ANTIBACTERIAL EFFICACY OF MANIBADRA CHOORNAYA AND ITS INDIVIDUAL INGREDIENTS

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ABSTRACT

The present study was carried out in order to find out the antibacterial activity of hot and cold extracts of Manibadra Choornaya and its ingredients; fruits of *Embelia ribes*, *Terminalia chebula* and *Emblica officinalis* and root of *Operculina turpethum* against *Staphylococcus aureus* (NCTC6571) and *Escherichia coli* (ATCC25922). Streptomycin and sterile distilled water were used as standard and control respectively. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were determined by macro broth dilution method. Phytochemical analysis was done to identify the presence of chemicals in each extracts. Quantitative and comparative evaluation of antibacterial properties showed that the hot and cold extracts of *Terminalia chebula* revealed significantly ($P < 0.05$) highest activity on both of the tested bacteria. Except *Operculina turpethum*, all the tested hot extracts including Manibadra Choornaya extracts exhibited better effect on *Escherichia coli* when compared with respective cold extracts. The same tendency was produced on *Staphylococcus aureus* by *Terminalia chebula* and the Manibadra Choornaya. *Terminalia chebula* hot extract showed the lowest MIC (7.8125 mg / ml) on both of the test bacteria and lowest MBC (15.625 mg / ml) on *E. coli*. Most of the remaining extracts had 31.25 mg / ml as their MIC and 62.5 mg / ml as MBC. The phytochemical analysis revealed the presence of tannins in all test extracts where as glycoside, saponins, phlobatannins, resins and terpenoids were found in some of the extracts. Present study prove the antibacterial activity of Manibadra Choornaya and its individual ingredients, further studies could provide more evidences for developing antimicrobial compounds from these extracts.

Keywords: Manibadra Choornaya, antibacterial activity, phytochemicals, *Escherichia coli*, *Staphylococcus aureus*, Medicinal plants.

INTRODUCTION

Herbal medicines are plant based preparations, which contain raw or processed ingredients from one or more plant parts. These natural products have received considerable attention in recent years not only in developing countries also in developed countries because of their wide bioactivities, higher safety margin and lower costs. Therefore, traditional plant based medical preparations and plant extracts are severely analyzed for their feasibility to treat various diseases and disorders¹. Manibadra Choornaya, an Ayurvedic preparation, is normally available in Ayurvedic pharmacies and prescribed to clear obstruction and normalizes the srotas². It is prepared by mixing the powders; roots of *Operculina turpethum*, pericarp of *Terminalia chebula*, seeded fruits of *Embelia ribes* and *Emblica officinalis*³. *Terminalia chebula*, a member of family Combretaceae, is a major constituent in many Ayurvedic preparations. The pulp of the fruit is given in piles, chronic diarrhea, dysentery, costiveness, flatulence, asthma, urinary disorders, vomiting, hiccup, intestinal worms, ascites and enlarged spleen and liver. Powder of the fruit is used in chronic ulcers and wounds, carious teeth and bleeding ulceration of the gums⁴. *Embelia ribes* is a large; scan dent shrub belongs to the family Myrsinaceae. The whole plant is used in the treatment of anti inflammatory to relieve rheumatism and fever⁵. The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice and mental disorders⁶. The dried berries are used along with other ingredients for treatment of fistula, piles, cough, enlarged spleen, abdominal dropsy and preventing effects of old age. A decoction of roasted fruit is a household remedy for worms and gripe in children, while the powdered fruit with bee's honey is given to prevent worm trouble⁷. Seeds are used as antibiotic, anthelmintic, anti-tuberculosis, alterative and stimulative⁸. *Operculina turpethum* (family Convolvulaceae) is a perennial, twinning climber with long, slender, fleshy, much branched roots. The fleshy roots are used along with other ingredients for fever, cough, asthma, skin diseases,

sores, boils, itches. The powdered root is given as a purgative with ginger; it is particularly beneficial in rheumatic and paralytic affections⁷. *Emblica officinalis* (family Euphorbiaceae) is a medium sized tree the fruit of which is used in many Ayurvedic preparations. It is useful in hemorrhage, leucorrhoea, menorrhagia, diarrhea and dysentery. Seed is used in asthma, bronchitis and biliousness. Leaves are also useful in conjunctivitis, inflammation, dyspepsia and dysentery. The bark is useful in gonorrhea, jaundice, diarrhea and myalgia. Liquor fermented from fruit is good for indigestion, anemia, jaundice, heart complaints, and for promoting urination⁴. The present study was undertaken to evaluate the antibacterial activity of hot and cold extracts of Manibadra choornaya and its individual ingredients against a Gram-positive and a Gram-negative bacteria and to detect available phytochemicals which may responsible for the bioactivity of the above extracts.

