

## Antimicrobial perspective of non-alcoholic beverages of Jimma, Ethiopia against *Streptococcus pyogenes*

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### Abstract

The objective of this study was to determine the antimicrobial activity of the selected non-alcoholic beverages of Jimma, Ethiopia against *Streptococcus pyogenes*. The susceptibility of *S. pyogenes* was evaluated by agar well diffusion and broth macro dilution assay. The results indicated that among the non-alcoholic beverages viz., black coffee, black tea, spreeze (mixture of black coffee & tea), keshar (ginger extract) and herbal teas (*Thymus schimperi* extract and a mix of *Thymus schimperi* and *Camellia sinensis*) tested, *S. pyogenes* was highly susceptible to the ethanol extract of *Thymus schimperi*. This activity was significantly higher than the antibiotic penicillin which served as positive control. Thus, Herbal remedies could restrain the burden of economically under privileged people in developing countries like Ethiopia as well as immunocompetent sufferers across the globe.

**Keywords:** ethno botanical survey, non-alcoholic beverages, *s. pyogenes*, *t. schimperi*

### 1. Introduction

*Streptococcus pyogenes* is one of the pathogens generally found in the decaying tooth, infected tonsils and oesophagus and cause frequent incidences of mild to severe strep-throat. Although strep-throat is a common illness, it causes severe health impact on the immunodeficiency individuals. Treating with antibiotics was unsuccessful in immune-compromised as well as lead to complications, side effects and drug resistance. At this junction, herbal remedies could restrain the burden of economically underprivileged people in developing countries like Ethiopia. Moreover, food we eat, the beverage we drink and the nutrients we consume have a direct link to the culture, customs and beliefs we have inherited over millennia<sup>[1]</sup>. In this context, the non-alcoholic beverages are stimulating, refreshing and consumed by most of the people<sup>[2]</sup> throughout the world. Non-alcoholic beverages like black and green tea used in different geographical locations were extensively studied and confirmed to possess antimicrobial activity against several pathogenic bacteria and fungi whereas the non-alcoholic beverages of Jimma, Ethiopia have not been exploited as a therapeutic medicine. Ethno botanical studies revealed the use of non-alcoholic beverages viz Coffee, Tea (*Camellia sinensis*), Spreez, Tosigni (*T. schimperi*) tea and Tosigni & tea (*T. schimperi* & *Camellia sinensis* mix)<sup>[3]</sup> and not evaluated for therapeutic value. Therefore the objective of this study was to study the antimicrobial activity of non-alcoholic beverages against *Streptococcus pyogenes* the causative organism for strep-throat.

### 2. Materials and methods

#### 2.1 Extract preparation

Non-alcoholic beverages i.e. roasted coffee powder (Abyssinia), commercial tea dust (wush wush), dry leaves of *Thymus schimperi*, and fresh rhizomes of *Z. officinalis* were collected from the local market of study area Jimma, Ethiopia and extracted by standard methods (Harbone, 1963). The extracts were dried at room temperature and stored at 4°C. Stock concentration of 100 mg/ml of crude extracts of *Coffea arabica*, *Camellia sinensis*, *Thymus schimperi* and *Zingiber officinalis* were prepared by reconstitution with dimethyl sulfoxid (DMSO).

Spreez (Coffee & tea mix) was prepared by mixing the extracts of roasted coffee and *Camellia sinensis* in the ratio of 1:1. *Camellia sinensis* & *Thymus schimperi* mix was prepared by mixing the respective extracts in the ratio 1:1.

a) The following plants used to prepare the non-alcoholic beverages (NAB) were chosen based on the ethnobotanical data collected.

1. a. *Coffea arabica* bean extract
2. b. Tea (*Camellia sinensis*) leaf extract
3. (a+b). Spreez (*Coffea arabica* and *Camellia sinensis*) mix - 1:1)
4. c *Thymus schimperi* leaf extract
5. (b+c) *T. schimperi* leaf extract with *Camellia sinensis* extract (1:1)
6. d *Zingiber officinalis* rhizome extract)

#### 2.2 Combination of extracts

The above mentioned extracts were mixed with cinnamon and brown & white honey to obtain the following combinations.

