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Original Research

Ethno botany and antimicrobial perspective of Spices and Honey against *Candida albicans*

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Abstract

Aim: In spite of obsessive use of spices in every Ethiopian meal, little has been investigated on the utilization of Ethiopian spices as a cure for oral opportunistic infections. Therefore, the aim was to identify spices used in Ethiopian food through ethno botanical survey and study their antifungal activity against *Candida albicans.*

Method: Ethno botanical survey of the selected Kebeles of Jimma, Ethiopia was conducted using a semi structured questionnaire from October 2006 to November 2007. Antifungal nature of the spices and combination of spices and honey were evaluated by agar well diffusion assay from September 2008 to July 2010.

Result: Ethno botanical survey indicated fourteen species of spices and honey play a major role in Ethiopian food & beverages. Single plant extract of *Trachyspermum copticum* showed highest activity against *C. albicans*. The same plant showed antagonistic effect when combined with brown and white honey. *Cinamomum zeylanicum* showed highest synergistic effect with both brown & white honey when compared to *Allium ursenum, Cuminum cyminum, Nigella sativa, Rosemarinus officinalis* and *Lippia adoensis*

Conclusion: Thus spices used in Ethiopian food could be a preventive as well as a cure for oral candidiasis caused by *C. albicans*.

INTRODUCTION

The burden of developing and under developed countries is tinted with day to day increase in endless list of opportunistic infections due to HIV/AIDS, diabetes, chemotherapy for cancer and contraceptive use. Immunosuppression and intolerance to antibiotics were the major constraints in managing oral opportunistic infections. Today, the introduction of highly active antiretroviral therapy (HAART) has dramatically reduced the incidence of opportunistic infections among HIV-positive people and Oropharyngeal candidiasis (OPC) with a shift in the spectrum of Candida species and remains the most frequent HIV-associated oral lesion in most developing countries, as well as underdeveloped countries like Ethiopia [1]. However, access to HAART is still limited in Ethiopia [2]. At this junction, herbal remedies can restrain the situation and sustain an immediate cure for the exploited. In addition, malevolent side effects, drug resistance and recurrence of infection will also be taken care of. Further, Ethno botanical survey of selected Kebeles of Jimma, Ethiopia, revealed obsessive use of spices and honey [3]. Herbs and spices have been used for their antimicrobial properties in preventing food deterioration and pathogenic diseases [4]. Previous researchers have confirmed the antifungal activity of honey against *C. albicans* [5], [6], [7] and Fluconazole resistant

C. albicans isolated from the oral cavity of AIDS patients [8]. Similarly, Spices of different parts of the world were studied for their anti-fungal activity. However, very little work has been reported so far

regarding the pharmacological properties of aromatic spices and herbs used in the Ethiopian traditional spiced food preparations [9]. Thus, an attempt was made to analyze the antifungal nature of selected spices and honey against *C. albicans* to suggest an immediate solution to the affected in Ethiopia as well as across the globe.

MATERIALS AND METHODS

Ethno botanical survey

The selected informants were interviewed repeatedly and the information regarding the usage of plants in day to day activities was collected through questionnaire based ethno botanical survey from October 2006 to November 2007. The data indicated that fourteen species of plants viz., *Afromomum angustifolium, Allium ursenum, Brassica oleraceae, Curcuma longa, Cuminum cyminum, Cinamomum zeylanicum, Lippia adoensis, Ocimum basilicum, Trachyspermum copticum, Nigella sativa, Rosemarinus officinalis, Ruta chalepensis, Thymus schimperi* and *Zingiber officinale* were used as spice [3] and the same were used for the current study.

Collection and extraction of spices and spice mixture

Plant parts used as spices identified through ethno botanical survey were collected from vendors of Jimma Market, extracted and stored in a refrigerator. Working concentration of 100mg/ml was prepared by reconstitution in Dimethyl sulfoxide (DMSO).

Traditionally prepared Ethiopian chilli spice mixture comprised of *Afromomum angustifolium, Allium ursenum, Cuminum cyminum, Lippia adoensis, Nigella sativa, Ocimum basilicum, Ruta chalepensis, Thymus schimperi* and Ethiopian chilli was extracted with 70% ethanol and the oily substance obtained was stored in the refrigerator at 4^0 C.

Honey

Un-processed raw honey was collected from Coffee estate in Jimma and working concentration was prepared by dissolving in known volume of water.