MATERIALS AND METHODS

Preparation of Test Extracts

The ingredients of Manibadra choornaya; Fruits of *Embelia ribes*, *Terminalia chebula* and *Emblica officinalis* and root of *Operculina turpethum* were collected from the local herbal medical shop in Jaffna, Sri Lanka. The shade dried fruits and root was powdered separately using mechanical grinder and sieved to get uniform particle size. The Manibadra choornaya was prepared by adding equal amount of above powders.

Cold Aqueous Extract Preparation

20 g of Manibadra choornaya and each of the ingredients of Manibadra choornaya were separately soaked in 60 ml sterile distilled water in a shaker for one hour at ambient temperature. Then they were filtered through double layered muslin cloth and resulting filtrates were further filtered by using What-man No1 filter paper. The filtrates were completely dried in an oven at 45°C⁹.

Hot Aqueous Extract Preparation

20 g of Manibadra Choornaya and each of the ingredients of Manibadra Choornaya were separately soaked in 60 ml boiling sterile distilled water for one hour. Then they were filtered through double layered muslin cloth and resulting filtrates were further filtered by using What-man No1 filter paper individually. The filtrates were completely dried in an oven at 45°C¹⁰.

Test Bacteria

The standard bacteria, *Staphylococcus aureus* (NCTC 6571) and *Escherichia coli* (ATCC 25922) were obtained from Department of Microbiology, Faculty of Medicine, University of Jaffna, Sri Lanka and they were stored on nutrient agar slants at 4°C until used for the assay.

Determination of Antibacterial Activity

Antibacterial activity of the extracts was tested by agar well diffusion method according to Jeyaseelan *et al*¹¹. 1 ml of the test culture [0.5 McFarland] was mixed with 20 ml sterile nutrient agar and allowed to solidify. Wells were then made using a sterile cork borer of 8 mm diameter. The test extracts were dissolved in sterile distilled water to get 250 mg / ml concentration. The wells were filled with 100 µl of test extracts, streptomycin (50 µg / 100 µl) and sterile distilled water. Streptomycin and sterile distilled water were used as standard and control respectively. The plates were incubated

at 37°C for 24 h. After the incubation, the antibacterial activity was determined by measuring the diameter of the clear zones around the well. Each experiment was repeated thrice.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC was determined by macro broth dilution method¹. 1 ml of 24 h culture of test organisms with 0.5 McFarland turbidity standards were incubated in different concentrations of 1 ml test extracts with concentration ranging from 250 to 7.8125 mg / ml in nutrient broth at 37°C for 24 h. The lowest concentration with no detectable bacterial growth was considered as MIC. For the determination of MBC, two loops full of culture were taken from each of the broth tubes that showed no growth on in the MIC tubes and inoculated onto fresh nutrient agar plates. After 24 h incubation at 37°C, the plates were observed for the growth of bacteria. The lowest concentration of the extracts that showed no growth was recorded as the MBC. Each experiment was repeated thrice.

Qualitative Phytochemical Analysis

Test samples were examined to find out the presence of tannins, terpenoids, saponins, flavonoids, cardiac glycosides, phlobatannins, alkaloids, resins and glycosides by using methods described by Trease and Evans¹².

Table 1: Antibacterial Activity of Hot and Cold Aqueous Extracts

Test Samples	Plant part	Extract	Diameter of inhibition zone (mm)*	
			<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>
<i>Emblica ribes</i>	Fruit	Hot	10.8 ± 0.8 ^{de}	-
		Cold	9.7 ± 0.6 ^f	-
<i>Terminalia chebula</i>	Fruit	Hot	27.0 ± 0.5 ^a	24.8 ± 0.8 ^a
		Cold	24.7 ± 0.8 ^b	18.7 ± 0.6 ^b
<i>Operculina turpethum</i>	Root	Hot	-	12.2 ± 0.3 ^{de}
		Cold	-	14.2 ± 0.3 ^c
<i>Emblica officinalis</i>	Fruit	Hot	14.5 ± 0.5 ^c	12.8 ± 0.8 ^d
		Cold	11.7 ± 0.6 ^d	12.0 ± 0.6 ^d
Mixture (Manibadra Choornaya)		Hot	12.2 ± 0.3 ^d	12.2 ± 0.3 ^{de}
		Cold	10.0 ± 0.4 ^{ef}	10.8 ± 0.8 ^e
Streptomycin			22.5 ± 0.7	15.2 ± 0.4

*Diameter of inhibition zones includes the diameter of well (8 mm); Values = mean ± SD; Values with different superscript in the same column differ significantly (P < 0.05); (-) – no clear zone.

