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Ethnobotanical note and antibacterial evaluation of "Punarnava churna" an ethnic formulation of Rayalaseema Nostrum

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Abstract---In this article, we document the ethnobotanical information and elucidate the antibacterial potency of ethnic formulation "*Punarnava churna*" (PC) prescribed for its immune boosting and strength enhancing property. "*Punarnava churna*" prescribed by Rayalaseema nostrum was examined against gram positive and gram negative human pathogenic bacteria by Agar well diffusion method. Results revealed that this ethnic formulation showed good antibacterial activity against bacterial strains (*Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia*) at 50 mg/ml. In conclusion, the findings of this research suggest ethic drug "*Punarnava churna*" effective against common human pathogens and can be trusted as antibacterial agent.

Keywords--- *Punarnava churna*, ethnic formulation, Rayalaseema Nostrum.

Introduction

In the current scenario of unpredicted health complications and very less significant roles of established drugs it's therefore became a need for new form of antidote against uncertain complications (Arulmozhi et al., 2018; Ajijolakewu et al., 2021). The plant derived formulations were well established in the forest sectors of Rayalaseema as antimicrobial agent against some digestive problems, worm infestations, skin eruptions. Sudarsanam et al., (2019) documented the ethnobotany of Kuppam in which ethnic formulation and dosages were well documented for this region. In our previous research, we have evaluated the

antioxidant and Neutraceutical potential of Nitya yevvana kashayanam (NYK), Mahabala Kashayam (MBK) which were widely used as its immune boosting and strength enhancing agents prescribed by ethnic healers of Rayalaseema districts of Andhra Pradesh (Sripriya et al.,2020, 2021).

The origin of unknown infections, illness necessitated the search to discover new alternatives to treat varied microbial infestation in humans. Ethnobotanical formulations obtained by infusions of plant parts forms the vast source of medications which has to be mined properly. Considering miscellaneous tribal formulation, *Punarnava churna* is one of the formulation which is prescribed by Rayalaseema nostrum especially in Kadhiri forest regions popularly known to cure indigestion, loss of appetite, strength giving and for immune booster. We assumed that this uninvestigated formulation may reveal significant therapeutic properties.To ensure the antimicrobial effect based on the ethnic usage of NYK and MBK in folk medicine, it is attractive to analyze the antimicrobial potential and therefore this study forms the earliest step to evaluate the antimicrobial activity. In this article, antibacterial activity of "*Punarnava churna*" an ethnic formulation was investigated based on the based on the ethnobotanical information and claim.

Materials and Methods

Collection of ethnobotanical drug - Punarnava churna

Ethic drug *Punarnava churna* was procured from the tribal healers and physicians from different regions of Rayalaseema region of Andhra Pradesh, India. Random study of ethnic information revealed some of the formation regarding the usage of the formulation.

Preparation of Plant Extracts

The powdered plant was extracted using various organic solvents in order of increasing polarity (hexane, chloroform, ethyl acetate, acetone and methanol). Separately, 500 grams of powder were macerated in 1500 mL hexane for three days with intermittent shaking. After that, it was filtered twice: once with muslin cloth and then again with Whatman no.1 filter paper. The residue was extracted twice with the same fresh solvent, and all of the filtrates were combined. The resultant residue was air dried before being extracted using chloroform, ethyl acetate, and acetone in the same way as the hexane extraction was done. Finally, the solvent was evaporated from each filtrate using a rotary evaporator at low pressure and temperature. Each extract's yield was weighed and stored at 4 °C until it was utilised.

Preparation of aqueous extract

The drugs were solidified by using hot air oven and subjected to extraction using soxhlet apparatus and stored at 4°C until further use in the equipment. Modified protocol was followed

Bacterial cultures

Human pathogens Gram-positive (*Staphylococcus aureus* (AB/Sa-MS198) and *Bacillus subtilis* (AB/Bs-MS215) and Gram-negative (Escherichia coli (AB/Ec-MS211) and *Klebsiella pneumonia* (AB/Kp-MS092) bacteria were used in this investigation. These selected bacterial cultures were obtained from Adhya Bioscience Pvt. Ltd, Visakhapatnam, Andhra Pradesh.

Inoculum preparation

Bacterial strains were sub cultured on overnight at 37°C in Muller Hinton agar slants. The colonies were transferred using 5 ml of sterile saline water, standardized at 350 nm (equivalent to half McFarland) which gave a stock suspension of microorganisms equal to 1×10^6 CFU/ml saline (Arulmozhi et al.,2018).

