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Microbiological, Pharmacological and Phytochemical Efficacy of *Catharanthus roseus* (L.) G. Don

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ABSTRACT

Madagascar periwinkle (Catharanthus roseus) is a medicinal plant of ethnopharmacological importance. The antimicrobial activity of all the extracts of C. roseus against S. typhi, X. citri, S. dysentriae, S. paratyphi B, A. niger and V. cholerae along with potentiation effect on Gentamicin is reported for the first time in literature to the best of our knowledge. Organic solvents with different polarity and dipole moment were used to extract the bioactive compound from dried leaves of C. roseus using soxhlet extraction. Extracts were evaluated for their antimicrobial susceptibility, synergistic and potentiation effect against the tested pathogens and their phytochemical contents were also analysed. Data interpretation indicates that the pattern of inhibition depends upon the plant part used, the extraction procedure, temperature, the assaying method and the organisms tested. It has been reported for the first time that polarity and dipole moment of a solvent plays an important part in the extraction of bioactive compound which in turn determines various activities being examined. X. citri was found to be most susceptible, while A. niger was resistant to all the organic extracts for the antimicrobial and synergistic activity. The combination of extracts tested for synergistic activity against the tested pathogens was found to be additive for bacterial pathogens and antagonistic in case of fungal pathogens. Blend of extracts with ethyl acetate was found to give better synergistic activity. Phytochemical analysis revealed alkaloids in all the extracts. Saponins, tannins and phytosterols were also present in some extracts. Activity of acetone extract was found to be maximum than ethyl acetate, followed by methanol, ethyl alcohol and chloroform.

Keywords: *C. roseus, Antimicrobial activity, Potentiation effect, Synergistic effect, Phytochemicals.*

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INTRODUCTION

Humans depend upon plant kingdom for their elemental needs like food, shelter, clothing, fertilizers, flavours and fragrances and, medicines. As the bacterial pathogens are thriving resistance against most of the currently accesible synthetic antimicrobial drugs, it is the need of an hour to produce new safer herbal antimicrobial medicines. India is graced with a rich wealth of medicinal plants. It possess around 47000 plant species and in the world biodiversity is 8th in rank and out of which, 8000 species are known to be medicinal. 2500 plant species are used by Indian system of medicine which belon to more than 1000 genera. Indians have always used herbs as the prime form of the medicine. Herbal medicine is usually called as botanical medicine or phytomedicine and it refers to using plants seeds, flowers, roots and other parts for medicinal purpose. Herbalism has a long tradition of use even outside of conventional medicine. Terrestrial plants, especially higher plants, have a long history of use in the treatment of human diseases. Enthralling research is being done all across the globe by various researchers on various aspects of medicinal plants. Plants especially those with ethnopharmacological uses, have been the primary sources of medicines for early drug discovery. The herbal medicines serve the health needs of about 80% of the World's population. More than 65% of the population globally uses medicinal plants as primary health care procedures[10]. Natural products or natural product derived drugs comprise about 28% of all new chemical entities launched onto the market. Plants have proved to be significant natural resources for medicines as documentation of their use in medicine originates from ancient times. Universal and Ethnobotanical plants provide a rich resource for natural drug research and development. On plant derived pharmaceuticals, 80% of World's population is dependent although unmet advancement in the field of medicine and molecular diagnosis has been made. WHO report depicts that plant based products or its derivatives accounts for around 28% of drugs available in the market. A large proportion of plant based compounds are used as lead molecules in discovery of drugs to produce molecular synthetic analogs that have similar skeletons yet intricate structures. Phytochemicals play a critical role in diversity oriented synthesis (DOS)[12], of natural product like pharma compounds[13,19]. Bacterial resistance to antibiotics has developed due to increasing use of antibiotics and poor patient compliance. As a result of this infectious diseases are becoming leading cause of worldwide mortality and morbidity especially in developing countries. Antibacterial activity of various plant parts like root, stem, leaves, seeds, flowers, fruits had been done for some of the medicinal plants in the past decades. The emerging drug resistant strains pose a threat to public. Therefore novel antimicrobial agents must be discovered[9]. A variety of biomolecules from various medicinal plants have antimicrobial action which can be a source for the development of new drugs. Medicinal plant based drugs owe the advantage of being simple, effective and exhibit a very broad spectrum of activity[4].

Catharanthus roseus is a short lived perennial with dark green and glossy leaves belonging to family Apocyanaceae[11,17]. It is popularly known as Madagascar periwinkle[5,20], as a species is endemic to Madagascar. It is a ubiquitous perennial evergreen shrub[15], growing one meter tall. It is an endangered plant in wild. It is also however widely cultivated and is naturalised in tropical and subtropical areas of the World. In Ayurveda the extracts of its roots and shoots are used against several ailments. In traditional Chinese medicine, extracts from it have been used against many diseases, including malaria, diabetes and Hodgkin's lymphoma. It is also used as an astringent, diuretic and expectorant[16]. Studies have found that *C. roseus* is potential source for anti-leukemic alkaloids. *C. roseus* has numerous effective invaluable therapeutic properties like anti-cancer, anti-diabetic, anti-microbial, anti-oxidant, anti-helminthic, anti-ulcer, anti hypertensive and anti diarrheal. It is also used in phytoremediation and has various ethnomedical (folkloric) uses.