Combination of spices and honey

Spice extracts were mixed with brown/white honey at 1: 1 concentration

Test organism

The referral strain, *C.albicans* ATTCC 10231 was obtained from Ethiopian Health and Nutrition Research Institute (EHNRI), was used in this experiment.

Antimicrobial activity

Antimicrobial activity was determined by agar well diffusion method [10]. Fluconazole as positive control and DMSO as negative control were included in all the experimental plates and each experiment was done in triplicates.

Agar well diffusion assay was repeated with spices and honey mixture. The effect of combination of plants extracts and honey was calculated using the following formula [11].

Calculated zone size = sum of zone size of both extracts /2

If,

1. Observed zone size = calculated zone size then the effect will be additive

2. Observed zone size > calculated zone size then the effect will be synergistic

3. Observed zone size < calculated zone size then the effect will be antagonistic

Minimum inhibitory concentration (MIC)

Modified micro dilution method [12] was employed to determine Minimum inhibitory concentration (MIC) of single spice plant extracts as well as combination of spices and honey. Instead of standard broth, PPG1% was used in the experiment. Colour change from red to yellow indicated fungal growth.

RESULTS

The results of this study documented low incidence of *C.albicans* among the selected informants (Table 1) of the study area. Ethno botanical survey of the Kebeles (2, 3 & 5) revealed that fourteen species of plants were used as spice in Ethiopian food, beverages and medicine. Generally, the spice plants (Table 2) were collected from home gardens (Fig. 1) and sold in the market (Fig. 2). *A.ursenum*, *R.chalepensis*, *R.officinalis*, and *Z.officinalis* were sold in fresh form, whereas *A.angustifolium* and *T.schimperi* are sold as fresh and in dried form. Rest of spices chosen in this study were sold in dried form only. Generally, all the spices were sold in the local market along with vegetables, butter and fruits. These plants belonged to six families and many of them were from Lamiaceae family. Specific part of the plant like leaf, stem, bark, bulb, rhizome, inflorescence and seed were used as spice. Seed was found to be the most used part followed by leaf. Spices were used in all Ethiopian sauces and dried *Ocimum* and *Lippia* leaves were added to butter to impart flavour (Table 2). Spices used in chilli spice mixture were roasted and coarsely ground at

home (Fig. 3) & then milled in the machine and used in all traditional Ethiopian food preparations especially in all sauces. Crushed seeds of *B.oleraceae* were wrapped in a cloth and smeared over the pan used for making injera (fermented pan cake) (Fig. 4).

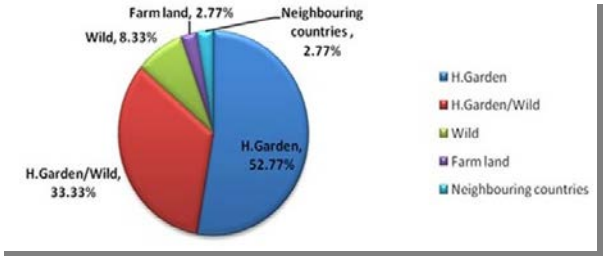

Fig. 1. Place of collection of medicinal plants by the herb sellers

Fig. 3. Pounding of spices with chilli

Fig. 2. Spice seller in Jimma market

Fig. 4. Crushed seeds of *B. oleraceae* applied on the injera making pan

Table 1. Details of Samples Collected

No	Clinical symptom	No. of samples Collected N (%)	No. of Samples positive for *C. albicans* N (%)
1.	Gum infection	45 (20.73)	-
2.	Halitosis	43 (19.81)	2 (00.92)
3.	Tonsillitis	43 (19.81)	3 (01.38)
4.	Tooth pain	43 (19.81)	-
5.	Throat pain	43 (19.81)	-
Total		**217 (99.97)**	**5 (2.30)**

