Research Paper

Volume : 3 | Issue : 4 | April 2014 • ISSN No 2277 - 8179

Biometric, Functional Group Analysis, Antibacterial and Antifungal Activity of Terminalia Bellirica Roxb and Phyllanthus Emblica L. Against Some Pathogenic Microorganism

Biochemistry

KEYWORDS : Terminalia bellirica, Phyllanthus emblica, Antimicrobial activity, Microorganism, FT-IR and Organic analysis, Poisoned Food Technique.

V. Eugin Amala — Research Scholar, Dept. of Biochemistry, Govt. Arts College (Autonomous) Kumbakonam, Tamilnadu , India.

Dr.Jeyaraj — Research Supervisor, Dept. of Biochemistry, Govt. Arts College, Kumbakonam.

ABSTRACT

Terminalia bellirica and Phyllanthus emblica has more bioactive principles and it constitutes rich source of medicine. It is locally known as Bahera and Amla, involves in holistic system of medicine. It has anticancer, antihypertensive and also protective effect of the organs. In co-operation with these plants these are having more phytochemical, neutraceutical, pharmacheutical, antimicrobial and antioxidant properties. Phytochemical screening was done to know the major phytoconstituents present in the plant material, organic analysis was done to detect compounds present, FT-IR carried out for the functional group identification, disc diffusion method and poisoned Food technique were done to make out the antibacterial susceptibility and antifungal activity; consequently this result helps further research to open up its beneficial values.

Introduction

Phyllanthus emblica is a fruit rich in vitamin C that builds immunity and an effective antioxidant that removes harmful toxins from the body. It has emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%) [1]. It also contains other polyphenols such as flavonoids, kaempferol, ellagic acid and gallic acid [2]. Phyllanthus muellerianus extracts are antimicrobial. [3] Phyllanthus amarus root and leaf extract showed significant hepatitis-C antiviral activity [4]. Phyllanthus niruri may possibly help prevent stone formation/urolithiasis [5]. These fruits are reputed to contain high amounts of ascorbic acid (vitamin C), 445 mg/100g the specific contents are disputed and the overall antioxidant strength of Amla may derive instead from its high density of ellagitannins [6].Terminalia bellerica fruits are astringent, acrid, digestive, antihelminthic, narcotic, ophthalmic, antipyretic and rejuvenating [7]. Glucoside , Gallo Tannic acid, Coloring matter, resins Ellargic acid, gallic acid, lignans, tannins, ellargic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyl emblin, mannitol, glucose, fructose and rhamnose [8]. The beneficial effects of the crude extracts of this fruits might be due to the synergistic action of some polyphenolic compounds that have glucose lowering activity [9].

According to World Health Organization (WHO) estimates more than 80% of the people in developing countries depend on the traditional medicine for their primary health needs [10]. Crude methanolic extract of the fruits of Terminalia belerica along with its various organic fractions elicited both invitro and invivo antioxidant and antimicrobial activity [11].

Materials and Methods

Sample Collection and authentication

Fresh fruits of Terminalia bellerica and Phyllanthus emblica were obtained from Therambattu, Vellore and Sirumalai, Dindugal the same was identified and authenticated by Dr. John Britto, Rapinet Herbarium, St. Joseph's College Trichy, Tamilnadu, India and given the Voucher Specimen No.VEA/002/2013,VEA/003/2003 respectively.

Preparation of extracts

Terminalia bellerica and Phyllanthus emblica fruits were shade dried, deseeded and pounded into fine powder using a stainless steel blender. Extracts were prepared by using Soxhlet extractor and 95% ethanol filtrates were individually pooled and each solvent removed at 40°C under reduced pressure by rotary evaporator [12]. The methanolic, acetoneic and aqueous extracts were subjected to preliminary screening of various plant constituents.

FT-IR analysis

Fourier Transform Infrared Spectroscopy (Thermo Scientific Nicolet 1S5 FTIR) study was carried out in the department of Chemistry, Annamalai University, Chidambaram, Tamilnadu, India, to identify the functional groups. The extracts of T.bellerica, P.emblica was mixed with KBr salt using mortar and pestle and compressed into a thin pellet. The adsorption capacity of adsorbent depends upon porosity as well as chemical reactivity of functional groups at the adsorbent surface [13] was recorded.

Preparation of Microorganism

The bacterial strains used in this study were gram positive bacteria Staphylococcus aureus (MTCC 3160) gram negative bacteria Psuedomonas aeruginosa (MTCC 1934) and Klebsiella pneumoniae (MTCC 4030). Fungal strains Aspergillus flavus (MTCC 277), Aspergillus niger (MTCC 282) and Candida albicans (MTCC 183) were procured from MTCC, Chandigarh, India. All chemicals media components and antibiotic impregnated discs used in this study from Hi Media, Mumbai, India.