From all the literature review that we garnered, following things were extrapolated:

- The *C. roseus* extracts were more efficient than the *C. alba* extracts prepared from various plant parts. *C. roseus* exhibited overall better antibiogram activity, so bioactive compounds of *C. roseus* could potentially be exploited as antimicrobial agents.
- *C. roseus* exhibits potent antimicrobial activity against various Human and Plant pathogens.
- The disk diffusion method was less efficient than the agar well diffusion method for Antimicrobial Susceptibility Testing (AST).
- Cold extraction procedure was less efficient than the hot extraction procedure.
- The leaves extract of *C. roseus* were having more antimicrobial activity as compared to other plant parts.
- The organic extracts were more efficient than the aqueous extracts.
- Extracts prepared in Organic Solvents like Methanol, Ethyl acetate, Acetone, Ethanol and Chloroform had good antimicrobial activity against the pathogens used.
- Synergistic activity can be used to detect whether the individual extracts are more potent or their combinations are.
- Potentiation activity of the extracts can be checked against a known antibiotic.
- A vast number of Phytochemicals are present in *C. roseus*.

Considering these conclusions drawn from the literature reviewed so far and a growing concern for the burgeoning MDR strains, this present work aims to determine antimicrobial susceptibility testing of dried leaves extracts prepared using Hot Extraction method, of *C. roseus*, in organic solvents against various Human and Plant pathogens by agar well diffusion method. Synergistic activity of leaves extracts against microbial pathogens and the Potentiation effect of the extracts on known antibiotic, Gentamicin were performed. The phytochemical analysis of the extracts was also done.

MATERIALS AND METHODS

Collection of plant materials

Disease free fresh plant material were gleaned from different localities of Nashik district (Maharashtra) randomly. The plant material was cleansed by washing it in running tap water and then in sterilised water[2,6]. The leaves were spread on a filter paper to remove excess water and then were dried in shade until complete dryness was observed[21]. The dried leaves were homogenised into fine powder in grinder. This fine powder was stored in a polythene bag and in dark under room temperature till future use.

Procurement of microorganisms

The Bacterial and Fungal pathogens used for various activities were collected from Dr. Vasantrao Pawar Medical College, Adgaon, Nashik and also from Culture collection centre of P.G. Department of Microbiology K.T.H.M. College, Nashik. These pathogens were- *Escherichia coli, Shigella flexneri, Klebsiella pneumoniae, Citrobacter divergens, Salmonella typhi, Salmonella paratyphiB, Pseudomonas aeruginosa, Vibrio cholerae, Candida albicans, Aspergillus niger, Proteus vulgaris, Bacillus subtilis* and *Xanthomonas citri.*

Chemicals and Medium

All the chemicals used for the extract preparation, antimicrobial activity, synergistic activity and phytochemical analysis were of analytical grade.

Preparation of Extracts

Five solvents were chosen for the preparation of the leaves extracts of *C.roseus.* These solvents were Chloroform, Ethyle acetate, Acetone, Ethanol and Methanol, chosen on the basis of polarity and dipole moment (Table 1).

Table 1 : Solvents on the basis of their polarity and dipole moment

Solvent	Polarity	Dipole moment
Chloroform	Non polar	1.04 D
Ethyl acetate	Polar aprotic	1.78 D
Acetone	Polar aprotic	2.88 D
Ethanol	Polar protic	1.69 D
Methanol	Polar protic	1.70 D

50gm of dried powder of *C. roseus* leaves was taken into a cotton thimble[7] and extracted with 100ml of organic solvents like Ethyl acetate, Methanol, Chloroform, Acetone and Ethyl alcohol using Soxhlet apparatus for 6-8 hours under respective boiling points of each solvent. The temperature parameter is of utmost importance for the activity of extracts as the higher temperature will adversely affect the extracts. Rotatory Vaccum Evaporator was used for evaporation of the solvent. The evaporation process resulted in complete pure dry extract. Proper care was taken for controlling the temperature here also so that the extract doesn't lose its activity or doesn't gets charred. Before making the extracts the initial weight of the flask was taken and then the final weight was taken after evaporation. The amount of extract obtained was assessed by examining the difference in weight (final-initial). Concentration of each extract was made to 300µg/ml, by dissolving it in respective quantity of DMSO[2]. The extracts were made sterile by vaccum filtration with a bacteriological filter. The extracts were stored in sterile amber colour glass bottle and kept in refrigerator until further use.

