ANTIMICROBIAL AND ANALGESIC ACTIVITY OF AMALAKYADI CHURNA AND ITS INGREDIENTS

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Summary

Aim: The alcoholic extracts of Amalakyadi churna and its ingredients were evaluated for antimicrobial and analgesic activities. **Introduction:** It is one of the Ayurvedic formulations, used in traditional Indian system of medicine, practiced against certain general health disorders such as carminative, appetizer, purgative etc. It is a powdered mixture of equiproportions of fruits of *Phyllanthus emblica* L (Euphorbiaceae), *Piper longum* L (Piperaceae), *Terminalia chebula* Retz (Combretaceae), roots of *Plumbago zeylanica* L (Plumbaginaceae) and rock salt. **Materials and Methods:** In the present study Amalakyadi churna and its individual components were screened for preliminary phytochemical studies and also tested against certain clinical bacterial and fungal isolates (*Escherichia coli, Staphylococcus aureus, Salmonella typhi, Pseudomonas aeruginosa, Proteus vulgaris, Aspergillus niger, A. fumigatus* and *A. flavus*) *invitro* agar well diffusion method and analgesic activity in Swiss albino mice by *invivo* Tail flick method. **Results:** Based on the preliminary phytochemical studies, it has been noticed that presence of alkaloids, flavonoids in *Piper longum*, phenols, tannins in *P emblica* and *T chebula*. Where as, quinones and steroids in *P zeylanica*. Amalakyadi churna and its ingredients exhibited promising antimicrobial activities, where as A. churna extract has shown to possess moderate analgesic activity. **Conclusion:** Obviously Amalakyadi churna is a mixed preparation of all these phytoconstituents of four different plant ingredients and rock salt, perhaps the combined effect of all these chemical groups might have synergistically exhibited the broad spectrum of antimicrobial and moderate analgesic activities. These principle constituents might be responsible for the folk use of this formulation.

Key words: Amalakyadi churna, Agar well diffusion technique, Tail-flick methods Antimicrobial, Analgesic

Introduction

Infectious diseases are as old as life. Antibiotics are molecular ammunition, which have proven to be a major asset in the fight against infectious bacteria by either killing them [bactericidal] or inhibiting their growth [bacteriostatic]

The resistance of pathogenic microorganisms to currently known antibiotics is constantly increasing due to a broad use of antimicrobials in medicine, animal husbandry and agriculture. If no preventive measures are taken, such events will certainly increase with time, this will inevitably lead to the development of novel antibiotics with alternative therapeutic strategies is essential. Among many proposed strategies, thus a good understanding of systematic screening of traditional system of medicine offers the potential of developing potent broad spectrum antibiotics. However, the systematic screening of medicinal plants for targeting microbials and their scientific documentation of such traditional drugs like as in modern medicine is necessary for future benefits. These documents opens up an expanding spectrum of applications and better understanding of diagnostic and therapeutic purposes to get effective antimicrobial agents. Hence, in this present research, same efforts are continued in the progression of searching novel therapeutics and probed their mechanism of action against antibiotic activity.