Antimicrobial assay

Agar well diffusion was used to evaluate the antimicrobial activity of each plant extract as reported by Valgas et al., (2007). Similarly, to the procedure used in well diffusion method according to the method followed by Radhika and Aneja (2009). The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire surface. Then, the Muller Hinton wells were dispensed with the respective aqueous extracts (25–75 mg/ml) as preferred concentration. Doxycycline (Doxy – Dr. Reddy's (4 µg) and Azithromycin (Azee-Cipla) (4 µg) were used as positive controls, aqueous extract as negative control. Then, agar plates were incubated for 24 hr. at 37 ± 2°C for bacterial strains and 30 ± 2°C. The assay was carried out as a colorimetric indicator of Bacterial cell viability and was done in triplicates. The measurement of zone inhibition was taken from the top of the well to the clear zone in millimeter (mm) with MIC Zone Scale (HI Media).

Results and Discussions

Ethnobotanical Information

The ethanobotanical information on the plants selected were also collected and these plants were used for indigestion, gastric problems. *Punarnava churna* was prepared in different combinations based on the ethnic healers' prescription. In this report the employed plants were fruits of *Alangium salvifolium*, bark decoction of *Terminala arjuna*, few powdered seeds of *Piper nigrum*, jaggery water with lemon juice according to the taste. Prepared as infusion or decoction. Whole mixture was preserved overnight in a mud pot. This formulation should be taken at early hours before sunrise. Dosage depend on the age group. For children half a glass and for adults it's a complete glass.

Ethnobotanical Claim

Punarnava churna is a mixture of above plant material prescribed for Strength, Irritation, skin eruptions, Indigestion, taken daily orally in liquid form in the morning after taking breakfast and at night before going to sleep.

Antimicrobial Screening

The antimicrobial screening of "*Punarnava churna*" (PC) against Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*E. coli* and *K. pneumoniae*) bacteria were assessed from the absence or growth of bacteria and zone of inhibition. Results reported that the antimicrobial activity of the aqueous extract of ethnic medication "*Punarnava churna*" was dose dependent as the concentration of PC increased the inhibition also increased.

Tablen1
Information on Ethnobotanical formulation of Drug
Punarnava churna (PC)

S.No	Name of the Plant	Family	Part used	Preparation/ Dosage
1.	*Alangium salvifolium* + *Carissa spinarum*	Alangiaceae / Asclepiadaceae	Fruits	Fruits crushed with moto and pestle. To this tamarind juice is added in sufficient amount.
2.	*Terminala arjuna* + *Pterocarpus santalinus*	Combretacea/ Fabaceae	Bark	Bark of the stems were collected and soaked in the water overnight, hot water extraction
3.	*Aegle marmelos* + *Citrus sps.*	Rutaceae	fruits	To add flavour based on the taste against vomitting
4.	*Piper nigrum* + *Zingiber officinale*	Zinziberaceae	Seed , Rhizomes	Piper nigrum seeds were crushed and Rhizome is squeezed with combination

The crude aqueous extract of "*Punarnava churna*" induced important inhibitory activities with inhibition zone diameters (measured in mm) varying from 1.5 to 22 mm and MIC at <25 mg/ml. (Figure 1). These bacteria were found to be sensitive with the zone of inhibition varying from 3 mm to 12 mm for *Bacillus subtilis*, 8 mm to 15 mm for *E. coli*, 6 mm to 18 mm for *S.aureus*, 1.5 mm to 22 mm for K. pneumoniae. *Klebsiella pneumoniae* is comparatively resistant against extract. So, the anti-bacterial activity of the *Klebsiella pneumoniae* is very less/ Furthermore, the best antibacterial results observed were provided by the extract obtained whose MIC ranged from 25mg/mL to 50 mg/ mL for all the selected strains. Here, MIC not less than or equal to 50 mg/mL as considerable inhibition. PC presented the best antibacterial activity against *E. coli* and presented the least antibacterial activity against *Klebsiella pneumoniae*. The highest zone of inhibition (mm) (15 □ 0.8 mm) zone was obtained against *E.coli* at the concentration of 75 mg/ml. The

inhibitory effect of PC started at 25 mg/mL against all the bacterial strains tested. PC suppressed all the strains at 75 mg/mL to a maximum level. But the inhibitory effect was dose dependent.

