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RESEARCH ARTICLES

Herbal antibacterial remedy against upper respiratory infection causing bacteria and in vivo safety analysis

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Abstract

In the present study, the antibacterial activity and in vivo acute toxicity of five different extracts was analyzed. The Minimum Inhibitory Concentration and antibacterial activity of five different extracts, including *Terminalia chebula*, *Camellia sinensis*, *Glycyrrhiza glabra*, *Vitis vinifera*, and *Terminalia arjuna*, against three different bacterial species, namely, *Streptococcus pyogenes*, *Haemophilus influenza*, and *Staphylococcus aureus* was analyzed by broth dilution method and agar well diffusion method, respectively. The in vivo acute oral toxicity was analyzed by using Wistar rats. The antibacterial activity of different extracts was calculated by analyzing the zone of inhibition, and the maximum zone of 22 mm and minimum zone of 10 mm were observed against the different upper respiratory infections causing bacteria. The lowest MIC (0.8 mg/ml) found was of *Camellia sinensis* against the *Streptococcus pyogenes* and *Staphylococcus aureus*. No sign of toxicity and mortality was observed during the 14 days of study. The in vitro results of the tested extracts indicate that they possessed antibacterial activity and were found non-toxic. So, it can be used as an alternative for the treatment of upper respiratory infection-causing bacteria.

Keywords *Streptococcus pyogenes, Haemophilus influenza, Staphylococcus aureus* · Upper respiratory tract infections · Antibacterial · Acute oral toxicity

Introduction

Nowadays, upper respiratory tract infection (URTI) is one of the common and acute problems among the common population and causes significant morbidity (Adeshina et al. 2014). Mostly the upper respiratory tract infections are self-limited and mild. A variety of bacteria can cause URTI (upper respiratory tract infections) which refer to infections such as laryngitis, tonsillitis, sinusitis, pharyngitis, otitis media, and common cold (Passioti et al. 2014). Some common symptoms of respiratory tract infections (RTIs) include sore throat, sneezing, coughing, headaches, fever, and nasal congestion.

Respiratory tract infections are responsible for more than 50 million deaths worldwide. The main reasons behind the high death rate are malnutrition, clinic visits, and poor immunity. Though the antibiotics treatment brings relief from bacterial infection but, over some decades, the increasing number of antibiotic-resistant bacteria is becoming a global problem (Kotwani and Holloway 2014).

To overcome these issues, researches have been focused on natural herbal supplements and medicines. More focused is on those natural antimicrobial agents who show the least adverse effects on human health (Mandal et al. 2010). Treatment by using traditional herbal medicine or plant extracts is increasingly acknowledged and accepted by the common population of both developing as well as developed countries (Bardia et al. 2007). However, the uses of herbs for the treatment of respiratory infections are common in some countries (Chen et al. 2007; Said et al. 2002). Therefore in this study, five different extracts, including *Glycyrrhiza glabra*, *Vitis vinifera*, *Terminalia chebula*, *Camellia sinensis*, and *Terminalia arjuna*, were screened for their possible antibacterial activity against some upper respiratory infection causing bacteria.

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Materials and methods

Plant extract collection

Standardized powdered extract of *Terminalia chebula*, *Camellia sinensis*, *Glycyrrhiza glabra*, *Terminalia arjuna*, and *Vitis Vinifera* were obtained from Saamir international Pvt. Ltd. (Delhi, India) and K. Patel Phyto Extractions Pvt. Ltd. (Gujarat, India). The powdered extracts were properly dissolved in an aqueous solvent and kept at -4 ℃ for further use.

Bacteria and growth condition

The bacterial strains of *Streptococcus pyogenes* (MTCC 1927), *Haemophilus influenza* (MTCC 3826), *Staphylococcus aureus* (MTCC 96) were procured from MTCC and were grown in brain heart infusion broth and nutrient broth media at 37 °C, pH 7.0. Media, including NB, BHI was procured from HI media.

MIC (Minimum Inhibitory Concentration)

The MIC (Minimum Inhibitory Concentration) of five different extracts against *Streptococcus pyogenes*, *Haemophilus influenza*, and *Staphylococcus aureus* was investigated by using the broth dilution method as per *NCCLS*, USA, 2006 guidelines. *Streptococcus pyogenes*, *Haemophilus influenza*, and *Staphylococcus aureus* bacterial culture (OD 600 nm = 1) were grown at 37 °C for 24 h in the Mueller-Hinton broth with or without extracts in 96 well plates. The concentration at which the bacterial growth was found completely inhibited was noted as the MIC for the extracts (Kalia et al. 2020).

Antibacterial activity (cut-well diffusion method) of extracts

In brief, the bacterial inoculums were spread on the prepared agar plate surface by using a sterile glass spreader. Then, aseptically a well is cut with the help of a sterile tip, and extracts sample (100 µL) is placed into their respective wells. Further, these agar plates are incubated at 37 °C for 24 h. The clear effective inhibition halo zone around the well was measured, showing the extracts antimicrobial activity against different strains. The diameter of the clear zone was noted in mm (Kalia et al. 2020).