Table 2. Spices used by the informants of selected Kebeles of Jimma - Ethiopia

No	Local name	Scientific name	Family	Part(s) of the plant used	Use in food	Ethnomedicinal use
1.	Besobila	*Ocimum basilicum L.*	Lamiaceae	Leaf	Sauce	Fever
2.	Nech Shinkurit	*Allium ursinum. L*	Liliaceae	Bulb	Sauce	Malaria, tooth pain, fever
3.	Gingibil	*Zingiber officinale Rose.*	Zingiberaceae	Rhizome	Sauce	Breathing difficulty, cough
4.	Gomenzer	*Brassica oleraceae L.var.botrytis*	Brassicaceae	Seed	Greasing injera pan	High body temperature
5.	Kereffa	*Cinnamomum zeylanicum*	Lauraceae	Bark	Tea	Itching
6.	Korarima	*Aframomum angustifolium Sonn*	Zingiberaceae	Seed	Sauce, milk	Chest congestion
7.	Kosaret	*Lippia adoensis*, *Forsk.*	Verbanaceae	Inflorescence/leaf	Clarification of butter & sauce	Fever
8.	Nech Azimud	*Trachyspermum Copticum L*	Apiaceae	Seed	Meat, chicken sauce, bread	Ulcer
9.	Rosemary	*Rosemarinus officinalis.L*	Lamiaceae	Leaf	Meat sauce	High body temperature
10.	Ten Adam	*Ruta chalepensis L.*	Rutaceae	Seed	Sauce	Evil eye
11.	Tiqur Azimud	*Nigella sativa*	Ranunculaceae	Seed	Meat, chicken sauce, bread	Gastritis, ulcer
12.	Tosigni	*Thymus schimperi*	Lamiaceae	Leaf	Sauce, tea	Fever
13.	Erid	*Curcuma longa L.*	Zingiberaceae	Rhizome	Sauce	-
14.	Zeera	*Cuminum cyminum*	Apiaceae	Seed	Sauce	-
15.	Chilli spice mixture	1,2,6,7,8,10,11,12, 14 + Berbere	-	-	All sauces	-

Antifungal activity of spices against *C. albicans* summarized in Fig. 5 indicated that *T. copticum* was highly reactive followed by *C. zeylanicum*, *N. sativa* and *R. chalepensis*. The pathogen was resistant to *A. angustifolium*, *B. oleraceae*, *C. longa*, *L. adoensis*, *R. officinalis*, and *Z. officinale*. Rest of plants selected in this study showed moderate activity against *C. albicans.* The resistance of the pathogen towards the spice plants used in the study was measured and found that *T. copticum* showed the lowest MIC followed by Chilli spice mixture (Fig 6).

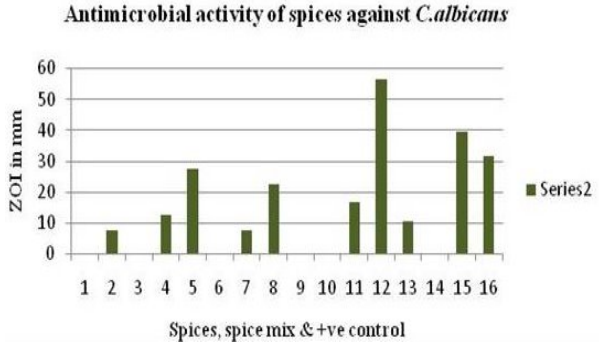

Fig. 5. Antimicrobial activity of spices against *C. Albicans*

The results summarized in Fig. 7 clearly indicated that the combination of spices and brown honey produced different levels of interaction; additive, synergistic, antagonistic or no activity. Antifungal activity of eight spices namely *A. ursenum*, *C. cyminum*, *C. zeylanicum*, *C. longa*, *N. sativa*, *L.adoensis*, *R. officinalis* and *R. chalepensis* was increased synergistically when combined with brown honey. *T. copticum*, *T. schimperi* and chilli spice mixture showed antagonistic activity when combined with brown honey, i.e. observed values were lesser than the expected value of the same combination. Though the activity of *T.copticum* was reduced by combining with brown honey, i.e. the ZOI produced by the combination of *T.copticum* and brown honey was higher than the rest of the spice plants used in this study. There was no antifungal activity in case of *A. angustifolium B. Oleraceae*, *O. basilicum* and *Z. officinale*, and their activity was also not enhanced by the addition of brown honey *i.e.* the compounds present in these spice plants were not synergistic, antagonistic nor additive.