Table 2: Minimum Inhibitory Concentration of Hot and Cold Extracts

Test plant	Minimum inhibitory concentration (mg/ml)			
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Hot extract	Cold extract	Hot extract	Cold extract
<i>Emblica ribes</i>	15.625	31.25	>250	>250
<i>Terminalia chebula</i>	7.8125	15.625	7.8125	31.25
<i>Operculina turpethum</i>	>250	>250	31.25	31.25
<i>Emblica officinalis</i>	31.25	31.25	31.25	31.25
Mixture	31.25	31.25	31.25	31.25

Table 3: Minimum Bactericidal Concentration of Hot and Cold Extracts

Test plant	Minimum bactericidal concentration (mg/ml)			
	<i>Escherichia coli</i>		<i>Staphylococcus aureus</i>	
	Hot	Cold	Hot	Cold
<i>Emblica ribes</i>	31.25	62.5	>250	>250
<i>Terminalia chebula</i>	15.625	62.5	31.25	62.5
<i>Operculina turpethum</i>	>250	>250	62.5	62.5
<i>Emblica officinalis</i>	62.5	62.5	62.5	62.5
Mixture of above	62.5	125	62.5	125

Table 4: Phytochemical Analysis of Hot and Cold Extracts of Different Plant Parts

Plant parts		Extract	Glycoside	Alkaloids	Flavonoids	Cardiac glycosides	Saponins
<i>Operculina turpethum</i>	Root	Hot	+	+	+	+	+
		Cold	-	+	+	-	+
<i>Emblica ribes</i>	Fruits	Hot	+	-	-	+	-
		Cold	-	-	-	+	+
<i>Emblica officinalis</i>	Fruits	Hot	-	-	+	+	+
		Cold	-	-	-	+	+
<i>Terminalia chebula</i>	Fruits	Hot	-	-	+	+	+
		Cold	-	-	+	+	+
Mixture (Manibadra Choornaya)		Hot	+	+	+	+	+
		Cold	-	-	+	+	+

(+) Presence

(-) Absence

Table 5: Phytochemical Analysis of Hot and Cold Extracts of Different Plant Parts

Plant parts		Extract	Tannins	Phlobatannins	Resins	Terpenoids
<i>Operculina turpethum</i>	Root	Hot	+	+	+	+
		Cold	+	+	+	-
<i>Emblica ribes</i>	Fruits	Hot	+	-	+	+
		Cold	+	+	+	+
<i>Emblica officinalis</i>	Fruits	Hot	+	-	+	+
		Cold	+	-	-	-
<i>Terminalia chebula</i>	Fruits	Hot	+	-	+	-
		Cold	+	-	-	-
Mixture (Manibadra Choorana)		Hot	+	-	+	+
		Cold	+	+	+	+

(+) Presence

(-) Absence

Statistical Analysis

Results were expressed as mean $\pm$ standard deviation (SD) of three replicates. The results of antibacterial activity were subjected for analysis of variance, Tukey test ($P = 0.05$) and t test (5 % significant level) using statistical software SPSS Windows version 13.0.

RESULTS

The antibacterial assay revealed that the hot and cold extracts of *Terminalia chebula*, *Emblica officinalis* and Manibadra Choornaya (mixture) were able to inhibit both *Escherichia coli* and *Staphylococcus aureus* at the tested concentration. The remaining hot and cold extracts of *Emblica ribes* and *Operculina turpethum* inhibited the growth of only *Escherichia coli* and *Staphylococcus aureus* respectively. Hot extract of *Terminalia chebula* expressed significantly ($P < 0.05$) highest antibacterial activity against both of the tested bacteria compared to other tested extracts and it was followed by the cold extract of the same plant. Both hot and cold extracts of *Terminalia chebula* revealed significantly higher effect on *Escherichia coli* than *Staphylococcus aureus*. Interestingly, Except *Operculina turpethum*, all the tested hot extracts including hot extract of mixture showed significantly higher inhibition on *Escherichia coli* when compared with respective cold extracts. Same tendency was also observed against *Staphylococcus aureus* by *Terminalia chebula* and the mixture but not in rest of the plant extracts. Hot and cold extracts of *Emblica ribes* produced lower inhibition on *Escherichia coli* and no effect on *Staphylococcus aureus*. On the other hand, even though *Operculina turpethum* failed to inhibit the growth of *Escherichia coli* it showed a moderate inhibition against *Staphylococcus aureus*. The Manibadra Choornaya showed relatively lower or equal antibacterial activity compared to the individual extracts except the results produced by the *Operculina turpethum* and *Emblica ribes* on *Escherichia coli* and *Staphylococcus aureus* respectively. The streptomycin had inhibition on both *Escherichia coli* and *Staphylococcus aureus* and the diameter of inhibition zone is higher than that produced by the test extracts, except

