiotics are associated with adverse effects, therefore, the search for new drugs from novel sources, such as plants, is necessary. Ithas been pointed out that more than 80% of worldis population depends on plants to meet their primary health care needs (1). Plants continue to be a major source of commercially consumed drugs. Even many synthetic drugs have their origin from natural plant products. The trend of using natural products has increased in recent years and the active plant extracts are frequently screened for new drug discoveries (2). *Aeglemarmelos*(Linn.) belongs to family *Rutaceae*, commonly known as bael (Hindi) and golden apple (English). It is found throughout India and is known from pre-historic time. *Aeglemarmelos*has been used from time immemorial in traditional systems of medicine for relieving constipation,diarrhoea, dysentery, peptic ulcer and respiratory infections (3). Several studies on differentparts of *Aeglemarmelos*showed that the plant possesses antidiarrhoeal (4), antidiabetic (5),anti-inflammatory, antipyretic, analgesic (6), anticancer (7), radioprotective (8) and antimicrobial activities (9, 10). Limited information is available regarding antimicrobial activity of *Aeglemarmelos*leaves; therefore, present study is carried out to investigate antimicrobial activity of serial extracts from leaves of *Aeglemarmelos*against various bacterial and fungal species. Preliminary phytochemical studies of these extracts are also undertaken to find out bioactive compounds having
antimicrobial activity.

II. Materials and methods

2.1Plant material

The leaves of *Aeglemarmelos*were collectedfrom their natural habitat from Delhi India.

2.2 Preparation of extract

The shade dried leaves bark and fruit were powdered using amechanical grinder and passed through 40 meshsieve. Powder (300 g) was successively extractedwith 1.5 L, chloroform in a Soxhlet apparatus at 60n70^{o}C eachfor 10n12 h consecutively. Solvents used were ofanalytical grade and removed from all the threeextracts under vacuum and a semisolid mass wasobtained . Extracts were stored in sterile ambercolored storage vials in refrigerator until used forexperiment.

2.3 Formulation of extract

Each extract was dissolved in 20% dimethylsulfoxide (DMSO) treated water and sterilized bypassing through membrane filter of 0.2 µm pore sizebefore antimicrobial testing.

2.4 Test microorganisms

Bacterial and fungal isolates used in the present study (bacteria*shigelladysenteriae,,shigellaflexneri, vibriocholeravibrio parahaemolyticus*, *Escherichia coli* , *Salmonella typhi,* were obtained from HiMedia Laboratories Pvt. Ltd. Navi Mumbai, culture collections of microbiology departments of All India Institute of Medical Sciences, New Delhi. The bacterial isolates were first subcultured in a nutrient broth and incubated at 37^0C for 18 h.

2.5Antimicrobial activity

The antimicrobial sensitivity patterns for the extracts were studied by disc diffusion method (13).Sterile discs (6 mm) prepared from Whatmanis filter paper no. 1 were made to absorb (500 µg) of the test samples. Discs were left to dry under laminar flow cabinet overnight. Standard reference antimicrobial discs with o-floxicine ,ciprofloxicine(30 µg) for bacteria were used as positive control and solvent discs were used as negative control. The microbial isolates were first grown in a nutrient broth for 18 h before use and standardized to 0.5 McFarland standards (1.5 ◊ 108 cfu/mL). Mueller-Hinton agar was prepared on the plates as the medium for the test organism. The microbial inoculum was spread evenly onto the surface of agar plate using the sterile cotton bud and then the extracts discs, 20% DMSO impregnated discs and standard antimicrobial discs were positioned on the inoculums agar surface. The antimicrobial activity was interpreted from the size of diameter of zone of inhibition measured to the nearest mm as observed from clear zone surrounding the disc. Each plant part extract was assayed in triplicate and the mean of the three values was taken and the pyto chemical study were tabulated in TABLE 3.

2.6 Determination of minimal inhibitory concentration(MIC)

The minimal inhibitory concentrations of different extracts were determined by twofold serial micro dilution method using sterile 96 well microliter plates (14). Hundred microliters of the test extracts at a final concentration ranging from 10 to 0.0049 mg/mL were introduced into the wells before 100 µL of standardized cell suspensions were added in each well. Microbial suspensions were used as a positive control and extract in broth was used as negative control. The MIC was taken as the lowest concentration of the extract in the well of microtitre plate that showed no turbidity after 24 h of incubation at 37^OC. The turbidity of the wells was interpreted as the visible growth of microorganism.

2.7 Determination of minimal microbial concentration (MMC)

The MMC of the extracts was determined by a modification of the method of Spencer and Spencer (15). Samples were taken from plates with no visible growth in the MIC assay and subcultured on freshly prepared nutrient agar plates, later incubated at 37^OC for 48 h for bacteria. MMC was taken as the concentration of the extract that did not show any visible growth on new set of agar plates.

III. Results

3.1Antibacterial activity

All the various parts of plant extracts showed varying degree of antibacterial activity against the test organisms (TABLE 1).