Amalakyadi churna is an Ayurvedic formulation, extensively used for treating all types of fever, carminative, appetizer and other general health disorders. It was prepared by an ancient Indian Physician Sarangadhara (1300 AD ~ 1400AD), and mentioned in Sarangadhara Samhita (1). It consists of fruits of *Phyllanthus emblica* L. (Euphorbiaceae), *Piper longum* L. (Piperaceae), *Terminalia chebula* Retz. (Combretaceae), roots of *Plumbago zeylanica* L. (Plumbaginaceae) and rock salt. All these plant materials are being used in traditional health care systems for the treatment of different kinds of disorders since antiquity. *T. chebula* extracts characterized by the presence of chebulininc acid, chebulanin, casuarinin, 1,6-di-O-galloyl-β-D-glucose (2-3). The *Plumbago zeylanica* has β-sitosterol, *β*-sitosteryl-3*β*-glucopyranoside, *β*-sitosteryl-3*β*-glucopyranoside-6′-*O*-palmitate, lupenone, lupeol acetate, plumbagin and trilinolein, *β*-sitosterol, and plumbagin (4), *Phyllanthus emblica* is one of the richest source of the vitamin-C, it also possess other active constituents like Emblicanins A and B, ellagitannins, putranjivan, flavonoid, quercetin (5-6). *Piper longum* has some of the chemical constituents guineensine, retrofracamide C, (2*E*,4*Z*,8*E*) – *N* - [9-(3,4-methylenedioxyphenyl) - 2, 4, 8-nonatrienoyl] piperidine, pipernonaline, piperrolein B, piperchabamide D, pellitorin, and dehydropipernonaline, flavones, flavonones, steroids, lignans (7-8).Two new alkaloids, piperlongumine and piperlonguminine are shown to be N-(3,4,5-trimethoxycinnamoyl)-Δ^5-piperidin-2-one and isobutylamide of piperic acid respectively, from the roots (9)**.** Hence, Amalakyadi churna has a rich pool of all these constituents, which holds tremendous therapeutic potentials. Several research groups in recent issues have clearly indicated the presence of antioxidant, free radical scavenging activity, antiulcer, hepatoprotective, anticancerous, hypolipidemic, antimutagenic, antifertility, and antimicrobial, immunomodulatory effects in these crude extracts and isolated active principles of *Phyllanthus emblica* (10-12) *Piper longum* (13-15), *Terminalia chebula* (16-17) and *Plumbago zeylanica* (18).

Materials and Methods

The present study was undertaken to assess the antibacterial and antifungal activities of different concentrations of Amalakyadi churna and its plant ingredients on highly pathogenic bacteria like *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi* and *Proteus vulgaris,* few pathogenic fungi such as *Aspergillus flavus, A. niger* and *A. fumigatus.* Analgesic activity studied for the maximum pain tolerance of Amalakyadi churna in pain induced mice.

Collection of Plant Materials: The plant materials of *Phyllanthus emblica* L (Euphorbiaceae), *Plumbago zeylanica* L (Plumbaginaceae) *Terminalia chebula* Retz (Combretaceae) were collected from Sandur, *Piper longum* L (Piperaceae) from GKVK, Bangalore (Karnataka, India) in October– November months and authenticated at the herbarium of Department of Botany, Gulbarga University, Gulbarga. The rock salt was purchased from the local Ayurvedic shop, Gulbarga.

Amalakyadi Churna: Amalakyadi churna was prepared by using standard formulation prepared by an ancient Indian Physician Sarangadhara (1300 AD ~ 1400AD), and mentioned in his treatise Sarangadhara Samhita (1). The plant materials and rock salt were powdered separately and sieved through muslin cloth. They were taken in equal proportions and mixed well. This Amalakyadi churna was stored in an airtight container for further processing.

Preparation of the Extracts: The 100g of Amalakyadi churna and its plant ingredients were extracted with 90% alcohol at 50 - 60°C in a soxhlet apparatus. The extract was concentrated to dryness in a flash evaporator (Buchi type) under reduced pressure and controlled temperature (40 - 50°C) and note down the yield of crude extract.

The Preliminary Phytochemical Studies: The alcoholic extracts of Amalakyadi churna and its extracts were tested by applying general chemical tests for alkaloids, glycosides, reducing sugars, tannins, steroids, terpenoids, phenols, flavonoids, proteins, saponins, amino acids, etc (19-23)

Microorganisms Used: Clinical laboratory bacterial isolates of *Staphylococcus aureus, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa* and *Proteus vulgaris* and pure fungal isolates like *Aspergillus niger, A flavus* and *A fumigatus* obtained from the Department of Microbiology, Gulbarga University, Gulbarga. Identity of these organisms was confirmed based on morphological, colony characteristics and biochemical tests.