Antimicrobial Susceptibility Testing

Agar well diffusion method was used to evaluate antimicrobial activity[1,22] of dried leave extracts of *C. roseus* against the pathogens. Using CLSI guidelines the suspension of pathogens were prepared using $BaSO_4$ turbidity standard equivalent to a 0.5 McFarland which contained 2 x 10^8 CFU/ml. On Sterile Mueller Hinton agar plates organisms were spread using sterile cotton swabs and the plates were kept inverted for 5 minutes in order for microorganisms to adhere and also for moisture removal. Then using a sterile borer, wells were digged and respective extracts (300µg/ml) were inoculated (25µl) in their allotted/ respective wells using a micropipette. DMSO was used as a negative control. The whole procedure was performed in duplicates and under sterile conditions in Laminar Air Flow. The plates were kept for prediffusion at 4^0C for 30min. The plates were then incubated at 37^0C for 24hrs. Observations for the inhibition zones were measured and recorded using digital vernier caliper.

Synergistic activity

Synergistic effect is the combination of two or more pharmacologically active compounds in order to check their mutual effect in comparison to their individual effect against a biologically active entity. The synergistic effect may be Antagonistic, Additive or Neutral. The use of synergistic combinations in antimicrobial chemotherapy is often used commercially for the treatment of various infections. Two plant extracts were amalgamated into one which was used to evaluate the synergistic effect against the different microbial pathogens. Well diffusion technique was performed to check synergistic effect of extracts on pathogens. All the extracts were mixed in equal proportions (1:1) aseptically[2,3]. The same procedure was used as described above for AST. The combinations of solvents were used as a control.

Potentiation effect

The difference between synergistic and potentiation effect is that synergistic is interaction of two or more substances while potentiation is about single substance and how it may act when in a synergy relationship. Potentiation effect is done to check whether the activity of known antibiotic is increasing or not when combined with pharmacologically active compounds against a pathogen. The plant extracts in combination with the antibiotic Gentamicin was used to evaluate the potentiation effect against the different microbial pathogens[2,3]. Gentamicin was used as it was effective in treating infections against a large spectrum of microbial infections. The concentration of antibiotic used was 25µg/ml. All the extracts were mixed with antibiotic in equal proportions (1:1) aseptically. The same procedure was used as described above for AST. Gentamicin was used as a control.

Phytochemical analysis

Diluted extracts were used for phytochemical analysis. Phytochemicals like alkaloids, saponins, tannins, triterpenes, flavonoids, anthraquinones, phytosterol, glycosides and steroids were determined by qualitative

method described by Bholay *et al*[3]., Kumari *et al*[12]., Prihanto *et al*[18]., and Mouafi *et al*[14].,

RESULTS AND DISCUSSIONS

Antimicrobial activity

The observation of antimicrobial activity (Table 2) was carefully scrutinized and the following results were made. Observations indicated that the maximum zone of inhibition was in acetone extract followed by Ethyl acetate, Methanol, Ethyl alcohol and Chloroform extracts. The maximum zone of inhibiton of acetone extract was observed against *Xanthomonas* followed by *P. aeruginosa, K. pneumoniae, V. cholerae, S. typhi, B. subtilis, S. paratyphi B, S. flexneri, P. vulgaris, E. coli* and *C. divergens.* For the ethyl acetate extract the maximum zone of inhibition was observed against *X. citri*, followed by *S. flexneri, V. cholerae, C. divergens, S. paratyphi B, P. vulgaris, E. coli, S .typhi, P. aeruginosa, B. subtilis,* C. *albicans, K. pneumoniae* and *A .niger*. Zone of inhibition for methanol extract was observed maximum for *X. citri* followed by *B. subtilis, V. cholerae, K. pneumoniae* and *C. divergens.* For the chloroform extract the maximum zone of inhibition was against *S. paratyphi B* followed by *X. citri, S. flexneri, V. cholerae, C. albicans, P. vulgaris, B. subtilis, P. aeruginosa, C. divergens, K .pneumoniae* and *E. coli*. For ethyl alcohol extract the maximum zone of inhibition was observed against *P. vulgaris*, followed by *X. citri, S. paratyphi B, C. albicans, S. flexneri, C. divergens, S. typhi, V. cholerae, K. pneumoniae, P. aeruginosa* and *B. subtilis*.

Table 2: Antimicrobial susceptibility testing of *C. roseus* leaves extracts. (Mean ± SD)

Solvents → / **Pathogens ↓**	**Methanol**	**Ethyl alcohol**	**Ethyl acetate**	**Chloroform**	**Acetone**
E. coli	-	-	18.5 ± 2.12	10.21±0.20	13.0 ± 0.20
S. flexneri	-	11.2 ± 1.41	24.2 ± 1.66	12.1±1.23	17±1.20
K. pneumoniae	11.5± 0.00	10.5 ± 0.71	13.5 ± 3.53	10.5 ± 0.00	23± 3.10
C. divergens	11.1 ± 0.00	12.5 ± 0.00	21.5 ± 2.12	10.3 ± 0.00	11.5 ± 2.40
S. typhi	10.6±0.00	12.6±0.00	15.1 ± 1.07	-	18.5 ± 0.71
S. paratyphiB	-	14.1±0.00	18.3 ± 2.16	**16.1±1.05**	15 ± 1.90
P. aeruginosa	-	10.5 ± 0.71	18.5 ± 2.82	10.2 ± 0.00	23 ± 2.70
V. cholera	11.4±0.71	11.4 ± 1.41	21.5 ± 2.12	11.5 ± 0.71	22 ± 0.00
C. albicans	-	13.1±0.00	15.6 ± 1.53	12.4±0.30	-
A. niger	-	-	13.2±1.54	-	-
P. vulgaris	-	**15.2 ± 1.41**	18.5 ± 2.82	11.31±0.21	17 ± 1.12
B. subtilis	12.2±0.28	11.1±0.00	19.5 ± 0.71	10.5±0.00	18 ± 3.01
X. citri	**18.1±0.00**	14.3 ± 1.66	**25.4 ±1.41**	15.4±2.24	**30 ± 1.01**

