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Antimicrobial activity of flavanoid sulphates and other fractions of *Argyreia speciosa* (Burm.f) Boj.

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Antimicrobial activity of flavanoid sulphates and different fractions of *A. speciosa* root was studied against bacteria, fungi and *Mycobacterium tuberculosis* H_{37} Rv sensitive strain by *in vitro* and *in vivo* assays. Flavanoid sulphates such as quercetin 3'7 di-O methyl 3- sulphate and kaempferol 7-O methyl 3-sulphate were isolated from the n-butanol fraction of 80% methanolic extract of the plant. The structures of the isolated flavanoids were confirmed by spectral studies. Ethyl acetate (EAAS) fraction and flavanoid sulphates inhibited the growth of *M. tuberculosis* Rv sensitive strain at MIC values 50 and 25 µg/ml, respectively. Ethanolic fraction (EtAS) showed significant inhibition of gram positive organism with a MIC of 31.25 µg/ml. More inhibition was observed with a less MIC (2 µg/ml) for flavanoid sulphates against *Klebsiella pneumoniae,* a gram negative organism and it is almost comparable with the standards. Interestingly, chloroform fraction alone exhibited significant antifungal activity with a MIC of 100 µg/ml. A synergistic effect between flavanoids sulphates and commercially available antitubercular drugs was observed with FIC index of 0.443±0.245, 0.487±0.247 for isoniazid and 0.468±0.333, 0.417±0.345 for rifampicin, whereas EAAS fraction showed partial synergistic effect. A synergistic effect was observed for EAAS fraction and flavanoids sulphates with FIC index < 0.5 with antibiotics. Hemolysis assay on RBCs suggested that EAAS and flavanoids sulphates exhibited least cellular toxicity to erythrocytes as compared to chloramphenicol. *In vivo* studies in mice infected with *K. pneumoniae* demonstrated that on day 10 post treatment of different fractions and isolated compounds of *A. speciosa*, about 60% of the animals treated with EAAS, 70% of animals treated with flavanoids sulphates and 40% of animals treated with EtAS were survived.

Keywords: Antifungal, Antimicrobial, Antitubercular, *Argyreia speciosa*, Flavanoid sulphates

Infectious diseases caused by bacteria, fungi, viruses and parasites are a major threat to public health despite tremendous growth in human chemotherapeutic medicine. Tuberculosis (TB), an infectious disease caused by different species of Mycobacterium, represents a worldwide public health problem and infects <30% of the global population[1]. Nearly 2 million people die of TB, with a global case fatality rate of 23% and reaching > 50% in some African countries due to high rates of coexisting HIV infection. Man infected with HIV is very susceptible to tuberculosis. Emergence of drug resistant strains of *Mycobacterium tuberculosis* has led to increased concern on current chemotherapy regimes[2]. Worldwide increase in the incidence of morbidity and mortality from tuberculosis prompted WHO to declare this disease a global emergency in the early 1990s[3]. The need for new antituberculosis agents is urgent due to increasing resistance of mycobacterium, together with increased incidence of severe disseminated infections produced by mycobacterium other than tuberculosis in immunocompromised patients, have prompted the search for new antimycobacterial agents, preferably those that can readily and simply be produced from some local natural plant sources. In addition to mycobacterial infectious diseases, other bacterial diseases and systemic mycoses are also difficult to medicate. Considering the increased incidence of severe opportunistic fungal and bacterial infections in immunologically deficient patients together with the development of resistance among pathogenic gram positive, gram negative bacteria and *Candida albicans,* there is a great need in finding new classes of natural products that may be effective against antibiotic-resistant bacteria and fungi. Natural products or their semisynthetic derivatives provide

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novel examples of such anti-infective drugs[4]. Because of the resistance against antibiotics, there is a great interest in search of new antimicrobial agents from the nature[5].