- Non-alcoholic beverages with brown honey (1:1)
- Non-alcoholic beverages with white honey (1:1)
- Non-alcoholic beverages with cinnamon (1:1)
- Non-alcoholic beverages with cinnamon and brown honey (1:1:1)
- Non-alcoholic beverages with cinnamon and white honey (1:1:1)

Water was used as a solvent in the preparation of honey involved combinations.

#### 2.3 Test organism

*S. pyogenes* ATCC19615 was obtained from Ethiopian Health and Nutrition Research Institute, Addis Ababa. The test organisms at 0.5 McFarlands standard was prepared in PPG1% [peptone water with red phenol supplemented with glucose 1% (w/v)]<sup>[4]</sup>.

#### 2.4 Antimicrobial activity

The antimicrobial activity of selected non-alcoholic beverages

was tested against *S. pyogenes* through agar well diffusion method (Perez *et al.*, 1990). The inoculated plates were incubated at 37°C for 24-48hrs. Penicillin antibiotic 10mg/ml served as positive control. The bacterial growth inhibition was determined as the diameter of the inhibition zone around the wells (mm). All the tests were performed in triplicate. The mean value was computed using SPSS 16.

**2.5 Combination effect**

The combined effect of each of the non-alcoholic beverages (a, b, a+b, c, b+c and d) with cinnamon and honey (brown/white) was calculated using the following formula (Mujeeb, 2010).

$$\text{Calculated zone size} = \text{sum of zone size of both extracts}/2$$

If,

- i) Observed zone size = calculated zone size then the effect will be additive
- ii) Observed zone size > calculated zone size then the effect will be synergistic
- iii) Observed zone size < calculated zone size then the effect will be antagonistic

The same formula was used to calculate the combination of three extracts.

$$\text{Calculated zone size} = \text{sum of zone size of three extracts}/3$$

**2.6 Minimum Inhibitory concentration**

The Minimum inhibitory concentration (MIC) for the combination of extracts was determined by broth macro dilution assay with a concentration range of 100 to 0.78 mg/ml using

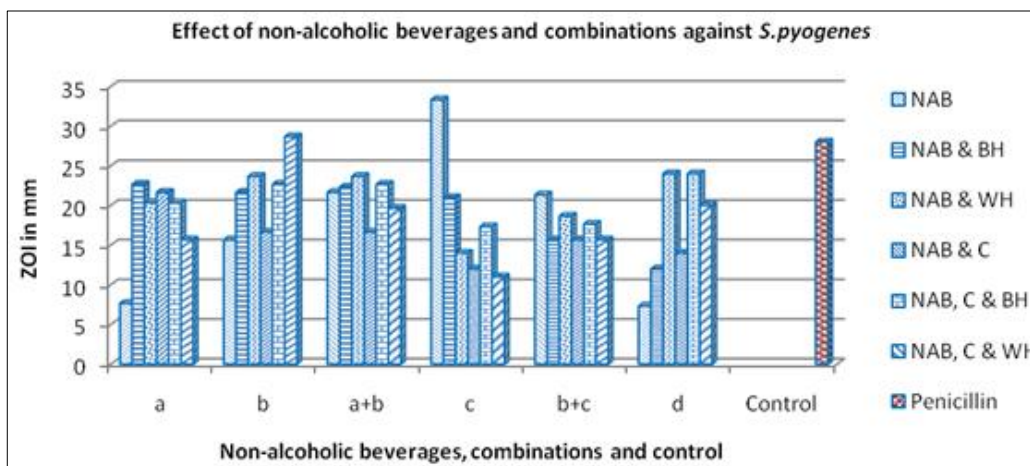
PPG1% broth. 50 µl of the respective extracts were loaded into Muller Hinton Agar plates swabbed with *S.pyogenes*. DMSO and water were included as negative controls. Penicillin 10 mg/ml was used as positive control. After incubation the test tubes were observed for colour change. Phenolphthalein indicator in PPG1% indicates the presence/ absence of bacterial growth. The wells which remained red indicated inhibition of bacterial growth and those turned yellow indicated microbial growth. Each of the tests was done in triplicates and the mean value was computed using SPSS.