Synergistic activity

Results of synergistic activity are shown in table 3. Among acetonic combinations (Figure 1), acetone+ethyl acetate had shown synergisitc effect against *B. subtilis* and *P. vulgaris* followed by low synergistic effect by acetone+chloroform and acetone+ethyl alcohol. Rest of the acetonic combinations had shown antagonistic effect. Among ethanolic combination (Figure 2) of all the extracts, maximum synergistic effect was seen in ethanol+ethyl acetate combination. Maximum inhibition was observed against *Xanthomonas citri*, followed by *Pseudomonas aeruginosa* and *S. typhi*. Ethanol+chloroform combination had shown low synergistic effect followed by ethanol+methanol as compared to ethanol alone.

Table 3: Synergistic effect of *C. roseus* extracts on different pathogens

Combinations → / **Pathogens ↓**	Ethanol + Ethyl acetate	Ethyl acetate+ Methanol	Ethanol + Acetone	Ethanol+ Chloroform	Ethanol+ Methanol	Acetone+ Chloroform	Acetone+ Methanol	Chloroform+ Methanol	Ethylacetate+ Chloroform	Ethylacetate+ Acetone
E. coli	17.5± 0.00	18.4± 3.24	13.5± 0.90	-	12.1± 1.12	10.2± 1.90	13.2± 0.58	14.2± 1.24	12.7± 1.87	13.4± 2.50
S. flexneri	21.2± 0.70	22.2± 0.12	13.2± 2.40	12.5± 0.45	13.5± 0.00	14.5± 2.11	11.4± 1.57	10.5± 0.00	-	12.6± 2.07
K. pneumoniae	20.2± 0.20	19.1± 3.01	15.2± 2.14	11.1± 1.41	10.1± 0.23	17.5± 2.41	14.7± 2.16	10.6± 0.52	18.1± 0.18	22.1± 1.80
C. divergens	21.1± 0.40	20.1± 1.12	13.7± 1.20	13.2± 0.21	15.5± 0.56	-	-	-	15.2± 0.68	19.5± 2.54
S. typhi	27.2± 0.00	25.1± 0.00	17.5± 1.06	19.2± 2.12	15.8± 0.78	17.6± 1.50	16.5± 3.24	10.4± 1.24	-	-
S. paratyphi B	19.4± 2.10	21.6± 0.56	12.3± 2.10	12.5± 1.61	11.2± 1.56	-	10.2± 0.17	11.6± 1.58	11.5± 0.45	12.3± 1.24
P. aeruginosa	28.2± 0.00	26.4± 0.75	**18.2± 1.26**	14.3± 1.56	17.3± 2.15	24.1± 0.14	15.1± 0.71	16.4± 1.69	13.2± 1.56	15.9± 2.59
V. cholera	19.1± 3.12	20.2± 1.25	15.4± 1.30	11.2± 2.01	10.4± 0.51	17.4± 2.01	16.3± 1.26	11.1± 0.27	14.6± 2.64	16.5± 1.80
C. albicans	-	-	-	-	-	-	-	-	-	-
A. niger	-	-	-	-	-	-	-	-	-	-
P. vulgaris	19.4± 2.56	21.5± 0.45	14.6± 2.20	12.3± 3.02	13.1± 1.54	17.5± 3.12	13.5± 2.01	10.1± 0.69	23.7± 1.75	**26.4± 0.45**
B. subtilis	16.1± 0.00	15.2± 0.32	13.5± 1.09	11.4± 2.14	10.1± 2.17	11.2± 1.02	12.5± 0.25	10.2± 1.10	20.6± 0.78	25.6± 1.90
X. citri	**29.1± 2.01**	**28.2± 0.00**	23.1± 1.50	19.1± 0.21	**20.3± 0.00**	**24.6± 1.80**	**19.6± 1.35**	**171± 1.25**	**23.9± 0.94**	26.3± 0.23