Preparation of Medium

Cultures were procured from MTCC, nutrient agar media had been used for Staphylococcus aureus and Psuedomonas aeruginosa, LB medium for Klebsiella pneumoniae and Czapek Yeast Extract Agar for Aspergillus flavus, Aspergillus niger and Candida albicans. These microbes were sub-cultured and used for the antimicrobial and antifungal activity.

Antibacterial Activity

Required numbers of petriplate were prepared and autoclaved at 121°C for 15 minutes and allowed to cool under laminar airflow chamber. Aseptically transferred about 20ml of media into each sterile petridish and allowed to solidify. 1ml inoculum suspension was spread equivalently over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with plant extract as triplicates and incubated at 5° C for 1 hour to permit good diffusion and then transferred to the incubator at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm) [14].

Antifungal Activity

Extracts were screened for antifungal activity by Food Poisoning method against A.flavus, A.niger, C.albicans. Extract concentration (1000µm) was mixed with sterilized medium in separate flasks and transferred into petriplates and allowed to solidify. In aseptic condition 6mm diameter of fungal culture disc were taken and inoculated to the center of petriplates containing extract asceptically and radial growth of colony was measured after incubation period[15].

Table:1 Phytochemical Screening of T. bellerica Roxb and P. emblica L. fruits extract

Phytochemicals	Terminalia bellirica	Phyllanthus emblica
Test for Alkaloids		
Wagners Test	-	++
Mayers Test	-	++

Test for Flavanoids Alkaline Reagent Test	-	++
Test for Carbohydrates Molisch's Test	++	+
Test for Glycosides Legal's Test	-	+
Test for Saponins Lead Acetate test	++	-
Test for Tannin Ferric Chloride Test Potassium Dichromate test	+++ ++	++ ++
Test for Protein Ninhydrin Test Aminoacid Test	+ +	++ ++
Test for Poly Phenol Ferric Chloride Test & Cyanide	-	++
Test for Quinone Reaction with NaoH	++	++
Test for Caumarin Reaction with NaoH	++	+

- absent, + less effective, ++ effective, +++ more effective

Table: 2 Systematic organic analysis for functional groups

S.No	Experiment	Terminalia bellerica	Phyllanthus emblica
1	Aromaticity	Aromatic (+++)	Aliphatic (++)
2	Solubility	Insoluble in $NaHCo_3$	Acidic
3	Test for Unsaturation	Phenolic or Aromatic Amine (+++)	Unsaturated (++)
4	Sodium Fusion Test	Nitrogen	-
5	Test for Acids	-	Acid
6	Test for Phenol	Phenol	Phenol
7	Test for Carbonyl compound	Aldehyde or Ketone	Aldehyde or Ketone
8	Fehling's Test	Aldehyde	-
9	Tollen's Reagent Test (Legal Test)	Ketone	Ketone
10	Test for Esters	Ester Aromatic Acid	-
11	Test for amine	Aromatic Primary amine (+++)	Aromatic Primary amine (+)
12	Test for amide	Amide	-
13	Test for diamide	Dimaide	-
14	Molisch's Test	Carbohydrate	Carbohydrate

- absent, ++ moderate, +++ high

Table: 3 FTIR peaks of Terminalia bellirica Roxb and Phyllanthus emblica L. fruit extract

Functional Group & Wave length	T. bellirica	P. emblica
OH Alcohol	3337.10	3293.33
CH Alkane	2925.50 2849.59	2922.95 2849.59
C=O Carbonyl Group	-	1724.46
O-H C-O Acid	2587.79 1284.52	-
NH Amide	1657.00 1642.23	-
NO Nitro	1530.08 1545.40	1567.64
C=C Aromatic	1461.81 1442.18	1540.92 1449.79
-C-H Alkane	1381.91	1916.46
CF Alkyl Halide	1326.51	1375.82 1236.34 1116.94 1000.13
C-O Ether	1158.33 1082.71 1045.70	-
=C-H Alkene	923.83 865.30 770.42 707.42	934.92 918.36 863.56 806.04 707.99 696.48

Table: 4 Antibacterial activity of T. bellirica from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Gram Positive		Gram Negative				Mean $\bar{x}$	S.D σ
	S. aureus	%	P. aeruginosa	%	K. pneumoniae	%		
Acetone	11.75	33.81	10.75	30.93	12.25	35.25	11.58	0.62
Methanol	10	33.61	10	33.61	9.75	32.77	9.91	0.12
Aqueous	9.8	32.88	10	33.55	10	33.55	9.93	0.09
Antibiotic	10	27.02	10	27.02	17	45.96	12.3	3.35