Animals

Healthy adult male rats were kept in the animal house of the United Institute of Pharmacy. Standard feed and water were provided *ad libitum* throughout the experimental period. The experiments were performed following the Institutional Animal Ethics Committee (IAEC) formed as per the direction of the CPCSEA Government of India, New Delhi. The research protocol was approved by the Institutional Animal Ethics Committee (IAEC) (UIP/IAEC/Nov.-2019/06) dated 15/12/2019, United Institute of Pharmacy (Allahabad). All the tests were performed during the light period (0800:1600 h).

Acute oral toxicity studies

The extracts acute oral toxicity was conducted as per the OECD (Organization for Economic Co-operation and Development) test guidelines 423 (OECD 2001). Single oral doses of aqueous extracts 5 mg/kg, 50 mg/kg, 300 mg/kg, and 2000 mg/kg body weight were administered to the overnight fasting rats (three animals per dose) except the control group rats. During 14 days of observation, the different wellness parameters, including mortality, skin and fur, body weight, food and water intake, diarrhea, hair loss, mortality, and behavioral pattern changes, were observed (Lipnick et al. 1995).

Statistical analysis

The analysis was performed in triplicate and expressed as average ± standard error. All the statistical analysis was performed by following ANOVA using Prism5. At $p \leq 0.05$, the significance value of the data was determined.

Results

The description related to the extracts are presented in Table 1.

Antibacterial activity (cut-well diffusion method) of extracts

The antibacterial activity of the extracts against the bacteria was calculated by measuring the clear halo zone around the cut wells. The antimicrobial activity of the different extracts was examined against *Streptococcus pyogenes*, *Haemophilus influenza*, *Staphylococcus aureus*, and a maximum clear halo inhibitory zone of 22.6 mm of *Terminalia arjuna*

Table 1 Showing the detailed description of the selected extracts used in the study

Extract	Part Used	Common Name/s	Traditional Medicinal Uses	Known Constituents	References
Terminalia chebula	Fruit	Harad	Diarrhea, gastroenteritis, constipation, asthma, ulcer, malabsorption syndrome, dyspnea, candidiasis, dyspepsia, hemorrhoids, cough, antiparasitic	Flavonoids, phenolic acids, tannins	Nigam et al. (2020)
Camellia sinensis	Leaf	Green tea	Improve heart health, promoting digestion, treating blood sugar stimulant, diuretic and astringent	Polyphenols, alkaloids	Chan et al. (2011)
Glycyrrhiza glabra	Root	Licorice or mulaithi	hyperdipsia, fever, sexual debility, stomach ulcers, hemorrhagic diseases, paralysis, rheumatism, skin diseases, and jaundice	Flavonoids, saponins, stilbenoids, coumarins, isoflavonoids	El-Saber Batiha et al. (2020)
Vitis vinifera	Fruit	Grape	Headache, skin disease, gonorrhea, scabies, dysuria, hemorrhoids, and vomiting	Anthocyanin, Procyanidins, Polyphenols, Flavonoids	Nassiri-Asl and Hosseinzadeh (2009)
Terminalia arjuna	Bark	Arjuna	Hypertension, anginal pain, dyslipidemia, and congestive heart failure	Triterpenoid, flavonoids, glycosides, β-sitosterol,	Dwivedi and Chopra (2014)

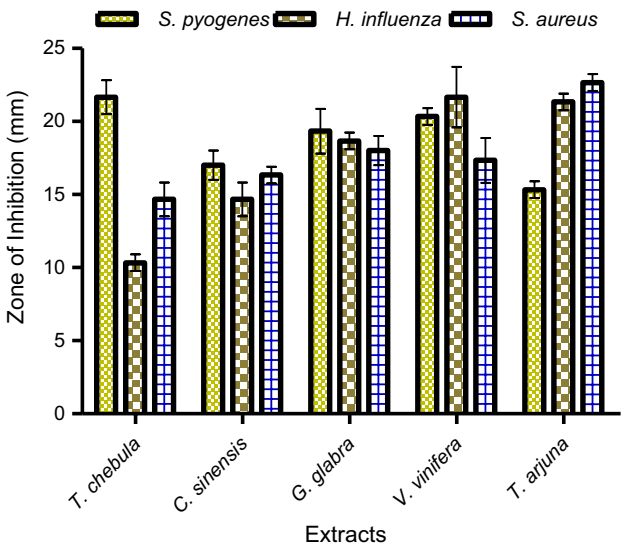

Fig. 1 Antibacterial activity by cut-well diffusion method

Table 2 Minimum inhibitory concentration value of different extracts

Botanical name	Family	MICs (mg/ml)		
		S. pyogenes	*H. influenza*	*S. aureus*
Terminalia chebula	*Combretaceae*	2.6	> 3	1.6
Camellia sinensis	*Theaceae*	0.8	> 3	0.8
Glycyrrhiza glabra	*Fabaceae*	1.6	> 3	2.4
Vitis vinifera	*Vitaceae*	0.8	> 3	1.2
Terminalia arjuna	*Combretaceae*	> 3	> 3	0.8

extract were observed against *Staphylococcus aureus*. The minimum inhibition zone of 10.3 mm of *Terminalia chebula* extract was observed against *Haemophilus influenza* (Fig. 1).