For the ethyl acetate combinations (Figure 3) it was observed that ethyl acetate+ethyl alcohol had high synergistic effect followed by ethyl acetate+methanol and ethyl acetate+acetone compared to ethyl acetate alone ethyl acetate+chloroform had shown antagonistic effect against all the tested pathogens except *P. vulgaris, B. subtilis* and *X. citri.* All the extracts had shown antagonistic effect against *A. niger* and *C. albicans.* From the chloroform combinations (Figure 4) it was seen that chloroform+acetone had shown higher synergistic effect as compared to chloroform alone. Chloroform+ethyl alcohol had shown low synergistic effect against *E. coli*, *S. paratyphi B,* and *V. cholerae. C*hloroform+methanol combination had shown similar effect against *X. citri, B. subtilis* and *K. pneumoniae* and antagonistic effect against *S. flexneri*, *C. divergens*, *P. vulgaris* and *V. cholerae.* As compared to antimicrobial activity of methanol alone (Figure 5) the combinations of methanol extracts have shown considerable additive effect against all the pathogens except chloroform and methanol combination. Among all the combinations of methanol extracts, ethyl acetate+methanol combination had shown maximum synergistic effect against all the tested pathogens as compared to methanol alone. Chloroform+methanol combination had shown antagonistic effect among all the methanolic combinations as compared with the standard.

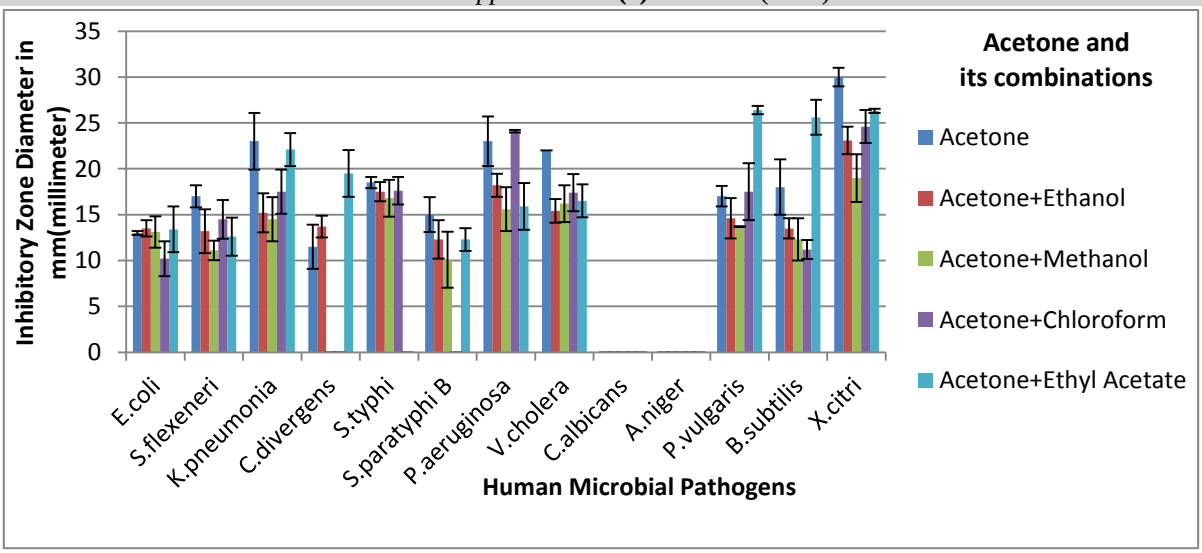

Fig. 1: Comparison of synergistic activity of acetone combinations with antimicrobial activity of acetone extract

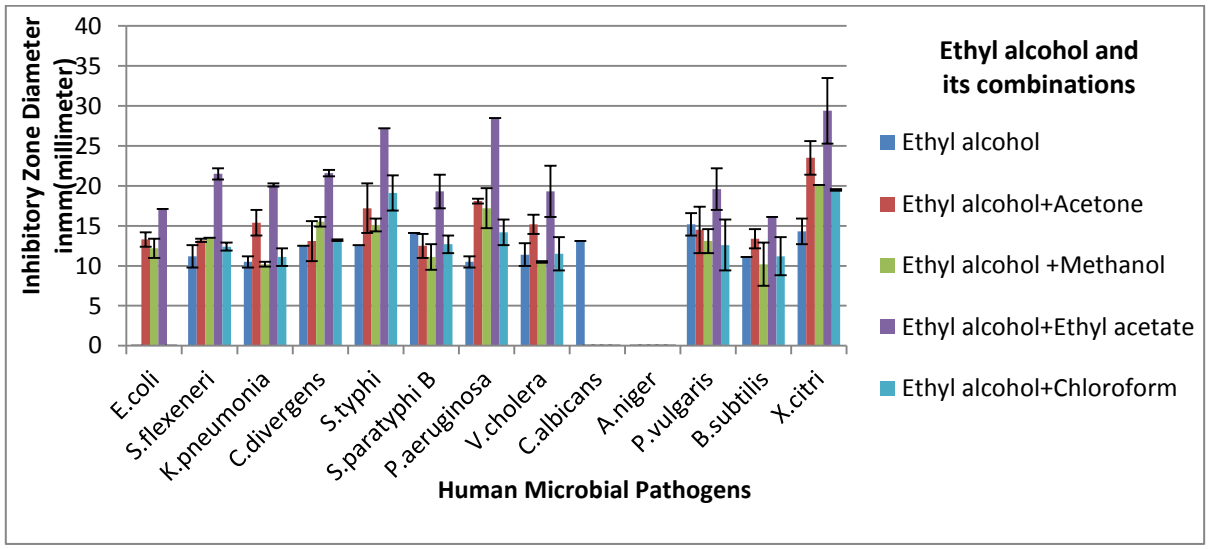

Fig. 2: Comparison of synergistic activity of Ethyl Alcohol combinations with antimicrobial activity of ethanol extract