Table: 5 Antibacterial activity of *Phyllanthus emblica* from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Gram Positive		Gram Negative				Mean $\bar{x}$	S.D σ
	S. aureus	%	P. aeruginosa	%	K. pneumoniae	%		
Acetone	11	33.58	11.75	35.87	10	30.53	10.92	0.72
Methanol	10	28.98	14.50	42.03	10	28.98	11.50	2.12
Aqueous	11	36.07	10	32.78	9.5	38.14	10.17	0.62
Antibiotic	10	27.02	10	27.02	17	45.94	12.3	3.35

Table: 6 Antifungal activity of T. bellirica from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Fungal Strain						Mean $\bar{x}$	S.D σ
	C.albicans	%	A.flavus	%	A.niger	%		
Acetone	9.1	29.2	8	25.72	14	45	10.4	3.44
Methanol	8.5	32.07	6	22.64	12	45.28	8.8	2.46
Aqueous	9.2	23.46	12	30.61	18	45.91	13.06	3.67
Antibiotic	10	29.4	9	26.47	15	44.1	11.3	2.62

Table:7 Antifungal activity of Phyllanthus emblica from various solvents

Test Samples and Extracts	Zone of Inhibition (mm)							
	Fungal Strain						Mean $\bar{x}$	S.D σ
	C.albicans	%	A.flavus	%	A.niger	%		
Acetone	10	32.3	10	32.3	11	35.5	10.3	0.47
Methanol	9	36.0	7	28.0	9	36	8.3	0.94
Aqueous	17	31.5	10	18.5	27	50	18	6.98
Antibiotic	10	29.4	9	26.47	15	44	11.3	2.62

Volume : 3 | Issue : 4 | April 2014 • ISSN No 2277 - 8179

Figure:1 FTIR study for KBr absorption from 600-4000nm (T. bellirica)

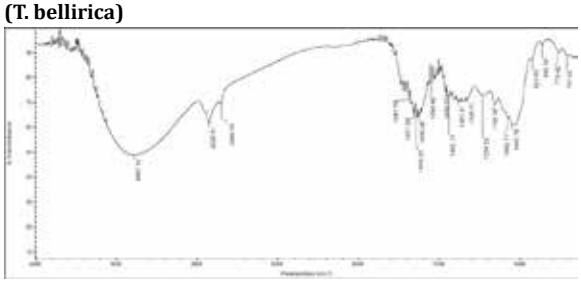
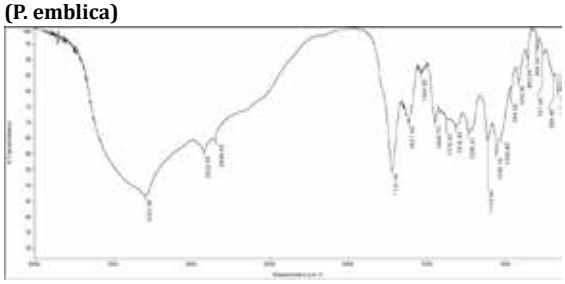


Figure:2 FTIR study for KBr absorption from 600-4000nm (P. emblica)



Results and Discussion

Present study illustrates the unique properties of phytoconstituents like Tanin, Quinone, Caumarin, Carbohydrate, Protein and aminoacids respectively. T.bellerica has saponin and P.emblica has greater amount of alkaloids, glycosides, and polyphenol reported in table:1. Terminalia bellerica and Phyllanthus emblica have been reported to contain β-sitosterol, gallic acid, ethyl gallate, chebulagic acid, galloyl glucose, mannitol, glucose, galactose, fructose, rhamnose [16].

Organic analysis and IR absorption were done to identify the functional groups. Peaks resembles in Alkane(CH), Alkene(=C-H), Alkane-C-H, Alcohol(OH), Nitro(NO), Aromatic(C=C), and Alkyl Halide. T.bellerica has the special quality of Acid(O-H,C-O), amide(NH) and ether(C-O) and emblica has CarbonylC=O are illustrated in table 2,3 and presented in figure 1,2. IR spectra of gallic acid showed typical frequencies due to –OH stretching vibration of both phenolic as well as hydroxyl group of carboxylic acid and carbonyl stretching frequencies [17].

Invitro preliminary screening of antimicrobial activity of the plant extracts of T.bellerica and P.emblica were studied against some microorganisms using antibacterial activity by disc diffusion and antifungal activity by food poisoning method exhibited in table 5&6. In that T.belerica has higher susceptibility in acetone solvent were summarized 35.25%,33.81%,30.93% and Standard deviation acetone 0.62,methanol 0.12, aqueous 0.09 to other solvents. P.emblica depicts higher effect in methanol extract 42%,29%,29% Standard deviation 2.12, 0.72. 0.62. Among these organisms P. aeruginosa has more efficiency to compare with the other solvents were similar to the Antimicrobial and anti HIV-1 activity [18]. Standard (Ceftriaxone-P.aeruginosa, Amoxyclav-S.aureus, Erythromycin-K.pneumoniae) antibacterial agents were more effective than all extracts.