**3. Results**

**3.1 Antimicrobial activity**

**3.1.1 Non-alcoholic beverages**

The plants used in the preparation of non-alcoholic beverages chosen for this study were subjected to antimicrobial study against *S.pyogenes*. The results indicated that Tosini (*T.schimperi* tea) produced the highest ZOI which is significantly higher than the positive control penicillin used in this study. Its activity was reduced when combined with brown/white honey/cinnamon. *C.arabica* showed increased activity when combined with cinnamon/brown/white honey in the ratio of 1:1. Similar result was observed for other non-alcoholic beverages viz., *Camilia sinensis*, *C.arabica* and *C. sinensis mix* and *Z.officinalis*. Activity of spreeze (coffee & tea mix) was significantly higher (P< 0.05) than the individual effect of coffee and tea (Fig.1).



a. *coffea arabica* bean extract and combinations,  
 b. Tea (*Camellia sinensis*) leaf extract and combinations,  
 (a+b). Spreeze (*Coffea arabica* and *Camellia sinensis*) mix - 1:1) and combinations  
 c. *Thymus schimperi* leaf extract and combinations,  
 (b+c) *T.schimperi* leaf extract with *Camellia sinensis* extract (1:1)and combinations  
 d. *Zingiber officinalis* rhizome extract and combinations  
 NAB-non-alcoholic beverage BH-brown honey WH – white honey Control - Penicillin

**Fig 1:** comparison of antimicrobial activity of non-alcoholic beverages, combinations and positive control Penicillin

**3.2 Minimum Inhibitory Concentration MIC**

The results of MIC studies confirmed the antibacterial activity of non-alcoholic beverages and its combination with honey, cinnamon and cinnamon and honey. The result indicated that lowest MIC of 1.56mg/ml was noted for *T.schimperi* extract among the six non-alcoholic beverages tested. Whereas, the

lowest (MIC) of 12.5µg/ml was observed for nine combinations among eighteen combinations tested i.e non-alcoholic beverages with brown/white honey/cinnamon at 1:1 ratio. In addition, 6.25mg/ml was observed for Tea in combination with cinnamon and brown honey at 1:1:1 combination (Fig.2).