Culture Media: The nutrient agar for antibacterial activity and Sabouraud's dextrose agar for antifungal activity (powdered form) were purchased from HiMedia, Laboratories Limited, Mumbai. Nystatin an antifungal agent, purchased from, Tocelo chemicals, Netherlands, Streptomycin sulphate another antibiotic from Nanjing Asian chemicals Co., Ltd., Analgin by Vani Pharma labs Limited, Hyderabad.

Antimicrobial activity: Here the *in vitro* antibacterial and antifungal activities were assayed by using agar well diffusion method. The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours, then subjected for optical density of the overnight incubated culture were adjusted to 0.1 at λ_{600} with sterile nutrient broth. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar and Sabouraud's dextrose agar plates for bacterial and fungal cultures, respectively. The wells were bored with 8mm borer in seeded agar, and then the particular concentrations (2mg/0.2ml to 10mg/0.2ml) of the extracts were added in each well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hr. After incubation period is completed, the zone of inhibition was measured and recorded (24)

Analgesic activity: The analgesic activity of the ethanolic extract of Amalakyadi churna was studied by tail flick method (25). Swiss albino mice weighing between 20 - 25g and heat sensitive were selected for experimental work. Then mice were randomly divided into five groups of 6 mice each. The first groups served as control and received the vehicle only (ie., 0.2ml of distilled water per animal). The second, third, fourth groups of mice have received the alcoholic extract of Amalakyadi churna suspended in distilled water at 100, 150, 200mg/kg b.wt. respectively. The fifth group received the standard analgin at a dose of 40mg/kg b.wt. All the treatments were administered intraperitoneally. The observations were made at 0, 30, 60 and 120 minutes.

Statistical Analyses: All the data are expressed as mean ± S.E.M. (standard error of the mean). Significance level in different groups were analyzed using student't' test. P values less than 0.05 were considered to be statistically significant

Results

Yield of Extract: The percentage of yield of the alcoholic extracts of Amalakyadi churna and its individual ingredients were: Amalakyadi churna (6.05%), *Phyllanthus emblica* (7.49%), *Piper longum* (6.6%), *Plumbago zeylanica* (4.05%) and *Terminalia chebula* (12.8%) weight / weight.

Preliminary Phytochemical Studies: The results of preliminary phytochemical studies revealed that the presence of phenols, alkaloids, flavonoids, tannins, saponins, reducing sugars, proteins and amino acids. But the extracts of *Piper longum* and *Plumbago zeylanica* showed negative for tannins, but in case of Amalakyadi churna, *Phyllanthus emblica* and *Terminalia chebula* shown positive to tannin test, where as the extract of *Plumbago zeylanica* has shown sparingly positive to saponins, but Amalakyadi churna exhibited positive response to all the above tests. All these chemicals hold tremendous therapeutic potential.

Antibacterial Activity: The different concentrations of Amalakyadi churna and its plant ingredients have shown promising antibacterial activity against all the bacteria tested. The zone of inhibition increased an increasing the concentration of the extract in well. This showed the concentration dependent activity. The different concentrations of alcoholic extract of Amalakyadi churna, showed maximum activity against *Pseudomonas aeruginosa* and *Salmonella typhi,* moderate to maximum against *Staphylococcus aureus,* but mild to moderate activity against *Proteus vulgaris* and *Escherichia coli*. Where as in case of the different concentrations of *P. emblica,* showed moderate to maximum against *P. aeruginosa* but less to moderate activity against other bacterial isolates. Similar observations were noticed even in *P. longum* and *P. zeylanica.* Where as, in case of *Terminalia chebula, P. aeruginosa, P. vulgaris* and *E. coli* were proved to be highly susceptible, but less susceptibility was noticed in *S. aureus,* and *S. typhi.*

An interesting observation was noticed in this present findings that, the growth of *P.aeruginosa* was effectively inhibited by Amalakyadi churna, *T. chebula, P. longum* but moderate activity noticed in *P. emblica, P. zeylanica* even at the concentrations of 0.8mg/0.2ml/well but at this same concentration, the streptomycin sulphate was failed to show effective activity against *P. aeruginosa,* This clearly indicates the development of antibiotic resistance in these organisms, hence activity at this dose was not shown, but when the concentration of this standard antibiotic was increased to 0.16mg/0.2ml, there was significant increase in the zone of inhibition against *P. aeruginosa* (Table-1).