Disc diffusion assay revealed maximum inhibition zones against Gram negative organisms *shigelladysenteriae, shigellaflexneri vibrio choleraevibrio parahaemolyticus*,*Salmonella typhi,* and *Escherichia coli* . chloroform leaves extract suggesting the highest antibacterial efficacy of other plant part extract against these organisms. Further, it compared favorably with standard antibacterial drug o-floxicine. Antibacterial activity of bark extract was moderateagainst *V.cholera*and mild against *Salmonella typhi*. *E.coli*showed maximal zone of inhibition with chloroform extract suggesting highantibacterial efficacy of leaf extract against these organisms. Further, it compared favorably with o-floxicine. The antibacterial activities of leaf extract were moderate against *v.cholera*and *Escherichia coli* and were mildagainst*Salmonella typhi*.bark extract showed maximum zone of inhibition against *Salmonellatyphi*suggesting highest efficacy against this organism. Further, it compared favorably with cefuroxime.The antibacterial activities of methanol extract were mild against the rest of the tested microorganisms.

The MIC of different extracts ranged from 1.25to 10 mg/mL and are shown in TABLE 2.The MIC *shigella*specious organisms were the lowest with bark and fruit extract suggesting that the smallest amount of this extract was required and was most potent. Also the MIC for control cefuroxime ranged from 0.0195 to 0.0391 mg/mL. The MIC for *vibrio* specious werethe lowest with fruit and bark extract suggesting that the

smallest amount of bark extract was required and was most potent. Also the MIC for control cefuroxime ranged from 0.0391 to 0.078 mg/mL. The MIC for *Salmonella typhi*was the lowest with leaf extract suggesting that the smallest amount of this extract was required and was most potent. The MIC of the standard drug cefuroximewas 0.078 mg/mL. The MMC of the petroleum ether, chloroform and methanol extracts for differentbacteria ranged from 2.5 to 20 mg/mL.

IV. Discussion

*Aeglemarmelos*leaf extracts showed varying degree of broad spectrum antimicrobial activities against tested bacterial species. Antimicrobial activities of leaf ,bark and fruit extracts could be attributed to the presence of phenols and sterols as such activities withthese compounds are reported [16, 17]. The antimicrobial activities of leaf extract may be due to the presence of tannins, triterpenoids and flavonoids.Tannins have been known to form irreversible complexes withprolene rich protein resulting in the inhibition of cell wall synthesis [18]. Triterpenoids are known to weaken the membranous tissue, which results in dissolving cell wall of microorganism [19]. Flavonoids, another constituentof methanolleaf extract, have exhibited a large number of biological activities like anti-inflammatory, antioxidant and antimicrobial properties [20]. Antifungal activity exhibited by methanol extracts of *Aeglemarmelos*leaves against all tested organisms be contributed due to the presence of coumarins. components of these extracts that showed these effects were not identified, yet the positive presence of antimicrobial active principles such as phenols, sterols, flavonoids, tannins, triterpenoids and coumarins seems to cause these activities. The ability of the leaf extracts of *Aeglemarmelos*to inhibit growth of bacteria is an indication of its broad spectrum antimicrobial activity, which may be employed as a source to develop new antimicrobialagents.

Table1. Antimicrobial activity of serial extracts from leaves bark and fruit of*Aeglemarmelos*

Test micro organisms	Zone of inhibition (mm) (the mean ± SD)		
	Leafextract(500 µg/mL)	Barkextract(500 µg/mL)	Fruitextract(500 µg/mL)
shigelladysenteriae	16±0.4	10±0.2	4±0.4
shigellaflexneri	18±0.8	11±0.2	5±0.5
vibrio cholerae	16±0.5	9±0.7	6±0.2
vibrio parahaemolyticus	15±0.2	8±0.2	4±0.5
Salmonella typhi	12±0.6	7±0.4	4±0.3
Escherichia coli	11±0.3	9±0.4	5±0.6

Table 2.MIC and MMC values of extracts from leaves of *Aeglemarmelos*and standard drugs in mg/mL.

Test organisms	Leaf extract		Barkextract		Fruitextract		Ofloxicine	ciprofloxicine
	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MIC
shigelladysenteriae	5	20	10	20	10	10	0.0391	0.0098
shigellaflexneri	5	10	10	20	5	2.5	0.0195	0.0049
vibrio cholerae	10	20	1.25	2.5	1.25	2.5	0.0195	0.0098
vibrio parahaemolyticus	5	10	5	10	5	5	0.0391	0.0098
Salmonella typhi	1.25	2.5	2.5	5	1.25	2.5	0.0391	0.0049
Escherichia coli	1.25	2.5	1.25	2.5	2.5	2.5	0.0391	-

MIC = minimum inhibitory concentration; MMC = minimum microbicidal concentration.

Table 3.Phytochemical screening of serial extracts from *Aeglemarmelos.*

Phyto chemical	Leaf extract	Bark extract	Fruit extract
Tannins	-	-	+
Flavonoids	-	-	+
Saponins	-	-	+
Phenols	+	+	+
Coumarins	-	-	+
Sterols	+	+	+
Triterpenoids	-	-	+

(+) present; (n) absent

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