Argyreia speciosa (Burm.f) Boj. (Convulvulaceae) is commonly known as *Vrudhadaruka* in Indian system of medicine. Roots of *A. speciosa* are used in ayurveda as aphrodisiac, rejuvenating, brain tonic, in the treatment of infected wounds, bronchitis, syphilis and pulmonary tuberculosis[6,7]. The plant has been screened for anti-inflammatory[8], immunomodulatory[9], nootropic[10] and hepatoprotective[11] activities. Flavonoid sulphates such as kaempferol 7-O methyl 3-sulphate, quercetin 3'7 di-O methyl 3- sulphate[12] and stigmasteryl p-hydroxycinnamate[13] have been reported from the roots. The aim of the present study is to understand the antimicrobial spectrum from natural resources and to support the traditional uses of *Argyreia speciosa* and its isolated compounds in the treatment of pulmonary tuberculosis and other infectious diseases.

Materials and Methods

Test microorganisms—In the present study strains used were, *Mycobacterium tuberculosis* H_{37} Rv sensitive strain ATCC 27294, Gram-positive bacteria-*Staphylococcus aureus* ATCC-11632, *Enterococcus feculis* ATCC-35550. Gram-negative bacteria-*Klebsiella pnuemoniae* ATCC-10031, *Escherichia coli* ATCC-10536, and fungi Candida albicans ATCC-2091, *Aspergelless fumigatus* ATCC-13073.

Culture medium—For *Mycobacterium tuberculosis* bioassay, Middle brook7H9 broth supplemented with 10% of albumin-dextrose-catalase and 0.2% of glycerol was used as culture medium[14]. Whereas, for the bioassay of other microorganisms, Mueller-Hinton agar for bacteria[2] and Sabraudus-dextrose agar medium for fungi was used[15]. All the test samples were sterilized by filtration using 13 mm nylon acrodiscs (0.22 μm pore size).

Culture and growth conditions—Stock strains of mycobacteria were maintained in 7H9 broth with 0.2% glycerol at -20°C. Subcultures of the microorganisms were made in Middlebrook 7H9 broth (Difco, Becton Dickinson and Co. USA) containing 10% ADC (albumin-dextrose-catalase) enrichment (Difco, BD, USA), 0.05% Tween 80, and 20 mg/ml of kanamycin (Sigma, St. Louis,USA.). Cultures of *Mycobacterium tuberculosis* were incubated for 24 hr at 37°C. Following incubation, the culture suspension was sonicated for 10 sec with a Sonicator (Cole-Parmer India). To prepare the inoculum, the sonicated culture was diluted in Middlebrook 7H9 broth without kanamycin to an absorbance at 540 nm of 0.05 absorbance. This procedure yielded a suspension containing approximately 10^5 CFU/ml. This diluted suspension was used to inoculate test trays as described below.

Plant material—Roots of *A. speciosa* were collected from hilly areas surrounding Dharwad, India and authenticated by Dr. G.R. Hegde, taxonomist, Karnataka University Dharwad, India. A voucher specimen was kept in the Department of Pharmacognosy (SETCPD/ pharmacog /33/herb/ 2006), SET's College of Pharmacy, Dharwad, India.

Preparation of extracts—Air-dried plant root (1.5 kg) was pulverized with a grinder. Approximately 1000 g of the pulverized plant part was extracted successively with petroleum ether (60°-80°C) (800 ml)/chloroform (800 ml)/ethyl acetate (800 ml)/95% ethanol (800 ml) respectively. The solutions were filtered through muslin cloth, concentrated under reduced pressure at 38°C with a rotary evaporator and stored at -20°C. Stock solution of the extract was prepared by dissolving preweighed samples of the extracts in dimethyl sulfoxide (DMSO) to attain final concentrations of 1 mg/ml. These stock solutions were stored at -20°C until further study.