Terminalia chebula. The control, sterile hot and cold distilled water did not show any effect on the bacterial growth (Table 1). The hot extract of *Terminalia chebula* revealed the lowest MIC (7.8125 mg / ml) on *Escherichia coli* and *Staphylococcus aureus*. Most of the remaining extracts had 31.25 mg / ml as their MIC value against tested bacteria (Table 2). MBC value of the tested extracts also showed more or less similar pattern, where the lowest MBC value was expressed against *Escherichia coli* (15.625 mg / ml) by the hot extract of *Terminalia chebula* (Table 3). Qualitative phytochemical analysis of cold and hot extracts of test plants and Manibadra Choornaya (mixture) showed the presence of different types of phytochemicals. Tannin was detected in all test extracts, cardiac glycosides and saponins were also present in all test extracts except cold extract of *Operculina turpethum* and hot extract of *Emblica ribes* respectively. The alkaloids and glycosides were detected only in few tested extracts. The alkaloids were found in both hot and cold extract of *Operculina turpethum* and the glycosides were in hot extract of Manibadra Choornaya, *Operculina turpethum* and *Emblica ribes* only. Among the tested extracts, *Operculina turpethum* hot extract showed positive results for all the phytochemicals tested. On the other hand, cold extract of *Emblica officinalis* possessed lowest number of phytochemicals. In general, cold extracts had relatively low number of phytochemicals than hot extracts, however, that pattern was not observed in the extracts of *Emblica ribes*. When compared the results obtained for the Manibadra Choornaya and its individual plant extracts, there are some variation in the phytochemical constituents. Even though some phytochemicals detected in individual extracts they were not in the Manibadra Choornaya (Table 4 and 5)

DISCUSSION

The present study was designed in order to check the feasibility of using Manibadra Choornaya and their individual ingredients as potential source for antibacterial agents. Because several studies with plant based preparations as well as individual plant extracts have been proved the antibacterial

activity against a wide range of bacteria. In this study, aqueous hot and cold extraction was carried out because in traditional medicine mostly water is used as solvent for the preparation of extracts or decoctions¹³. Hot and cold extractions were performed, because to check whether variation in the extraction temperature causes any influences in the antibacterial activity and the phytochemical constituent of the extract. According to the results obtained in the present study it is clear that the extracting temperature may play a role in the bioactivity of the extract, however, that may not be applicable to all plant extracts. Because, some previous studies reported that the extracting at high temperature may leads to reduction of active compounds¹⁴. However, in the present study hot extracts revealed presence of higher number of phytochemicals and better antibacterial activity than cold extracts. Even though the hot and cold extracts of *Operculina turpethum* had higher number of phytochemicals compared to *Terminalia chebula* they expressed lower antibacterial activity. This may be due to the antagonistic effect of bio-molecules or the available chemicals may be in lower concentration. In a crude mixture, the activity of bio-molecule can be influenced by the other molecules¹⁵. Water is a polar universal solvent therefore the aqueous extracts might be with variety of bio-molecules. The lower antibacterial activity of the mixture and absence of some phytochemicals in the mixture those were detected in individual extracts may be due to the low amount of active ingredients in the extracts. Because the mixture was prepared by combination of one forth of each plant powder, but the other extracts have only the individual powder. The phytochemicals tannins, saponins, cardiac glycosides, resins and terpenoids were detected in at least six of the tested ten extracts. However, glycosides, alkaloids and phlobatannins were detected only in few tested extracts. The variation of phytochemical content depends on the inherent property of the plant. Phytochemicals are secondary metabolic products of plants and they play several key roles in plant including providing protection from pests and pathogenic organisms. In microorganisms like bacteria different phytochemicals show different mode of action. Flavonoids have ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. In addition to that, some more lipophilic flavonoids may also disrupt microbial membrane¹⁶. Saponins are surface active agents which interfere with or alter the permeability of the cell wall. Therefore, this facilitates the entry of toxic materials or leakages of vital constituents from the cell. Tannins act by coagulating the cell wall proteins¹⁷. The alkaloids have ability to intercalate with DNA¹⁸.

CONCLUSION

Present *in vitro* antibacterial activity of hot and cold extracts of Manibadra Choomaya and its individual ingredients revealed diverse antibacterial activity on tested bacteria. The results of this study validate the traditional usage of medicinal plant preparations on microbial control. Among the tested extracts, *Terminalia chebula* expressed more effect on

Escherichia coli and *Staphylococcus aureus*. Therefore, this extract is a better source for further identification of bioactive natural products which may lead to the development of new antimicrobial drugs.

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