Antifungal activity: Similarly, the results demonstrated that all these test samples (0.8mg/ 0.2ml) have potent antifungal activity against different *Aspergillus* species tested here. The alcoholic extract of *T. chebula* was significantly inhibited the growth of all the *Aspergillus* species, where as considerable inhibition in the growth rate of all the *Aspergillus* species were noticed in Amalakyadi churna as well as in *Piper longum,* but less to moderate activity was noticed in *P. emblica,* but *P. zeylanica* was failed to inhibit the growth rate of *Aspergillus* species. An interesting observation was noticed that, the extract of *T.chebula* was most effectively inhibited the growth rate of all these *Aspergillus* species almost equal to the standard Nystatin drug (Table-2).

Analgesic activity: The results indicated that, the alcoholic extract of Amalakyadi churna at different concentrations of 105, 160 and 210 mg/kg b.wt. showed mild to moderate analgesic activity after 30, 60 and 120 minutes of treatment compared with vehicle control and standard analgin treated groups of mice (Table-3). An increase in the pain tolerance capacity was observed at 0 to 30 minutes in all the post treatment groups of mice, which exhibited up to maximum of 5 seconds in case of A. churna treated groups and 7.65 seconds in analgin treated group, where as significant increase in the pain tolerance capacity was observed in mice after 60 minutes post treatment period, which exhibited the tolerance of pain up to 5.2 to 6.1 seconds in A. churna treated groups but 9.4 seconds in analgin treated group. Where as, mild decrease in the analgesic activity was observed at 60 – 120 minutes in all post treatment groups of mice, which was found to be 5.8 to 6.0 seconds. However, after 120 minutes of post treatment period in all the treated groups of mice showed gradual decrease in the pain tolerance capacity.

Table-1 Antibacterial activity of the alcoholic extracts of Amalakyadi churna and its ingredients