Isolation of flavanoid sulphates[12]—Flavanoid sulphates were isolated by the method as reported earlier. Root powder was extracted with 80% methanol at room temperature. After evaporation the residue was dissolved in water and extracted successively with CH_2Cl_2 and *n*-butanol. Dried *n*-butanol fraction of *A. speciosa* was chromatographed over silica gel column (27×3cm) using EtOAc-MeOH-H_2O (80:10:10) as eluent. Flavanoids compounds were eluted after 270-420 ml. These sub-fractions were further chromatographed on Sephadex LH-20 by stepwise gradient elution with H_2O- MeOH. The isolation was subjected to TLC using silica gel GF_{254} EtOAc-Me-CO-Et-HCOOH-H_2O (5:3:1:1). The TLC plates were checked under UV light (254 nm) and then sprayed with 2-aminoethyl diphenyl borinate and observed for fluorescent spots at 366 nm. The structures of flavanoids were confirmed by spectral studies and co-TLC with reference compounds. Stock solutions of the isolated compounds were prepared by dissolving in DMSO to attain a final concentration of

1 mg/ml. These stock solutions were stored at -20°C until further analysis.

Antimycobacterial activity—Antitubercular screening of plant extracts and isolated compounds was obtained for *M. tuberculosis* H_{37} Rv ATCC 27294 sensitive strain by broth dilution assay[16,17]. A frozen culture in Middle brook 7H9 broth supplemented with 10% albumin-dextrose-catalase and 0.2% glycerol was thawed and diluted in broth to 10^5 CFU/ml for *M. tuberculosis* and used as the inoculum. For assay, U-tubes (1 ml) were used to accommodate test extracts and isolated compounds in the concentrations of 10, 25, 50 and 100 µg/ml. Each U-tube was then inoculated with 0.05 ml of standardized culture and then incubated at 37°C for 21 days. The Bacterial growth in U-tubes was compared with visibility against positive control (without drug), negative control (without drug and inoculum) and with standard isoniazid (INH) and rifampacin. INH, rifampacin, were solubilized in distilled water and stored at -20°C.

Antibacterial activity—Antibacterial activity of different fractions and isolated compounds of *A. speciosa* was carried out by broth microdilution method[18]. Serial dilutions of the test fractions, isolated compounds and reference drugs were prepared in DMSO to attain a final concentration of 1 mg/ml. Further progressive dilutions with Mueller-Hinton agar were performed to obtain the required concentrations of 1, 2, 4, 16, 31.25, 62.5, 125, 250 and 500 µg/ml. The tubes were inoculated with 10^5 cfu/ml (colony forming unit/ml) of each microorganism and incubated at 37°C for 18 hr. To ensure that whether solvent had any effect on the bacterial growth, a respective parallel control was performed. Minimum inhibitory concentration (MIC) of the fractions was determined. Ciprofloxacin and norfloxacin were used as standards to compare the antibacterial activity of the fractions of the plant.

Antifungal activity—Antifungal activity of different fractions and isolated compounds of *A. speciosa* was carried out by broth microdilution method[19]. Serial dilutions of the test fractions, isolated compounds and reference drugs were prepared in DMSO to attain a concentration of 1mg/ml. Fungal growth inhibition was determined at 25, 50, 100, 250 and 500 µg/ml concentrations The tubes were inoculated with 10^5cfu/ml of each microorganism and incubated at 37°C for 18 hr. To ensure that solvent had no effect on fungal growth, a respective control was performed. Minimum inhibitory concentration (MIC) of the fractions was determined. Flucanazole was used as standard to compare the antifungal activity.

Synergism between flavanoid sulphates and antitubercular drugs—Solutions of flavanoid sulphates alone (50% dimethyl sulfoxide in water), and flavanoid sulphates in combination with respective antitubercular drugs were prepared by the doubling dilution method with sterilized water and were poured into petridishes separately. Sterilized Mueller-Hinton agar (8 ml) was poured into the above petridishes and mixed. MIC of flavanoid sulphates alone, antitubercular compounds alone and flavanoid sulphates in combination with each drug were determined. Fraction inhibitory concentration was calculated and the interactive effects between the flavanoid sulphates and antitubercular drugs were examined[20].