MICs (minimum inhibitory concentrations)

The MICs (minimum inhibitory concentrations) were calculated by using the microdilution method in 96 well microplates. According to the obtained result, the lowest MIC (0.8 mg/ml) was found for *Camellia sinensis* against the *Streptococcus pyogenes* and *Staphylococcus aureus* (Table 2).

Acute oral toxicity

When the single oral dose of herbal extracts including *Terminalia chebula*, *Camellia sinensis*, *Glycyrrhiza glabra*,

Table 3 The effect of doses on the animal's physical parameters during ld50 analysis

Parameters	Acute toxicity dose			
	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg
Mortality	No	No	No	No
Bodyweight	Gradual increase	Gradual increase	Gradual increase	Gradual increase
Food intake	Normal	Normal	Normal	Normal
Water intake	Normal	Normal	Normal	Normal
Hair loss	Nil	Nil	Nil	Nil
Fur	Normal	Normal	Normal	Normal
Feces color	Normal	Normal	Normal	Normal
Behavior	Normal	Normal	Normal	Normal
Diarrhea	No	No	No	No
Urination	Normal	Normal	Normal	Normal

Terminalia arjuna, and *Vitis Vinifera* was orally administered to rats (n = 5), no significant changes were observed during 14 days of observations (Table 3).

Discussion

Upper respiratory tract infections (URTIs) are the most common problem among the general population (Goossens et al. 2005). Bacteria, including *Streptococcus pyogenes*, *Haemophilus influenza*, *Staphylococcus aureus*, are usually responsible for causing upper respiratory tract infections. Many times antibiotics are prescribed for the treatment of respiratory tract infections. However, bacteria developing resistance against antibiotics are the major threats for public health worldwide (Vervloet et al. 2016). Plant extracts contain the phytochemical compounds that employ the therapeutic effects on health and pose antimicrobial activity (Heinonen 2007). Herbal remedies have started gaining greater attention in this modern era than ever before, just because of their easy availability, least side effects, efficacy, and low cost when compared with the market available drugs (Mohan et al. 2011).

Respiratory tract infection was selected in this study because it is a common disease, especially among children, and is directly correlated with air pollution and industrialization. *S. aureus* causes severe bacterial pneumonia, secondary disease, to viral infections, which causes widespread mortality and morbidity (Kumar et al. 2009). *H influenza* infection causes swelling of the epiglottis to ensue and progressive erythema. *S. pyogenes* infections normally take the form of fever (rash), pharyngitis, scarlet, and impetigo (Wong and Yuen 2012). This study aims to screen the antimicrobial activity of five different herbal extracts: *Terminalia chebula*, *Camellia sinensis*, *Glycyrrhiza glabra*, *Terminalia arjuna*, and *Vitis Vinifera* against some upper respiratory infection causing bacteria.

MIC determination is a simple and easy method for calculating the inhibitory doses of herbal extracts (an antibacterial agent) against the particular bacteria. All five extracts showed successful bacterial growth inhibition. On the other side zone of inhibition was calculated to determine the antibacterial activity of the different extracts. All the extracts showed antibacterial activity (Kalia et al. 2020; Chan et al. 2011; Pradhan and Dubey 2020). The maximum zone of inhibition was shown by *T. arjuna* against *S. aureus* (22 mm) and *H. influenza* (21 mm), followed by *V. vinifera* against *S. pyogenes* (20 mm), *H. influenza* (21 mm), and *S. aureus* (18 mm) and *T. chebula* against *S. pyogenes* (21 mm). Simultaneously, a minimum inhibitory zone of *T. chebula* was observed against *H. influenza* (10 mm) and *S. aureus* (14 mm). The zone of inhibition result suggests that the extract contains bioactive compounds that will directly affect the *S. pyogenes, H. influenza*, and *S. aureus* destruction (Adeshina et al. 2014; Bardia et al. 2007; Kaushik 2019; Pradhan and Dubey 2020).

Further, the in vivo acute oral toxicity of the five extracts was analyzed following the OECD 423 guidelines. The results showed that all the five extracts are found non-toxic when given up to the dose of 2000 mg/kg. During 14 days of acute oral toxicity study, no mortality, no signs of toxicity, and no behavioral changes were observed (Lipnick et al. 1995; Limsuwan et al. 2020).

Conclusions

Herbal extracts are the potent antibacterial and can be used to decrease the antibiotic resistance problem. However, the use of CAM (complementary and alternative medicine) therapies, including herbal extracts, herbal formulations, could reduce prescribing different antibiotics in the condition when there is no clinical indication, like viral infections. The five different extracts screened in the study have the

potential and can be used as an alternative remedy for treating upper respiratory infection. In the future, these results may be considered for in vivo and clinical analysis.

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Declarations

Conflict of interest The authors declare no conflict of interest exists.

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