Plant Materials	Dose (mg/0.2ml)	*Pseudomonas aeruginosa* (mm)	*Staphylococcus aureus* (mm)	*Salmonella typhi* (mm)	*Proteus vulgaris* (mm)	*Escherichia coli* (mm)
Phyllanthus emblica L.	0.8	18.0± 0.45	13 ± 0.577	13.5 ± 0.49	13.5 ± 1.50	15.5 ± 0.17
	1.6	20.5 ±0.50	14.3 ± 0.58	18.0 ± 1.00	16.6 ± 0.24	16.3 ± 0.81
	2.4	21.0 ± 0.54	21.0 ± 0.55	20.0 ± 1.00	17.6 ± 0.25	19.5 ± 0.32
	3.2	23.0 ± 0.65*	23.0 ± 0.65*	21.5 ± 0.50*	19.3 ± 0.37*	20.5 ± 0.32*
	4.0	25.3 ± 0.63*	20.5 ± 0.71*	22.0 ± 0.01*	20.8 ± 0.37*	22.0 ± 0.45*
Piper longum L	0.8	22.0 ± 1.15	12.6 ± 0.41	12.5 ± 0.45	14.4 ± 0.25	15.1 ± 0.33
	1.6	27.5 ± 0.65	15.6 ± 0.33	13.5 ± 0.40	15.6 ± 0.79	16.5 ± 0.35
	2.4	30.0 ± 0.91	18.6 ± 0.33	13.5 ± 0.50	17.8 ± 0.49	17.6 ± 0.81
	3.2	31.7 ± 0.85*	22.3 ± 0.88*	14.0 ± 1.00*	19.4 ± 0.40	18.3 ± 0.49*
	4.0	32.5 ± 1.15*	23.0 ± 0.52*	16.5 ± 0.49*	20.4 ± 0.24*	20.0 ± 1.41*
Plumbago zeylanica L	0.8	19.5 ± 0.25	11.6 ± 0.13	11.4 ± 0.25	11.5 ± 0.50	11.5 ± 0.17
	1.6	20.7 ± 0.25	13.2 ± 0.20	12.6 ± 0.25	18.0 ± 1.27	13.5 ± 0.50
	2.4	24.2 ± 0.47	14.4 ± 0.24	16.5 ± 0.25	20.5 ± 0.32	15.5 ± 0.50
	3.2	25.2 ± 0.48*	15.4 ± 0.51*	17.8 ± 0.79*	22.0 ± 1.08	18.5 ± 0.50*
	4.0	27.5 ± 2.90*	17.6 ± 0.93*	20.0 ± 1.14*	24.5 ± 0.50*	19.0 ± 1.00*
Terminalia chebula Retz.	0.8	20.2 ± 0.38	13.0 ± 0.32	11.6 ± 0.24	18.4 ± 0.96	19.8 ± 0.30
	1.6	22.4 ± 0.51	14.5 ± 0.50	14.2 ± 0.20	20.0 ± 0.32	20.5 ± 0.22
	2.4	23.0 ± 0.64	16.3 ±0.22	16.4 ± 0.24	22.6 ± 0.24	22.5 ± 0.22
	3.2	26.0 ± 0.63*	18.0 ± 0.32	18.4 ± 0.24*	25.2 ± 0.37*	23.1 ± 0.18*
	4.0	26.2 ± 1.72*	19.8 ± 0.31*	19.8 ± 0.36*	28.8 ± 0.38*	24.5 ± 0.22*
Amalakyadi churna	0.8	21.6 ± 0.98	15.5 ± 0.49	20.2 ± 0.58	11.5 ± 0.29	09.3 ± 0.13
	1.6	23.5 ± 0.28	17.3 ± 0.25	21.6 ± 0.24	12.5 ± 0.29	11.0 ± 0.28
	2.4	24.5 ± 0.49	18.6 ± 0.24	22.0 ± 0.55	13.8 ± 0.37	13.3 ± 0.43
	3.2	25.4 ± 0.93*	20.4 ± 0.24*	25.0 ± 0.55*	15.6 ± 0.60*	15.3 ± 0.45*
	4.0	28.2 ± 0.49*	21.8 ± 1.16*	26.6 ± 0.25*	17.8 ± 0.37*	18.3 ± 0.91*
Streptomycin sulphate (std)	0.4	11.0 ± 0.01	39.0 ± 0.38	21.0 ± 0.91	30.7 ± 0.50	38.2 ± 0.63
	0.8	13 .0± 0.01	40.7 ± 0.27	26.0 ± 0.29	34.7 ± 0.58	39.7 ± 0.27
	1.6	44.2 ± 0.75	41.0 ± 0.38	38.0 ± 0.77	47.5 ± 1.75	40.0 ± 0.90
Control (dw)	0.2 ml	--	--	--	--	--

Note: * = significant compared to 0.8mg/0.2 with all other doses (a = $P<0.05$);
dw=distilled water; std=standard antibiotic

Table-2 Antifungal activity of the alcoholic extracts of Amalakyadi churna and its ingredients

Plant materials (0.8mg/0.2ml)	*Aspergillus niger* (mm)	*Aspergillus flavus* (mm)	*Aspergillus fumigatus* (mm)
Amalakyadi churna	16.0 ± 0.335	18.0 ± 0.706	19.0 ± 0.334
Phyllanthus emblica	11.0 ± 0.333	11.3 ± 0.509	13.6 ± 0.509
Piper longum	17.6 ± 0.297	15.5 ± 0.352	19.5 ± 0.352
Plumbago zeylanica	06.0 ± 0.706	07.5 ± 0.351	05.5 ± 0.351
Terminalia chebula	20.0 ± 0.335	21.5 ± 0.352	25.0 ± 0.333
Nystatin (0.2mg/0.2ml)	23.7 ± 0.370	24.0 ± 0.704	26.5 ± 0.349
Control (0.2 ml of distilled water)	--	--	--