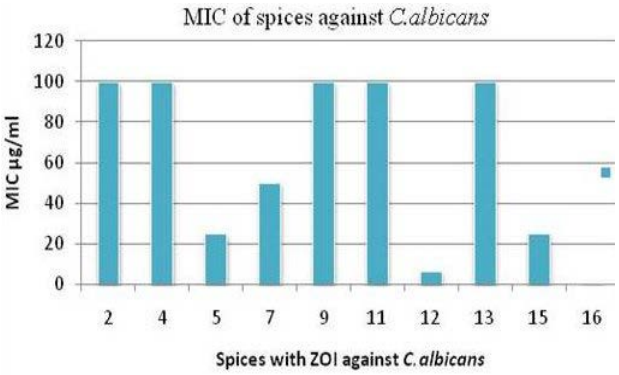

Fig. 6. MIC of spices against *C. albicans*

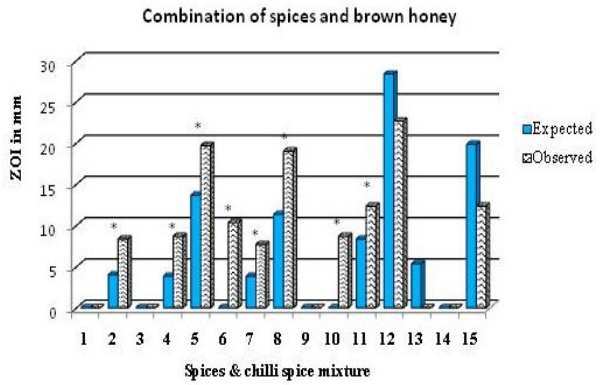

Fig. 7. Combination of spices and brown honey

R. chalepensis, *Trachyspermum copticum* and chilli spice mixture worked antagonistically with white honey whereas *A. ursenum*, *C. cyminum*, *C. zeylanicum*, *C. longa*, *N. sativa*, *L.adoensis*, *R. officinalis* and *Thymus schimperi* were synergistic. *C. albicans* was resistant to combination of white honey with *Afromomum angustifolium*, *Brassica oleraceae*, *Curcuma longa*, *Ocimum basilicum*, and *Zingiber officinale* (Fig. 8).

DISCUSSIONS

Ethno botany of spices of Jimma

Indigenous people possess immense knowledge of their environments [13], and they are skilled in careful selection and proper utilisation of plants in food and beverages as preventive and protective action against several diseases. Therefore, in recent years, ethno botanical and traditional uses of natural compounds especially of plant origin received much attention as they are well tested for their efficacy and generally believed to be safe for human use [14]. Cultivation and use of spices, herbs and medicinal and other essential oil bearing plants is not new to Ethiopia. It is as old as the crop themselves and its history can be traced back to the reign of Queen Sheeba (ca 992B.C).

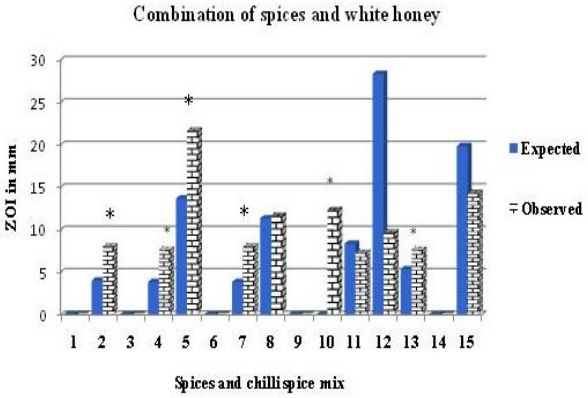

Fig. 8. Combination of spices and white honey

1. *A. angustifolium*, 2. *A.ursinum*, 3. *B. Oleraceae*, 4. *C. cyminum*, 5. *C. zeylanicum*, 6. *C. longa*, 7. *L.adoensis* 8. *N. sativa*, 9. *O.basilicum*, 10. *R. officinalis*, 11. *R. chalepensis*, 12. *T. copticum*, 13. *T. schimperi*, 14. *Z. officinalis*, 15. Chilli spice mixture

Positive control – Fluconazole: 31.66±0.57I

* = statistically significant synergistic activity

ZOI in mm = Mean ± Standard deviation

Brown honey - 0.00 mm White honey - 0.00 mm

Fig. 9. Foot note for Fig. 5, 6, 7 & 8

The informants of this study were willing to share their cultural knowledge of plants used in food and beverages without any bias. This enabled the authors to find out the similarities in Ethiopian and Indian scenario regarding spice usage in food. For example, in India, leaves of *Moringa oleifera* or *Murraya koenigii* (curry leaf) were added to clarify and impart flavour to butter, which was similar to the addition of *Ocimum* leaves for the same reason in Ethiopia. It is also known as Al-Rehan (In Arabic) which has received a great deal of attention over the past decades around the world [15].