Synergism between flavanoid sulphates and fractions with antibiotics—Solutions of flavanoid sulphates alone (50% dimethyl sulfoxide in water), and flavanoid sulphates in combination with respective antibiotics (ciprofloxacin and norfloxacin), other fractions alone and other fractions with antibiotics were prepared by the doubling dilution method with sterilized water and were poured into petridishes separately. Sterilized Mueller-Hinton agar (8 ml) was poured in to the above petridishes and mixed. MIC of flavanoid sulphates alone, antibiotics alone, other fractions of the plant alone and other fractions in combination with antibiotics were examined and fraction inhibitory concentration were determined.

Determination of cellular toxicity to human erythrocytes—Since ethylacetate fraction and isolated flavanoids showed good antibacterial activity, the cellular toxicity of RBCs was investigated. Blood was obtained from blood bank of Karanataka Medical College, Hospital and Research Centre, Hubli, India. Human erythrocytes were isolated from the blood by removing buffy coat and suspended in PBS (10 m*M* phosphate, 150 m*M* sodium chloride, *p*H 7.4) which were dispensed in sugar tubes (10^{10} cells/500µl/tube). The serial dilutions of EAAS and flavanoids sulphates were made and mixed with erythrocytes keeping final volume of 1 ml. The cells were incubated for 1 hr at 37°C and finally centrifuged at 1500 *g* for 10 min. Lysis of the cells was observed by determining

absorbance at 600 nm using colorimeter. The respective dilutions of test compounds (without erythrocytes) were used as blank for determination of absorbance. The erythrocytes were completely lysed by treatment with 1% Triton-X100 and absorbance of the released hemoglobin was taken as 100% lysis[21].

Assessment of in vivo antimicrobial activity—Since petroleum ether (PEAS), ethylacetate (EAAS), ethanolic fraction (ELAS) and isolated flavanoids sulphates showed good activity during *in vitro* studies against *Klebseilla pnuemoniae*, the activity of these compounds was accessed using animals. Swiss mice of either sex (20-22 g) were used in the study. All the animals were given a standard pellet diet (Hindustan Lever Ltd) and water *ad libitum*. Animals were checked daily for their mortality and morbidity prior to commencement of the study and only healthy animals were included in the experiment. Techniques used for the bleeding, injection as well as sacrifice of the animals were approved by the Animals Ethics Committee as per CPCSEA guidelines. Each animal was challenged by 5×10^5 viable *Klebseilla pnuemoniae* bacteria in 200 µl of normal saline (0.9%) through intravenous route. The drug treatment was started 24 h post infection. Suspension of PEAS, EAAS and EtAS was prepared in Tween 80 and administered orally at a dose of 100 mg/kg body weight, whereas the isolated flavanoid sulphates were dissolved in DMSO and administered at a dose of 2 µg/ml. Control group animals were given normal saline. All the test extracts were administered for 7 days and necessary precautions were taken to administer specified dose of the drug to the experimental animals.

Statistical analysis—Effect of treatment on the survival rate of the animals was tested by Mantel Haenzel test[22]. $P<0.05$ was considered statistically significant.

Results

Yield of petroleum ether fraction (PEAS), chloroform fraction (CAS), ethyl acetate fraction (EAAS) and ethanol (EtAS) fraction after successive extraction of *A. speciosa* root powder was 0.12, 0.4, 0.8 and 1.75% respectively. Amount of flavanoid sulphates isolated was 15 and 10 mg for quercetin 3'7 di-O methyl 3-sulphate and as kaempferol 7-O methyl 3-sulphate, respectively. EAAS fraction and flavanoid sulphates inhibited the growth of *M. tuberculosis* H_{37} Rv ATCC 27294 sensitive strain at MIC values 50 and 25 µg/ml, respectively (Table 1). The tested fractions inhibited the growth of bacteria at different MIC values. Among the four fractions EAAS and EtAS fractions and flavanoids of the plant showed significant activity. MIC values of fractions and flavanoid sulphates for antibacterial and antifungal activity have been represented in Table 2. All the tested fractions and flavanoid sulphates showed better activity against *K. pneumoniae* than other organisms tested. Among the fractions tested against fungi, chloroform fraction showed significant inhibition with a lesser MIC (100 µg/ml) compared to other fractions against both the tested fungi, whereas flavanoid sulphates of the plant did not show any inhibition against fungi tested. Since quercetin 3'7 di-O methyl 3-sulphate, kaempferol 7-O methyl 3-sulphate and EAAS fraction showed active against *M. tuberculosis*, the investigation was extended to study the synergetic effect between these active compounds and commercially available antitubercular drugs. FIC index calculations, which are widely accepted method to evaluate *in vitro* synergistic studies between antitubercular compounds used in the present experiments and the results, have been given in Table 3. A synergistic effect between flavanoids sulphates and commercially available antitubercular drugs was observed having FIC index of 0.443±0.245, 0.487±0.247 for isoniazid and 0.468±0.333, 0.417±0.345 for rifampicin whereas EAAS fraction showed partial synergistic effect having FIC index of 0.612±0.204 and 0.735± 0.247 for isoniazid and rifampicin, respectively.