Table -3 Analgesic activity of the alcoholic extracts of Amalakyadi churna

Plant materials	Dose (mg/ kg b.wt ip)	0 min	30 min	60 min	120 min
Amalakyadi churna	105	2.8 ± 0.21	4.5 ± 0.22	5.2 ± 0.54	5.1 ± 0.12
	160	2.5 ± 0.26	4.8 ± 0.32	5.8 ± 0.08	5.3 ± 0.15
	210	2.8 ± 0.26	5.0 ± 0.92	6.6 ± 0.22 a	6.2 ± 0.20 a
Analgin	40	4.3 ± 0.81	7.7 ± 0.91a	9.4 ± 0.50 a	8.7 ± 0.14 a
Control	0.2 ml	2.7 ± 0.17	2.8 ± 0.16	2.8 ± 0.15	2.8 ± 0.15

Note: a= significant compared to control (a = $P<0.05$);

Discussion

The results presented that the alcoholic extract of *Amalakyadi* churna and its plant ingredients have the potentiality in combating clinical drug resistance bacteria. Many prior experimental evidences have also clearly shown that the controlling of bacterial growth by some of these ingredients of Amalakyadi churna. The inhibition of plasmid DNA (carrying ampicillin, kanamycin, cotrimoxazole multi antibiotic genes) replication in *E. coli* by plumbagin (an active principle of *Plumbago zeylanica)*, through the mediation of its DNA intercalating property and thereby suppressing the multidrug antibiotic resistant bacteria (26). Spencer *et al* (1985) (27) have exposed actively growing *E coli* to plumbagin, a redox cycling quinine that increases the flux of O^{-}_{2} radicals in the cell, were mutaginized and then killed by this treatment.

Tannins in the fruits of *Terminalia chebula* serve as a natural defense mechanism against microbial infections. It has been reported that tannic acid and propyl gallate-E were shown effective against growth inhibition of bacteria (17), *Piper longum* is responsible for immunomodulatory activity (14). Piperine an active principle of *P. longum* has many pharmacological actions such as antifungal, anti-inflammatory and anticancerous activities (28). The fruits of *Piper longum* are attributed with numerous medicinal uses particularly antifungal, antibacterial as well as analgesic activities (29)

However, the present phytochemical screening of Amalakyadi churna and its plant ingredients revealed that the presence of alkaloids predominantly found in the alcoholic extract of *Piper longum,* where as the presence of polyphenols, flavonoids, tannins in *P. emblica* and *T. chebula,* occurrence of naphthoquinones, terpenoids in *P. zeylanica,* and also traces amount of steroids, saponins found in all the five extracts. But the Amalakyadi churna has shown the presence of all these above useful phytoconstituents in it. Several of isolated compounds from these plants have been already evidenced for antimicrobial activity. Perhaps the obtained activities might be due to the combined effect of these constituents. Saponins are characterized by their surface active properties and which have been implicated as a bioactive antibacterial agent of plants (30-31).

Conculsion

The results of all the above studies demonstrated that, the role of these natural products ie. Amalakyadi churna and its ingredients acted as an excellent antimicrobial activity against some of the pathogenic bacteria and fungi tested and it has also revealed the moderate analgesic activity. Comparatively all the individual ingredients, Amalakyadi churna extract has shown antimicrobial activity against broader range of pathogenic bacteria and fungi, perhaps this might be due to the combined effect of many of these constituents present together. This study would provide the preliminary scientific evidence for the folkloric, ethnobotanical and traditional use of this churna for destruction of pathogenic microbes, increase pain tolerance and other health benefits.

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