Antifungal activity of spices

Addition of some spices to foods could not only impart flavour and pungent stimuli but also would provide antimicrobial property [5]. Although many plants have been investigated for their antifungal activity against *C. albicans*, the search is still on to find long term prevention and cure with medicinal herbs available in each locality. The results of this study correlated with the previous researchers who proved that the growth of *C. albicans* was controlled by *A. ursenum* [16] *C. zeylanicum* [17], *C. cyminum* [18], *N. sativa* [19] [20] and *R. chalepensis* [21] and contradicted with Hussien, (2011), in case of *T. schimperi* [9].

According to Rasooli, (2008), ajowan oil contained thymol (37.2%), and p-cymene (32.3%) [22], whereas, Chialva, (1993) reported (61%) thymol, (15.6%) p-cymene and (11.9%) γ-terpinene [23]. Therefore, it is evident that the constituents of a plant greatly differ with geographical location and thereby in their bioactivities. Cox, (2001) found that particularly γ-terpinene in ajowan oil was effective against *C. albicans* [24] and Hammer 1999 recorded the lowest MIC value of 0.03% v/v for thymol oil against *C. albicans* [25] and Giordani 2004 further insists that Thyme is a potent antifungal, being of particular benefit in oral candidiasis [26]. In addition, ethno botanical survey of this study indicated that whole seeds of *T. copticum* were used in the preparation of bread and in sauces. Thus *T. copticum* could have directly or indirectly inhibited *C. albicans* and resulted in low incidence of oral thrush caused by *C. albicans*.

Honey

Antimicrobial activity of honey is primarily due to hydrogen peroxide generated by the action of an enzyme that bees add to the nectar, but there are some floral sources which provide additional antimicrobial compounds [27]. Though many researchers have confirmed that honey inhibit the growth of *C. albicans*, results of Kothai, (2012) revealed that Honey collected from Jimma Market was not effective against *C. albicans* [28] and correlated with the results of Moussa, (2012) who found that *C. albicans* was resistant to all concentrations (10, 30, 50 & 70) % of undiluted honey samples collected in Algeria [29].

Combination of Spices and honey

Secondary metabolites and volatile compounds in *A. ursenum*, *C. cyminum*, *C. zeylanicum*, *N. sativa*, *R. officinalis* and *L. adoensis*, might have worked synergistically with both brown and white honey and produced a broad spectrum antifungal activity. There was no report on the use of these spices in combination with honey. Further exploration will lead to an efficient herbal remedy for oral Candida infection.

Chilli spice mixture

A spice mixture with a combination of Cinnamon, clove and ginger, showed higher activity against *C. albicans* than the individual effect [5]. Similarly, Ethiopian chilli spice mixture exhibited high antifungal activity than the individual effect of most of the spices chosen for this study. Chilli spice mixture used in this study was prepared traditionally in which *Afromomum angustifolium*, *Allium ursenum*, *Cuminum cyminum*, *Ocimum basilicum*, *Lippia adoensis*, *Nigella sativa*, *Ruta chalepensis*, *Thymus schimperi* and Ethiopian chilli were combined in a specific quantity and the exact reason was un-known. Exuberant use of this mix in everyday dishes could be also another reason for low incidence of *C. albicans* which needs further exploration.

Synergistic activity

High degree of synergistic activity due to the wide variety of organic compounds present in either single plant or multi plant extracts could be a major reason for low incidence of *C. albicans* among the inhabitants irrespective of their HIV status. Thus, the burden in using the synthetic antifungal agents which bring serious side effects, drug resistance and resurgence of Candida infection could be effectively managed with natural food based therapy.

CONCLUSION

Plant based cure through spiced food which are consumed in a locality and within their cultural context might be one of the simple way to meet the immediate health care need of the economically downtrodden in Ethiopia as well as across the globe. Unknowingly the use of spices have already reduced the burden of oral candidiasis caused *C.albicans* and the current study has scientifically validated the folkloric usage of Ethiopian spices which further deepen the association between the plants and inhabitants. Therefore, this study insists on regular use of Ethiopian spices may prevent oral candidiasis caused by *C. albiacns* and may alleviate the same where access to HAART is limited. As the effect is dose dependent, further research on the standardisation on the quantity of spice used in each food is necessary to get the desired effect against the fore said pathogen.

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