Synergism study for different fractions and isolated compounds was also studied with antibiotics (ciprofloxacin and norfloxacin). A synergistic effect was observed for EAAS fraction and flavanoids sulphates having FIC index < 0.5 and partial synergism with antibiotics for other fractions having

Table 1—Antitubercular activity of flavanoid sulphates and different fractions of *Argyreia speciosa* against *M. tuberculosis* H_{37} Rv strain

Plant fraction/isolated compounds	MIC in µg /ml.
PEAS	>100
CAS	>100
EAAS	50
EtAS	>100
Isoniazid	0.25
Rifampacin	0.1
Quercetin 3'7 di-O methyl 3- sulphate	25
Kaempferol 7-O methyl 3-sulphate	25

PEAS: Petroleum Ether fraction, CAS: Chloroform fraction, EAAS: Ethyl acetate fraction, EtAS: Ethanol fraction.

FIC index between 0.5 and 1.0. The results have been summarized in Table 4.

Results of hemolysis assay suggested that EAAS and flavanoids sulphates caused least hemolysis of erythrocytes as compared to chloramphenicol (Fig. 1). Survival data showed in Table 5 clearly demonstrated that on day 10th of post treatment of different fractions and isolated compounds of *A. speciosa*, about 60% of the animals treated with EAAS, 40% of animals treated with EtAS and 70% of animals treated with flavanoids sulphates were survived. All the control animals were died within 6 days.

Discussion

Antimicrobial activities of various plants have been reported[20,21,23]. Plant derived compounds have been attracting much attention as potent alternatives for infectious diseases. Phytoconstituents present in plant extracts namely polyphenols, flavonoids, flavones, quinones, alkaloids, tannins, triterpenoids, lectins, latex, lignan, lactones, resins, monosaccharide, organic acid, coumarin, polypeptides and essential oils are providing excellent opportunity for the expansion of modern chemotherapies against wide range of resistant microorganisms[24-26]. Quercetin and kaempferol are widely distributed polyphenolic flavanoid compounds in nature. These flavanols possess anti-inflammatory[27] analgesic[28], cytotoxic[29], antioxidant and antimicrobial[30,31] activity. These compounds possessed significant action against variety of gram positive and gram negative microbes. Further, antimicrobial combinations of quercetin with

Table 3—Synergism between potential fractions of *A. speciosa* and commercially available antitubercular drugs against *Mycobacterium tuberculosis* strain
[Values are mean ± SE of 2 replications]

Plant fraction/isolated compounds	FIC index	
	Isoniazid	Rifampicin
EAAS	0.612±0.204	0.735±0.242
Quercetin 3'7 di-O methyl 3-sulphate	0.443±0.245	0.487±0.333
Kaempferol 7-O methyl 3-sulphate	0.487±0.247	0.467±0.345

Synergistic effect: FIC index ≤ 0.5, Partially synergistic effect: 0.5 < FIC index < 1.0, no synergistic effect: FIC index >1.0, Antagonistic effect: FIC index ≥ 2.0.

