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Antimicrobial activity of drug Naru-3 On Gram Positive and Gram Negative Micro organisms

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Abstract: The present study describes the antimicrobial activity of Naru-3 extract against microorganism. Staphylococcus aureus, Escherichia coli, Salmonella typhi, Brucella melitensis. For this purpose extract of drug Naru-3 were prepared and tested by "Disc Diffusion Method". As a result of this study it was found that the extract of Naru-3 generally revealed antimicrobial activity against both gram positive bacteria (Staphylococcus aureus)and gram-negative bacteria (Escherichia coli, Salmonella typhi, Brucella melitensis).
Keywords: Antimicrobial activity, disc diffusion method, NARU-3.

Introduction

Plant produces a wide variety of secondary metabolites which are used either directly as precursors or as lead compounds in the pharmaceutical industry. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant microbial pathogens. However, very little information is available on such activity of medicinal plants and out of the 4,00,000 plant species on earth, only a small number has been systematically investigated for their antimicrobial activities[1]. Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

NARU-3 is a Traditional Mongolian medicine used traditionally for several decades in Mongolia for the treatment of rheumatoid arthritis and antibacterial activity. It consists of fine powders of Terminalia chebula, Piper longum, and Aconitum Kuznezofii [2].

Terminila chebula Retz (Combretaeae) is medicinal plant widely distributed through out India, Burma and Srilanka. The dried ripe fruit T.chebula also know as black myrobalan has widely been used in the treatment of asthma, sore throat, vomiting, hiccough, bleeding, piles, diarrhoea, gout, heart and bladder diseases[3].

Piper longum, known as long pepper is a native of north-east India and an important traditional medicinal plant. The plant has tremendous medicinal values and a known curing agent against cough, leprosy, diabetes, piles, cardiac diseases, chronic fever and to improve appetite to name a few [4].Various pharmacological activities including anti-allergy, antibacterial, anti-hepatitis and anti-tubercular have been reported from long pepper.

Aconite(wrestler) was usedsince antiquityas an externalanalgesicfor gout, rheumatism, neuralgia, migraine, sciatica, in feverish conditions, toothache. When applied to theskin ofall kinds ofaconitecause itching, followed by anesthesia.
Above literature revealed that there is a need of the hour to search the new secondary antimicrobial metabolite from the plants. The present study was to evaluate the antimicrobial activity of Naru-3 extracts.

Material and Methods

Drug material

NARU-3 herbal medicine was prepared in the traditional medical factory of Traditional Medical Science Technology and Production Corporation of Mongolia. NARU-3 herbal medicine contains medicinal herbs such as the powders of Terminalia chebula 220 mg, Piper longum 620 mg, and Aconitum kuznezofii 156 mg.

Preparations of extracts

The samples were carefully washed under running tap water followed by sterile distilled water. These were air dried at room temperature (30 °C) for two days and pulverized to a fine powder using a sterilized mixer grinder and stored in airtight bottles. Three different solvents namely ethanol, methanol and aqueous were used for extraction. The 10 g of pulverized fruit was separately soaked in 100 mL of ethanol, methanol and cold sterile distilled water for 24 h. Each preparation was filtered through a sterilized Whatman No. 1 filter paper and the filtered extract was evaporated in water bath at 60 ºC till to finally get dry powder [5,6].

Tested Microorganisms

The test microorganisms were *Staphylococcus aureus*-MTCC435 (Gram positive), *Salmonella* typhi-MTCC3216, *Escherichia coli*-MTCC723, Brucella melitensis-MTCC526 (Gram negative).

Anti-microbial activity

The antimicrobial activity of the ethanol, methanol and aqueous extracts was carried by disc diffusion method [7]. A suspension of tested microorganisms was spread on Tryptic Soy Agar (TSA) (Difco) medium. The filter paper discs (8mm in diameter) were individually impregnated with different concentration of extract and then placed into the agar plats which had previously been inoculated with the tested microorganisms. The plates were subsequently incubated at 37^{o}C for 24 hrs. Antimicrobial activity was recorded if the zone of inhibition was greater than 8 mm[8]. Commercially available antibiotics chloramphenicol, kanamycin, ampicillin, penicillin, gentamicin, cefazolin, sulphamethoxazole, erythromycin, doxycycline, tetracycline were used as positive control.