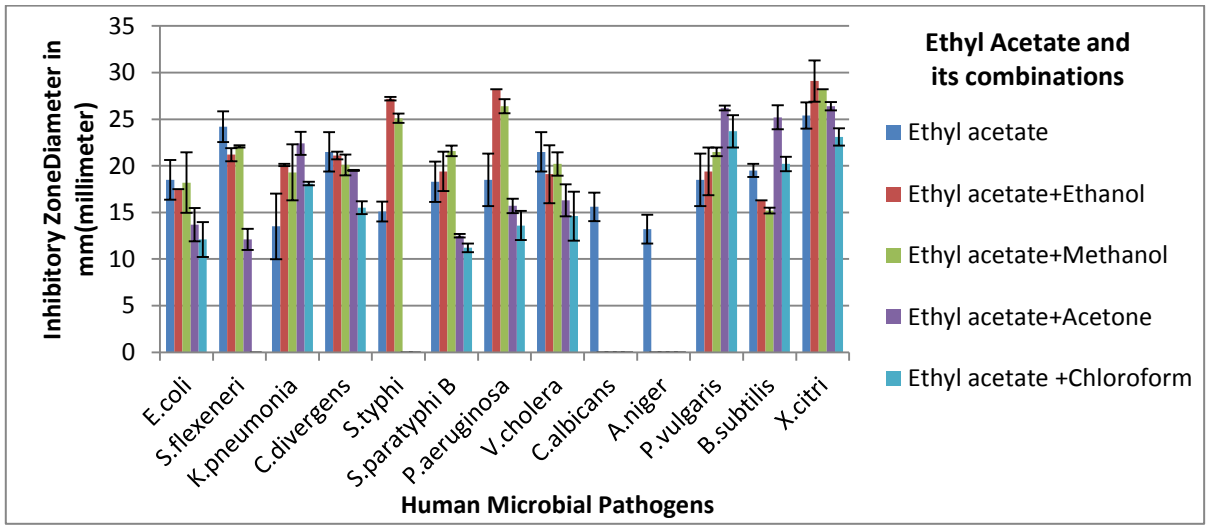

Fig. 3: Comparison of synergistic activity of ethyl acetate combinations with antimicrobial activity of ethyl acetate extract

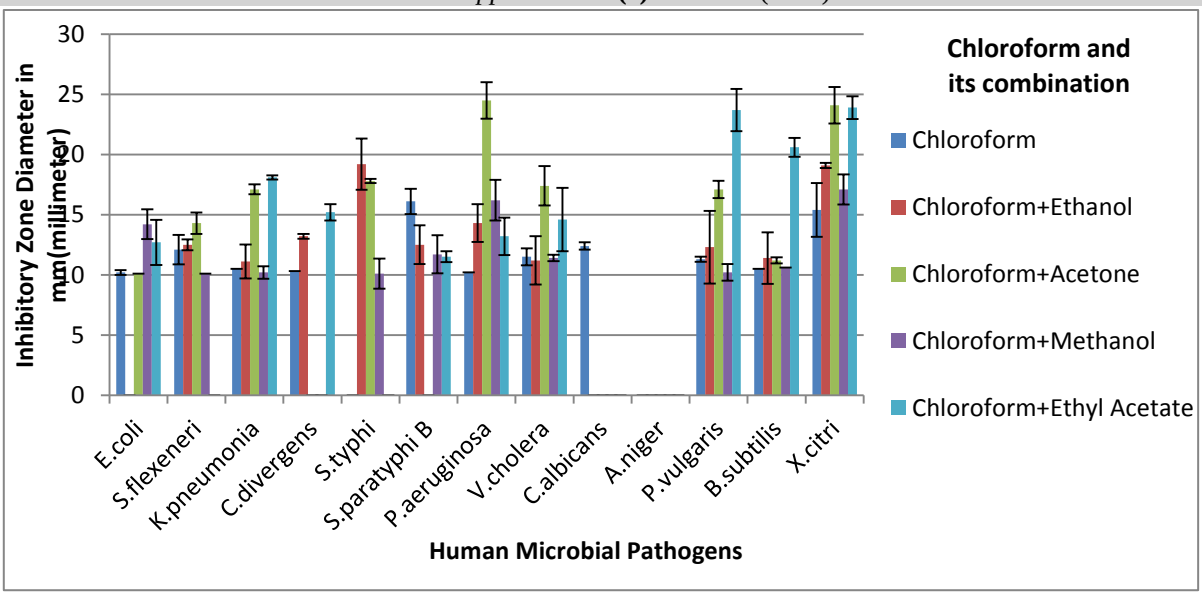

Fig. 4: Comparison of synergistic activity of chloroform combinations with antimicrobial activity of chloroform extract