E. coli and *B.subtilis* found to be sensitive at all the concentrations. There is no much difference between 50 to 75 mg/mL in the inhibition. *E. coli* was sensitive to all the concentrations than other bacteria. The *K.pneumoniae* was found to be resistant and very minimal inhibition was shown when zones were compared. *S.aureus* was moderately inhibited and the growth of inhibition was maximum at 50 mg/mL while the zone of inhibition is almost equal in case of 75mg/mL of PC. The inhibition zones of control were compared to the zone obtained with 5 µg of Doxycyclin and Azithromycin (Standard antibiotics –used as control). Whereas Azithromycin showed greater potency in inhibiting all the selected bacteria than Doxycylin. Graph.1 depicts the zones of inhibition with comparision of PC with Antibiotics. These are highly used antibiotics in COVID -19 treatment which were used as control. The other pertinent details of zones of inhibition against various strains (*S.aureus*, *P. aeruginosa*, *K. pneumonia* and *E. coli*) are provided (Table 1, Graph 1). Similar reports on the selected strains against aqueous extracts of medicinal plants was reported by Bhalodia and Shukla, (2011) and Gonelimali et al.,2018). The bacterial strains selected for the study induce human gastroenteritis diseases (Gupta et al. 2010; Pandey and Singh, 2011; Mostafa et al.,2018).

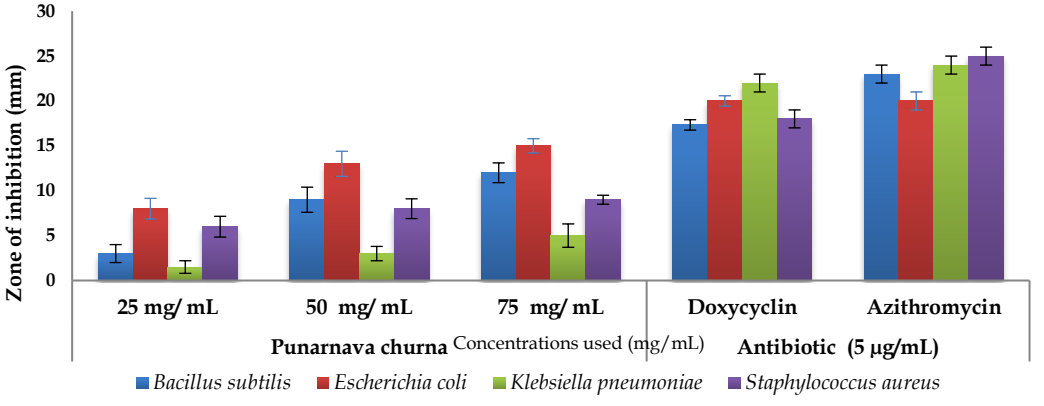

Figure 1. Antibacterial screening of Punarnava churna against bacterial strains

Indeed, our previous reports on this ethnic formulation reported good quantity of terpenoids (Sripriya 2020, 2021) whereas Ramos et al., (2021) reported that terpenoids cause the antibacterial efficacy. Purification of the bioactive components from the extracts has to be investigated which may improve our understanding of possible antimicrobial activities (Kothari et al., 2010; SreeLatha and Basha (2013). Phytochemical constituents such as saponins, tannins, alkaloids, terpenoids, anthraquinones, phenolic compounds, cardiac glycosides, and flavonoids are identified to be antimicrobial agents (Kebede et al., 2021). In our previous research it was reported ethnic formulations NYK, MBK possess saponins, phenolic rich bio constituents (Sripriya 2021,2022). The presence of therapeutic phytoconstituents like phenolic (polyphenols, flavonoids, tannins),

terpenes, pro anthocyanidins and anthocyanidins were found to be present which may possess antimicrobial effect. Several researchers investigated the efficiency of plant extracts and their effective compounds as antimicrobial agents to control growth of bacteria (Gill and Holley, 2006; Tiwari et al., 2009;). We opine that precursor molecules have to be instigated promptly for new effective antimicrobials and further studies should be done on clinical efficacy trial, safety, toxicity of this pure compounds. Based on the zone of inhibition it was evident that this ethnic drugs possess bioactive compounds which are responsible for acting as antimicrobial agent and should be tested for pharmacological efficacy.

Conclusion

From this study we can suggest that the traditional use of this ethnic drug "*Punarnava churna*" for the treatment of infectious diseases is promising alternate supplement against pathogenic bacteria.

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References

Ajijolakewu, K.A., Ayoola, A.S., Agbabiaka, T.O. et al. (2021). A review of the ethnomedicinal, antimicrobial, and phytochemical properties of Musa paradisiaca (plantain). Bulletin of the National Research Centre 45, 86

Arulmozhi, P., Vijayakumar, S., Kumar, T (2018). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. Microbial Pathogenesis. 123: 219–226.