Results and Discussion

In vitro preliminary screening of the antimicrobial activity of the plant extracts from Naru-3 was studied against some micro-organisms using the filter paper disc diffusion method.

The antimicrobial affect of plant extract against the different strains are illustrated in Table 1 and Figures 1.

The antimicrobial activity was determined by measuring the diameter of zone of inhibition recorded. The ethanol and methanol extracts of all the plants were found to have maximum antimicrobial activity in comparison to aqueous extracts.This indicates that ethanol and methanol extracts are better as compared to aqueous extract. These observations may be attributed to two reasons; firstly, due to the nature of biologically active components (alkaloids, flavonoids, quinine, tannins etc.) which might be enhanced in the presence of ethanol and methanol [9]. It has been documented that alkaloids, flavonoids and tannins are plants metabolites well known for their antimicrobial activity [10]. Secondly, the stronger extraction capacity of ethanol could have produced a greater number of active constituents responsible for antibacterial activity.

Table 1: Antimicrobial activity of drugs and raw materials three extracts of different microorganisms.

Samples of drugs and raw materials	Micro-organisms (inhibition zone in mm)			
	Gram-positive	Gram negative		
	S.aureus	S.typhi	E.coli	B.melitensis
Naru-3 aqueous	-	-	-	13
Naru-3 methanol	-	12	10	11
Naru-3-ethanol	12	13	-	16
T.chebula aqueous	-	16	14	16
T.chebula methanol	-	20	14	18
T.chebula ethanol	-	16	13	20
P.longum aqueous	-	-	-	-
P.longum methanol	-	-	-	-
P.longum ethanol	-	-	-	12
Aconitum aqueous	-	-	-	-
Aconitum methanol	-	-	-	12
Aconitum ethanol	-	-	-	-

(a) (b) (c) (d)

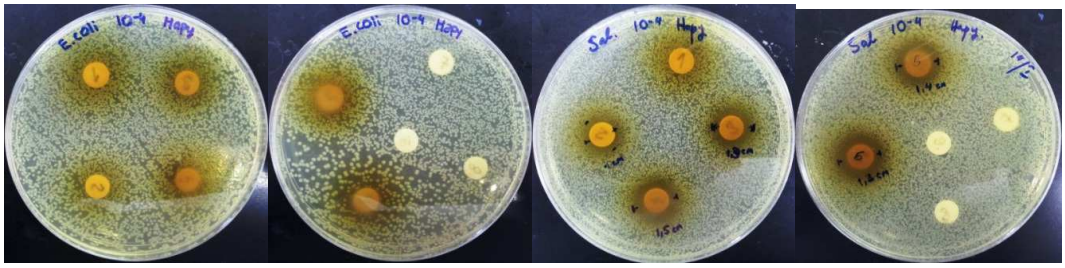
(e) (f) (g)

Fig.1: Photographs showing the zone of inhibition of drugs and raw materials extracts against various microorganisms E.coli (a),(b), S.typhi (c),(d), S.aureus (e), B.melitenses (f),(g)