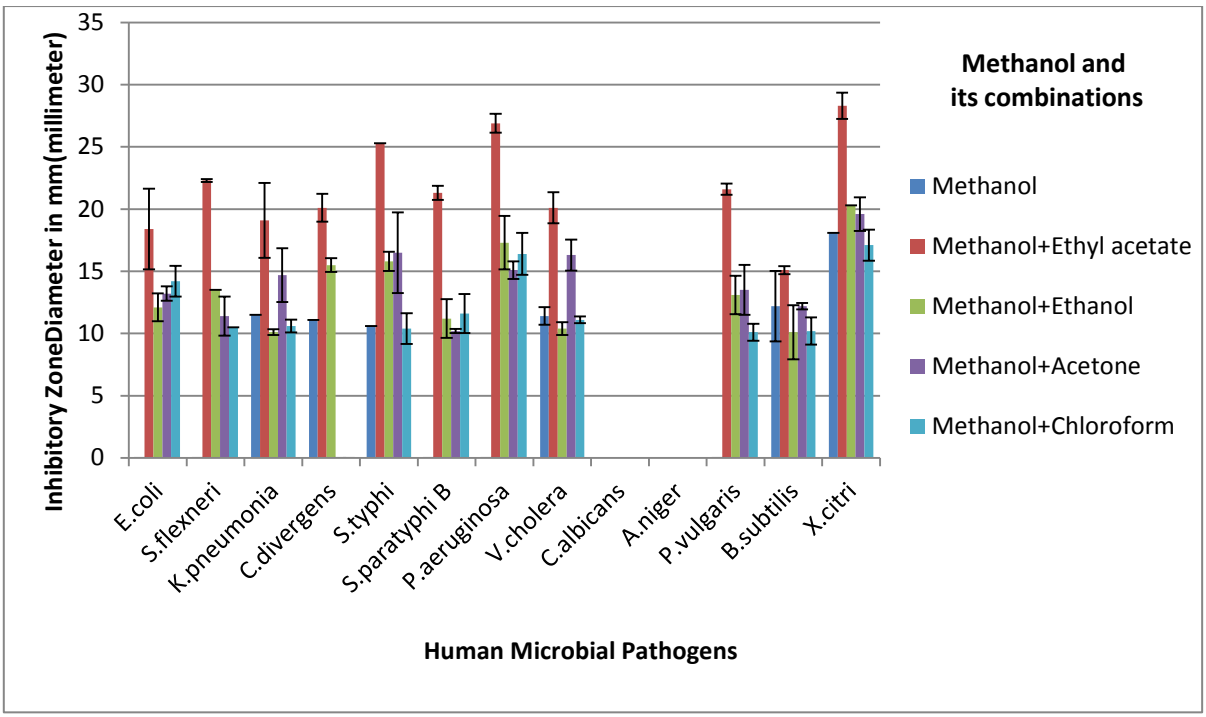

Fig. 5 : Comparison of synergistic activity of methanol combinations with antimicrobial activity of methanol extract

Potentiation effect

The results of the potentiation effect of gentamicin are shown in table 4. These results indicate that the extracts have nullified the effect of Gentamicin instead of potentiating it, only acetone extract was able to augment the effect against a single pathogen i.e. *Proteus vulgaris*, a marginal improvement.

Table 4: Potentiation effect of *C. roseus* leaves extracts on a standard antibiotic, Gentamicin. (Mean value ± standard deviation)

Extract → / Pathogens ↓	+ Methanol Gentamicin	+ Ethyl alcohol Gentamicin	+ Ethyl acetate Gentamicin	+ Chloroform Gentamicin	+ Acetone Gentamicin	Gentamicin
E. coli	27.5 ± 0.71	27.5 ± 0.71	26.3 ± 1.41	27.5 ± 0.71	27.5 ± 0.71	32.1 ± 0.00
S. flexneri	29.1± 1.41	29.5 ± 0.71	28.34 ±0.71	28.01 ± 0.71	30.1 ± 0.00	3.02 ± 0.00
K. pneumoniae	26.4 ± 0.00	25.5 ± 0.71	26.01 ±1.41	26.32 ± 1.41	27.3 ± 1.41	29.1 ± 1.41
C. divergens	18.12±0.71	17.5 ± 0.71	18.12 ±0.71	18.2 ± 1.41	25.5 ± 3.53	26.5 ± 0.71
S. typhi	-	-	-	-	-	-
S. paratyphi B	23.3 ± 2.81	24.2 ± 1.41	24.02 ±0.00	24.5 ± 0.71	24.4 ± 1.41	27.5 ± 0.71
P. aeruginosa	21.1 ± 0.00	21.12 ± 0.00	20.5 ± 0.71	20.01 ± 0.71	22.1 ± 0.71	27.2 ± 0.00
V. cholerae	24.22±0.71	24.5± 0.00	23.2 ± 0.71	24.2 ± 0.71	24.5 ± 0.71	27.5 ± 0.71
C. albicans	-	-	-	-	-	-
A. niger	-	-	-	15.3 ± 1.41	16.23± 0.00	20.1 ± 0.00
P. vulgaris	**31.4 ± 0.71**	**31.2 ± 0.71**	31.04 ±1.41	-	**32.5 ± 0.71**	32.5 ± 0.00
B. subtilis	30.5 ± 0.71	31.1 ± 0.71	**31.4 ± 0.00**	**30.01 ± 0.71**	31.2 ± 0.00	**32.25 ±0.00**
X. citri	28.01 ± 0.71	28.2 ± 1.41	29.1 ± 0.00	28.5 ± 0.71	30.1 ± 2.12	32.13 ±0.00