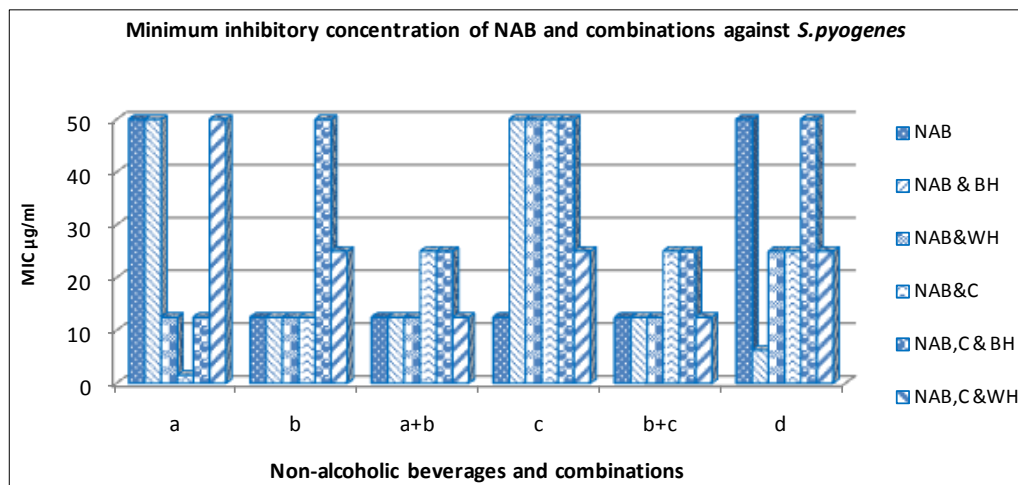


Fig 2

#### 4. Discussion

*S. pyogenes* causes several oral, dermal and systemic diseases, in particular pharyngotonsillitis in children [5]. Usually strep-throat/tonsillitis is treated with antibiotics as well as with home remedies. Generally, Immune depression resists the antibiotic treatments. Moreover, vast majority of Ethiopia's population lives in rural areas where the health care coverage is low and the existing public sector resources are being stretched to the limits. One of the greatest challenges faced by the country is to determine, how best to narrow the gap between the existing services and the population whose access to the existing services is very limited [6]. In a nutshell, antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases, while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds were mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, flavonoids, steroids, resins fatty acids gums which are capable of producing definite physiological action on body [7]. Today, many scientists have realized that the study of native culture can provide valuable clues for natural remedies in such cases. In addition to the ethno botanical data documented by previous researchers [8, 9], present study has given due recognition of the non-alcoholic beverages of Jimma, Ethiopia. Some of the non-alcoholic beverages like spreez (mixture of black coffee & tea, *Thymus schimperi* (tosigni) tea and *Thymus schimperi* & *Camellia sinensis* mix are unique to Ethiopia. It was observed that the natives consume all the non-alcoholic beverages mentioned in this study without milk [3]. Antimicrobial perspective of non-alcoholic beverages of Jimma was not studied before. The antimicrobial activity of NAB (Non-alcoholic beverage) alone as well in combination with cinnamon and honey were discussed in the following section along with the cultural, social and scientific validation.

##### 4.1 Coffee

Study area is situated in Keffa region which is the birth place of Arabica coffee. In addition, coffee was also mentioned as additives in few Ethiopian traditional medicine recipes. Coffee brew is a widely consumed beverage with multiple biological

activities due to both naturally occurring components and hundreds of chemicals that are formed during the roasting process [10]. Previous research found that roasted coffee powder showed wide range of antibacterial activity against a gram positive microorganisms [12], whereas green coffee extract exhibits no such activity [13]. The increase in antimicrobial activity of coffee against *S. pyogenes* might be due to synergistic activity of the active ingredients of coffee and cinnamon.

##### 4.2 Tea

The popularity of tea is attributed to its pleasant flavour, combined with its stimulating effect. Tea leaves are known to possess antibacterial activity against many microorganisms [14]. Nanyingi, (2008), found that, both black and green teas completely inhibited the growth of *S. pyogenes* at a concentration of 12.5mg/ml after seven hours and at 25mg/ml after three hours. In addition, the author also found that the concentration of gallic acid in different tea varieties has curative effects therefore, study of teas as a nutritional supplement to cure certain infectious diseases could be the subject of future studies [15]. The results of this study revealed that the activity of tea was improved by combining with cinnamon and white honey. Besides documentation, this study highlights the potential of cinnamon infused tea as a cure for strep-throat caused by *S. pyogenes*.

##### 4.3 Spreez –Coffee & tea mix

Spreez is a mixture of black coffee & tea, consumed as a beverage in Ethiopia. Consumption of such mixture of beverages was not reported elsewhere and not evaluated for its antimicrobial activity against *S. pyogenes* before. Spreez showed significant ( $p < 0.05$ ) antimicrobial activity and synergistic effect with white honey in controlling the growth of *S. pyogenes*.