Antifungal substances present in the plant extracts were fungistatic at lower concentration while become fungicidal at higher concentration. Fruit extract of T.belirica and P.emblica showed toxicity towards Candida albicans, A.flavus, A.niger at higher concentration. Methanol solvent has more growth inhibitory effect in T.bellirica extract and it has the higher activity in A.flavus 22.64%, 32.07%, 45.28 and standard deviation 2.46, 3.44, 3.67 respectively. In P.emblica methanol has 28%,36%36% and Standard deviation 0.47, 0.94, 6.98 was revealed in table:6&7 [19]. Methanol extracts reported more effective than the standard (Penicillin) used.

Conclusion

Results of the present investigation clearly indicate the presence of bioactive compounds, organic analysis, functional group identification using FTIR, susceptibility of bacterial cultures using antibacterial activity and radial growth of different fungi inhibited by the extract was studied in antifungal activity. Hence these extracts definitely possess potent therapeutical activity and this can serve as an important platform for industrial drug formulation and toxicity studies.

REFERENCE

1. Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya S. K. (1999). "Antioxidant activity of active tannoid principles of Emblica officinalis (amla)". Indian journal of experimental biology 37 (7): 676–680. | 2. Habib-ur-Rehman, Yasin KA, Choudhary MA, et al. (Jul 2007). "Studies on the chemical constituents of Phyllanthus emblica". Nat. Prod. Res. 21 (9): 775–81. | 3. Brusotti G, Cesari I, Frassà G, Grisoli P, Dacarro C, Caccialanza G"Antimicrobial properties of stem bark extracts from Phyllanthus muellerianus (Kuntze) Excell." J Ethnopharmacol. 2011 Jun 1;135(3):797-800 | 4. Ravikumar YS, Ray U, Nandhitha M, Perween A, Raja Naika H, Khanna N, Das S.,"Inhibition of hepatitis C virus replication by herbal extract: Phyllanthus amarus as potent natural source." Virus Res. 2011 Jun;158(1-2):89-97 | 5. Boim MA, Heilberg IP, Schor N "Phyllanthus niruri as a promising alternative treatment for nephrolithiasis." Int Braz J Urol. 2010 Nov-Dec;36(6):657-64; discussion 664 | 6. Dharmananda S. Emblic Myrobalans: Amla, Institute of Traditional Medicine. | | 7. Vaidyaratnam PS. Varier's Indian Medicinal Plants, Published by Orient Lonhman Private Ltd. Chennai.2004:05:258-262 | 8. Nadkararni KM. Indian Materia Medica, Published by Ramdas Bhatkal for popular Prakasn Pvt. Ltd.Mumbai. 2002:1:2012-1205 | 9. Iwai K 2008, Antidiabetic and antioxidant effects of poly phenols in brown alga Ecklonia stolonifera in genetically diabetic KK-A mice. Plant food hum. Nutr.63:163-169 | 10. Madhuri Sharma and Padney Govind. Ethanomedicinal Plants for prevention and treatment of tumours, International Journal of Green Pharmacy.2009;2-5 | 11. Badrul Alam. Antioxidant, antimicrobial and toxicity studies of the different fractions of fruits of Terminalia belerica Roxb. Global Journal of Pharmacology. 2011;5(1):07-17 | 12. Kokate C.K., 2001, Practical Pharmacognosy, Vallabh Prakashan, pp. 218. 16. Mohammed A., 1994, Text Book of Pharmacognosy, CBS Publishers and Distributors... | 13. Muruganantham.S, Anbalagan.G and Ramamoorthy N. FT-IR and SEM-EDS comparative analysis of medicinal plants. Eclipta alba & Eclipta prostrate. Romanian J. Biophysics.2009,19,285-294. | 14. Bayer et AL 1966. Antibiotic susceptibility testing by standard single disc 13-18. | 15. Schmitz H. Food poisoned technique. Industrial Engineering Chemical Analyst Education;P.361-363. | 16. Row, L.R and P.S Murty, 1970. Chemical examination of Terminalia belerica. Indian J.Chen.,8:1047-1048 | 17. Bahulikar AS, Kashalkar RV, Pundlik MD Infrared spectrometry in studies on herbal drug Triphala churna, Asian J Chem.,15(2) (2003) 851. | 18. Valsaraj, R.,P. Pushpangadan, U.W.Smitt, A.Adersen ans S.B Christensen et al., 1997. New antiHIV-1, antimalarial and antifungal compounds from Terminalia bellerica J.Nat.Prod.60:739-742 | 19. Ambasta .S.P(1992) the useful plants OF India; Raw material V-E, C.S.I.R Publication, New Delhi. |