Phytochemical analysis

These observations indicate that the phytochemical constituents (Table 5) of extracts varied significantly depending upon the type of solvent used i.e. Methanol, Ethanol, Acetone, Ethyl acetate and Chloroform. Methanol extract showed the prescence of alkaloids, tannins, flavonoids, anthraquinones, phytosterols and cardiac glycosides. Acetone extract showed the prescence of alkaloids, saponins, tannins, phytosterols, steroids and flavonoids. Alkaloids and saponins were also present in ethanol, chloroform and ethyl acetate extracts. These results indicate that the prescence of different bioactive principles in different extracts could be due to the solubility of the phytochemical compounds in different solvents, as a simple rule is followed "like dissolves like."

Table 5: Phytochemical analysis of various leaves extracts of *C. roseus* (Present'+' and Absent '-')

Extracts → / Phytochemicals ↓	Methanol	Ethanol	Ethyl Acetate	Acetone	Chloroform
Alkaloids	+	+	+	+	+
Saponins	-	+	+	+	-
Tannins	+	-	-	+	+
Triterpenes	-	-	+	-	-
Flavonoids	+	+	-	+	-
Anthraquinones	+	-	-	-	-
Phytosterols	+	-	+	+	+
Glycosides	+	-	-	-	-
Steroids	-	-	+	+	+

CONCLUSIONS

Among all the pathogens tested against all the organic extracts of dried leaves of *C. roseus*, *Xanthomonas* was found to be most sensitive and *Aspergillus* was resistant to them. This result indicates that the antimicrobial activity (zone of inhibitions) varied depending upon the type of solvent used. Acetone extract exhibited maximum antimicrobial activity than the other solvent extracts of *C. roseus.* From

the results obtained it was concluded that the combinations of ethyl acetate with every extract had shown highest synergistic effect against *X. citri, B. subtilis, P. vulgaris, and P. aeruginosa.* Antagonistic effect was observed against the fungal pathogens used for synergistic combinations. Chloroform combinations had shown lower synergistic effect against the bacterial pathogens. In conclusion the synergistic effect of leaves extracts combinations of *C. roseus* against pathogens was observed and was additive against the pathogens as compared to antimicrobial activity of extracts alone. Our observations and results indicate that none of the extracts were able to withstand to potentiate the activity of antibiotic used, gentamicin. Only acetonic combination against a single pathogen, *P. vulgaris,* was able to potentiate the effect of gentamicin. The extracts showed an increasing deleterious effect on the antibiotic in the order – Acetone, Ethyl alcohol, Methanol, Ethyl acetate and Chloroform. From these observations and result it can be concluded that the combinations of extract didn't had additive interaction on gentamicn, in contrast they were proven to be showing antagonistic effect in combination with gentamicin. All the extracts showed the prescence of alkaloids, which indicates its higher solubility in different solvents. The interpretation of results indicates that *C. roseus* is highly rich in the diversity of phytochemicals constituents. Plants, including most food and feed plants, produce a broad range of bioactive chemical compounds via their so called secondary metabolism. Plants with potent bioactive compounds are often characterised as both poisonous and medicinal, and a beneficial or an adverse result may depend on the amount eaten and the context of intake. For typical food and feed plants with bioactive compounds with less pronounced effects, the intakes can be regarded as beneficial.

These solvents were chosen on the basis of their polarity and the dipole moment. From these results it was observed that activity of organic extract increased when the dipole moment increased viz in the order choloroform, ethyl alcohol, methanol, ethyl acetate and acetone. All antimicrobial activity were observed to be concentration dependent (dose dependent). Gram negative bacteria are more sensitive than the Gram positive, this may be due to the difference in chemical composition and the structure of cell wall of both type of microorganisms[8]. So we can conclude that acetone extract was more suitable solvent for maximum extraction of active metabolites which are responsible for antimicrobial activity. In this present study there is not such significant potentiation effect seen, but potentiation effect can be checked for other antibiotics as well which may prove fruitful. Future research can be carried out for identification of active principles which could possibly be exploited for pharmaceutical use.

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