Table 4—Synergism between potential fractions and flavanoids sulphates of *A. speciosa* with commercially available antibiotics against bacteria
[Values are mean ± SE of 2 replications]

Plant fraction/isolated compounds	FIC index	
	Ciprofloxacin	Norfloxacin
PEAS	0.842±0.322	0.752±0.208
CAS	0.654±0.291	0.545±0.372
EAAS	0.423±0.191	0.542±0.195
EtAS	0.673±0.303	0.726±0.274
Quercetin 3'7 di-O methyl 3-sulphate	0.324±0.246	0.425±0.328
Kaempferol 7-O methyl 3-sulphate	0.343±.354	0.453±0.292

PEAS: Petroleum Ether fraction, CAS: Chloroform fraction, EAAS: Ethyl acetate fraction, EtAS: Ethanol fraction, Synergistic effect: FIC index ≤ 0.5, Partially synergistic effect: 0.5 < FIC index < 1.0, no synergistic effect: FIC index >1.0, Antagonistic effect: FIC index ≥ 2.0.

Table 2—Efficacy of *A. speciosa* fractions and flavanoids sulphates against bacteria and fungi
[Values are mean of two replications]

Plant fraction/isolated compounds	MIC (µg /ml)					
	Bacteria				Fungi	
	Staphylococcus aureus	*Enterococcus feculis*	*Klebsiella pnuemoniae*	*Escherichia coli*	*Candida albicans*	*Aspergelless fumigatus*
PEAS	250	>500	4	250	>500	>500
CAS	125	62.5	31.25	250	100	100
EAAS	62.5	31.25	4	62.5	500	500
EtAS	31.25	31.25	4	62.5	250	250
Quercetin 3'7 di-O methyl 3-sulphate	62.5	125	2	62.5	nil	nil
Kaempferol 7-O methyl 3-sulphate	62.5	125	2	62.5	nil	nil
Ciprofloxacin	<5	<5	≤1	≤1	-	-
Norfloxacin	<5	<5	≤1	≤1	-	-
Flucanazole	-	-	-	-	8	8

PEAS: Petroleum ether fraction, CAS: Chloroform fraction, EAAS: Ethyl acetate fraction, EtAS: Ethanol fraction.

Table 5—Efficacy of *A. speciosa* root fractions and isolated compounds on *K. pneumoniae* infection in mice

Plant fraction/isolated compounds	No of days/ Percentage of protection										
	0	1	2	3	4	5	6	7	8	9	10
Control	100	100	100	80	60	20	NS	NS	NS	NS	NS
PEAS	100	100	100	80	60	60	40	20	NS	NS	NS
EAAS	100	100	100	100	100	100	80	60	60	60	60
EtAS	100	100	100	100	100	80	60	60	60	40	40
Quercetin 3'7 di-O methyl 3- sulphate (QS)	100	100	100	100	100	100	80	80	80	70	70
Kaempferol 7-O methyl 3-sulphate (KS)	100	100	100	100	100	100	90	80	80	70	70

Swiss albino mice (n=60) were challenged with 5×10^5 cfu of *K. pneumoniae*. The animals were treated orally with different fractions of *A. speciosa* (100 mg/kg body weight) and flavanoids sulphates (2 mg /kg) daily for 7 days. *P* value: Control vs. EAAS, $P < 0.001$; Control vs. QS, $P < 0.001$; Control vs. KS, $P < 0.001$. NS: Not survived. PEAS: Petroleum Ether fraction, CAS: Chloroform fraction, EAAS: Ethyl acetate fraction, EtAS: Ethanol fraction