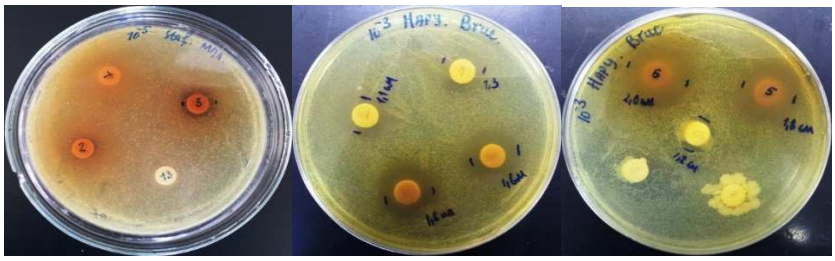
The antimicrobial activity of methanolic extract using disc diffusion method, Naru-3 showed maximum zone of inhibition (12 mm) against S.typhi followed by B.melitensis (11 mm), E.coli (10 mm). The antimicrobial activity of methanolic extract using disc diffusion method, T.chebula showed maximum zone of inhibition (20 mm) against S.typhi followed by B.melitensis (23 mm), and E.coli (14 mm) (Table 1). The antimicrobial activity of Terminalia chebula fruit extracts may be due to the presence of flavanoid, poly-urinoides, saponin, and tannin. Not a single research paper has been found for the antimicrobial activity of Terminalia chebula against B. amyloliquefaciens, S. epidermidis, S. enterica ser. Typhi, Aspergillus fumigatus according to [11]. The ethanol extract of Naru-3 was strongly inhibited to S.aureus, forming zone of inhibition i.e. 12 mm in disc diffusion technique. On comparing the inhibition zone of the extract to that of standart antibiotics extract showed better activity than chloramphenicol, erythromycin, doxycycline and cephalexin. However, extract is not potent than sulphamethoxazole and tetracycline in these condition. In the end of study we have found the extracts Naru-3 and T.chebula revealed antimicrobial activities against microorganisms (Table 2).

Table 2: Antimicrobial activity of antibiotics on different micro organisms.

Antibiotics	Micro-organisms (inhibition zone in mm)			
	Gram-positive	Gram negative		
	S.aureus	S.typhi	E.coli	B.melitensis
Chloramphenicol	23	25	28	30
Kanamycin	14	23	16	28
Ampicillin	10	14	-	21
Penicillin	26	12	13	30
Gentamicin	9	25	18	26
Cefazolin	-	10	18	30
Sulphamethoxazole	-	-	-	-
Erythromycin	25	-	8	36
Doxycycline	19	19	11	29
Tetracycline	11	15	8	-

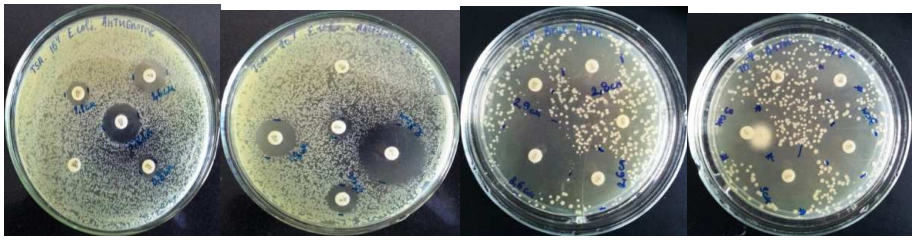


(b) (b) (c) (d)

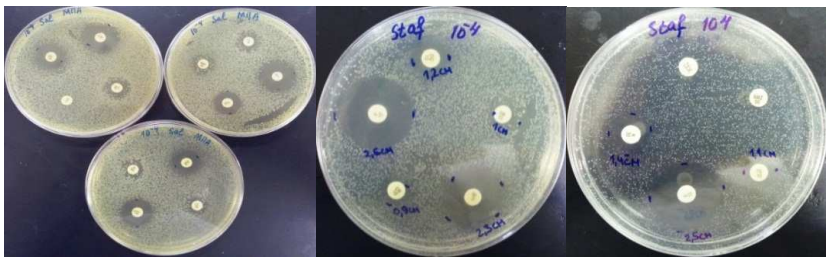


(e) (f) (g)

Fig.1: Photographs showing the zone of inhibition of drugs and raw materials extracts against various microorganisms E.coli (a),(b), S.typhi (c),(d), S.aureus (e), B.melitenses (f),(g)



(a) (b) (c) (d)



(e) (f) (g)

Fig.2: Antimicrobial activity of standard antibiotic against different microorganism: E.coli (a), (b), B.melitenses (c), (d), S.typhi (e), S.aureus (f), (g)

Conclusion

The plants have traditionally provided a source of hope for novel drug compounds, as plant herbal mixtures have made large contributions to human health and well being. The use of plant extracts with known antimicrobial properties can be of great significance for therapeutic treatment.

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