Bhalodia, N.R., and Shukla, V.J. (2011). Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: an ethnomedicinal plant. Journal of Advanced Pharmaceutical Technology & Research. 2: 104–109.

Chandra sekhara and Sripriya (2020). Evaluation of Radical Scavenging Activity of Ethnomedicinal Syrup "Nitya Yevvana Kashayam"Annals of Plant Sciences. 9(6) : 3899-3907.

Gonelimali, F.D., Lin, J., Miao, W.,Xuan, J., Charles, F., Chen, M., and Hatab, S.R (2018). Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Frontiers in Microbiology. 9:1639.

Gupta, R.N., Kartik, V., Manoj, P., Singh, P.S., Alka, G., 2010. Antibacterial activities of ethanolic extracts of plants used in flok medicine. International Journal of Research in Ayurveda and Pharmacy. 1 (2), 529–535.

Kebede, T., Gadisa, E., Tufa, A. (2021.) Antimicrobial activities evaluation and phytochemical screening of some selected medicinal plants: A possible alternative in the treatment of multidrug-resistant microbes. PLoS ONE 16(3): e0249253.

Kothari V, Shah A, Gupta S, Punjabi A and Ranka A (2010). Revealing the antimicrobial potential of plants. International Journal of BioSciences and Technology. 3(1) 1-20.

Kusumawati, A. H., Wulan, I. R., & Ridwanuloh, D. (2020). Formulation and physical evaluation sheet mask from red rice (Oryza Nivara) and virgin coconut oil (Cocos Nucifera L). International Journal of Health & Medical Sciences, 3(1), 60-64. https://doi.org/10.31295/ijhms.v3n1.148

Mostafa AA., Al-Askar AA., Almaary KS., Dawoud T M., Sholkamy E N., Bakri MM. (2018). Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences. 25 : 361–366

Pandey, A., Singh, P., 2011. Antibacterial activity of Syzygium aromaticum (Clove) with metal ion effect against food borne pathogens. Asian Journal of Plant Science & Research. 1 (2), 69–80

Radhika J. and K. Aneja, (2009). "Antimicrobial activity of Amomum subulatum and Elettaria cardamomum against dental caries causing microorganisms," Ethnobotanical Leaflets, 13: 840–849,

Ramos GF, Amponsah IK, Harley BK, Jibira Y, Baah MK, Adjei S, Armah FA, Mensah AY. (2021). Triterpenoids mediate the antimicrobial, antioxidant, and anti-inflammatory activities of the stem bark of Reissantia indica. Journal of Applied Pharmaceutical Science, 11(05):039–048.

SreeLatha Devi RK and Basha SKM. (2013). Antimicrobial activity of Indigofera barberi, a high valued endemic herb of tirumala hills. Indian Journal of Fundamental and Applied Life Sciences. 3 (3) : 683-687

Sripriya D. and Sekhara M. C (2022). Phytochemical screening and free radical scavenging Activity of "Amrutha Mahabala Kashayam"- an ethnobotanical formulation. International Journal of Creative Research Thoughts . 10 (3) : d563-d572

Sripriya D. (2017) Ethno-Botanical uses of some Plants by Tribes in AP, India. International Journal of Pharmacy and Biological Sciences. 2017. 7. 4 67-74.

Sripriya D. (2018) Traditionally Used Medicinal Plants for Wound Healing in the Chittoor District, Andhra Pradesh (India). International Journal of Current Research in Life Sciences, 7. 2 : 980 – 985.

Sripriya D. and Naik MRB. (2019a), Ethnomedico botanical studies of high Potential medicinal plants of seshachalam biosphere reserve, A.P, India. International Journal of Engineering & Science Research. 9(5):22-27.

Sripriya D. and Naik MRB. (2019b) Ethno Medicinal plants of Seshachalam Biosphere Reserve, Andhra Pradesh, India. India. International Journal of Pharmacy and Biological Sciences. 9 (2): 18-22.

Sudarsanam G., Pratap G. P and Nagaraju V. (2019). Ethnobotany of Kuppam. AkiNik Publications. Pp140.

Suryasa, I. W., Rodríguez-Gámez, M., & Koldoris, T. (2022). Post-pandemic health and its sustainability: Educational situation. *International Journal of Health Sciences*, *6*(1), i-v. https://doi.org/10.53730/ijhs.v6n1.5949

Valgas C., M. Simon, F.A. Elza, J.R. Smania, Artur Smania, (2007). Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology 38:369-380.