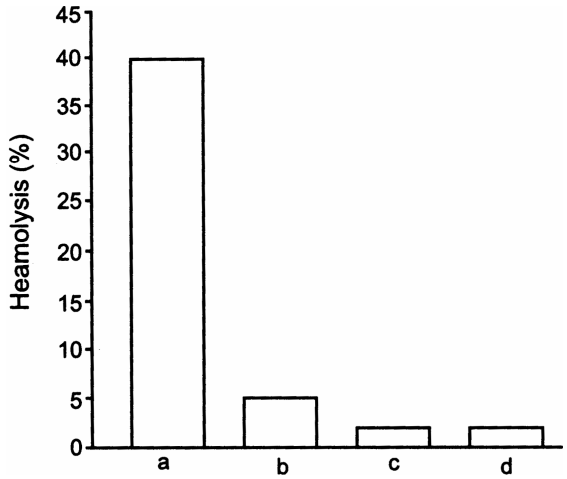

Fig. 1—Cellular toxicity of ethyl acetate fraction and flavanoids of *A. speciosa* [a=chloramphenicol (100 mcg/ml), b= EAAS (200 mcg/ml), c= quercetin sulphate (100 mcg/ml), d= keamferol sulphate (100 mcg/ml)]

antibiotics resulted in synergism without any antagonism. In the present study, gram positive and gram negative bacteria, *M. tuberculosis* and fungal strains were selected for the screening of antimicrobial effect of *A. speciosa* extracts and flavanoid sulphates to perceive the antimicrobial spectrum as well to validate ethnomedicinal assertion. Fractions were considered as active if they gave MIC≤ 500 µg/ml against bacteria and a MIC ≤ 200 µg/ml against fungal strains and a MIC ≤ 100 µg/ml against the strain of *M. tuberculosis*. Although, some consensus on these values can be found in the literature concerning *M. tuberculosis,* in the case of other microorganisms tested, there does not appear to be a clear criterion for determining the lower concentrations of plant extracts that can be considered as having an adequate antibacterial activity. In a recent review Rios[32] has suggested to avoid experiments with quantities higher than 1000 µg/ml. *M. tuberculosis* H_{37} Rv strain is a standard strain used around the world to study the preliminary antituberculosis activity of different chemical entities and plant extracts. In the present study, EAAS and flavanoid sulphates isolated from the *n*-butanol fraction of *A. speciosa* showed better activity at MIC value of 50 and 25 µg/ml, respectively. *Staphylococcus aureus,* and *Klebesiella pneumonia* were included in the study, because these species are some of the most common bacterial agents for pneumonia. Treatment of patients infected with these strains is difficult because bacteria are resistant to variety of penicillins[33, 34]. *Enterococcus feculis* and *Escherichia coli* are the common bacterial strains causing GIT infections and other diseases. EAAS and EtAS fractions of *A. speciosa* showed best activity against these organisms at acceptable MIC values (4-62.5 µg/ml).

Combination of antimicrobial agents with different modes of action is useful in the treatment of infectious diseases. One benefit is decrease of the administration dose of each individual agent due to synergetic effect, reducing the appearance of side effects and resistant mutants[35,36]. Fraction inhibitory concentration (FIC) was determined to study synergistic effects of active fractions and flavanoids sulphates with commercially available antitubercular drugs and antibiotics against *M. tuberculosis* and other organisms. The EAAS fraction and flavanoids sulphates showed better synergism with drugs and organism studied. Keeping in to consideration the fact that antibiotics exert serious untoward effects to the host tissues leading to the systemic toxicity, we performed hemolysis assay which revealed that administration of *A. speciosa* fraction and isolated compounds did not lead to the unfavorable biochemical changes against human erythrocytes[21]. This study was extended in animal system as well and established the potential of

A. speciosa fractions and isolated compounds to cure experimentally induced pneumonia infection in mice. The results clearly demonstrated that EAAS and flavanoids sulphates were significantly active against experimental pneumonia.

Hence, the results of the present investigation revealed that *A. speciosa* had antibacterial, antifungal, and antituberculosis activity. Although phytotoxic hexadecanyl p-hydroxy cinnamate and scopoletin have been isolated from the plant, this is the first report on antimicrobial, antitubercolsis activity of flavanoid sulphates from the plant. Results of the study show a good correlation between the reported uses of *Argyreia speciosa* roots for respiratory and other infections in Indian system of medicine. Finally it can be concluded that the active chemical compounds present in *A. speciosa* are useful for the treatment of bacterial infections, particularly pulmonary tuberculosis and pneumococcal infections.

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