##### 4.4 *Thymus schimperi* (Tosigni) - tea

*T. schimperi* is called Tosigni in Local language. GC-MS analysis of the leaf essential oil of *T. schimperi* collected from Dinshu and around Addis Ababa [16, 17, 18]. Lattaoui, (1994) found to contain 1: 3-octanone (1.6%) 2: myrcene (1.6%) 3: p-cymene (4.3%) 4: gama-terpinene (3.3%) 5: alpha-terpinene (1.2%) 6: linalool (8.2%) 7: thymol (59.3%) (14) 8: carvacrol (14.9%) [18]. the same compounds may be the reason for the wide spectrum antimicrobial activity of *T. schimperi* tea against *S. pyogenes*.

#### 4.5 *T. schimperi* - tea mix

Dried leaves of *T. schimperi* were mixed with ground tea dust and consumed as tea. The drink was not popular in the study area. The antimicrobial activity of such mix showed similar result with spreeze (coffee and tea mix). The results of this study confirm the use of combination of plants in traditional medicine. The antimicrobial activity of tea against many gram-positive bacteria including *S. pyogenes* may be due to poly phenols<sup>[19]</sup> flavonols, quercetin, kaempferol, myricetin<sup>[20]</sup> and catechin<sup>[21]</sup>. These compounds could have synergistically worked with the active ingredients of *T. schimperi*.

#### 4.6 Kesher

The rhizome of the Zingiberaceae family is widely used as spice in many African & Asian countries, and their medicinal properties have been broadly discussed and accepted in many traditional recipes. Spices have been shown to possess medicinal value; in particular, antimicrobial activity<sup>[22]</sup>. The antimicrobial activity of kesher in the current study corroborates with previous studies<sup>[23, 24, 25]</sup>. The antimicrobial activity of ginger was more significantly enhanced by the combination with white honey than brown honey (Fig. 1). There are no reports of severe toxicity of ginger on humans<sup>[26]</sup>, therefore the drink can be consumed with honey to get the desirable natural immune boost and as a cure for strep-throat caused by *S. pyogenes*.

#### 4.7 Honey

Generally, traditional healers prefer to use additives such as honey to mask the bitter taste or to enhance their activity. It also plays a dominant role in Ethiopian food and beverages<sup>[3]</sup>. Flavonoids are ubiquitous in photosynthesising cells and are commonly found in honey. Current research also confirm the additive effect of Honey.

#### 4.8 Cinnamon

Often cinnamon is used as a stimulant to other herbs enabling the herbal remedies to work faster (<http://www.wic. Herbal encyclopaedia - on line>). Current research also found that stem bark extract of cinnamon has enhanced the activity of coffee. In contrast, it was found that cinnamon was found to be antagonistic with *T. schimperi* extract.

#### 4.9 Combination effect of cinnamon and honey

Active compounds produced during secondary vegetal metabolism are usually responsible for the biological properties of some plant species used throughout the globe for various purposes, including treatment of infectious diseases<sup>[10]</sup>. The combination of certain plants produced synergistic effect while others were antagonistic or additive. Therefore, it is clearly evident that the active ingredients of a plant vary with one another because of the unique combination within. Increasingly, this class of natural products is becoming the subject of anti-infective research<sup>[27]</sup>. This combination study on non-alcoholic beverages with cinnamon and honey has not been reported elsewhere.

#### 5. Conclusion

Black coffee, tea and many herbal teas are regularly consumed in many Asian & African tropical countries and gaining more importance. The scientific validation of non-alcoholic beverages of Jimma, Ethiopia as well its combination with cinnamon and honey can be of great significance in natural therapeutic

treatment of strep-throat in immune suppressed across the world. As a whole, non-alcoholic beverages of Jimma, Ethiopia, chosen in this study were individually as well as in selected combination with cinnamon and honey were able to inhibit the oral pathogen *S. pyogenes*. Much scientific exploration is needed on the frequency of dose needed to control *S. pyogenes* thereby strep-throat.

#### 6. Acknowledgement

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