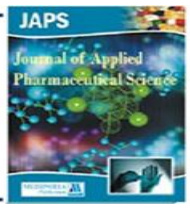
Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 14-01-2012
Revised on: 20-01-2012
Accepted on: 25-01-2012

Antimicrobial properties of Ethiopian chewing sticks against *Candida albicans*

Kothai Seshathri

Kothai Seshathri
Department of Biotechnology, Bharathidasan University, Tiruchirappalli - 620 024, Tamil Nadu, India.

ABSTRACT

Ethnobotanical survey was done in Jimma, Ethiopia, to identify the plants used for oral hygiene and evaluate the same against a selected oral pathogen. The survey revealed the use of chewing sticks to manage oral hygiene/infection. In spite of their wide use, very little has been done to evaluate their antimicrobial activity. Hence 11 chewing stick plants were chosen for antimicrobial study against an oral pathogen – *Candida albicans* the causative organism for oral candidiasis by agar well diffusion (Perez, 1991). The results revealed that *Olea europaea* extract showed maximum inhibition on its own. The activity of *Justicia schimperiana* was increased to three fold when combined with cinnamon and brown honey. The research documents the use of chewing sticks to manage oral infection caused by *Candida albicans*, which will be of immense assistance to developing countries with financial constraints and limited health care facilities.

Keywords: chewing sticks, antimicrobial property, oral candidiasis, *Justicia schimperiana*, *Olea europaea*, *Candida albicans*

INTRODUCTION

Oral Candidosis has become a major health problem as an opportunistic infection of HIV/AIDS. According to UNAIDS, Ethiopia has an estimated 1.1 million people living with HIV/AIDS. Up to 90% of HIV-infected individuals suffer at least one episode of oral candidiasis during the course of their disease (Vazquez, 2000). Oral candidosis was not focused as a serious health issue in Ethiopia since the country's burden and battle is dominated with limited medical supplies. In spite of the availability of modern medicines, people living with HIV continue to depend greatly on Traditional Health Practitioners (THP) and herbal treatments for health information (Jaco, 2003). The published and documented Ethiopian traditional prescriptions till now did not focus its attention towards the opportunistic infections of HIV except tuberculosis. The available antifungal agents are not only limited in number (Mehta *et al.*, 2002), but many are toxic and very expensive (Mehta *et al.*, 2002). Relapse of Candida infections is very common (Debruyne, 1997) and this increases the burden of managing this opportunistic infection (Runyoro, 2006). Thus the need of the hour is to find an inexpensive, affordable & available plant based cure to manage the oral candidiasis and to overcome the problems associated with modern medicines. Ethnobotanical survey in Jimma revealed the use of chewing sticks to maintain oral hygiene/infections which were not scientifically evaluated against oral pathogens especially *C. albicans*.

For Correspondence
Kothai Seshathri
Email: kothaiseshathri@yahoo.co.in

In addition Wu, (2001) suggested that anti-microbial substances that naturally protect plants against various invading microorganisms or other parasites may leach out into the oral cavity from the chewing sticks and these compounds may benefit the users by protection against oral pathogens. Hence an attempt was made to evaluate the antifungal nature of chewing sticks used by the inhabitants of Jimma-Ethiopia.

MATERIALS AND METHODS

Survey

Ethanobotanical survey was conducted from October 2006 to December 2007 and semi-structured questionnaire was used to collect the data.

Collection and extraction of chewing sticks

Fresh twigs were obtained from home gardens, nearby villages and from the vendors in the market. The plants were identified and confirmed by the herbarium unit of Addis Ababa University, Addis Ababa, Ethiopia. Extracts were obtained by standard method (Harbone, 1963)**.** Working concentration (100mg/ml) of chewing sticks and cinnamon were prepared by reconstitution in DMSO. Water was used in case of honey. For the combination test, chewing stick extracts were mixed with cinnamon at 1:1 ratio (v/v) and chewing stick, Cinnamon and brown or white honey in the ratio of 1:1:1 (v/v).

Test organism

24 hrs old test organism - *Candida albicans* (clinical isolate and referral strain ATCC 10231) was grown in PPG1% agar (peptone water with red phenol supplemented with glucose 1% (w/v). After 4 hours, the cells were suspended in normal saline, adjusted to 0.5 Mc Farland standard turbidity with PPG 1% to give a final concentration of $5x10^5$CFU/ml.

Antimicrobial property

The antimicrobial activity of selected chewing stick plants, cinnamon and un-processed honey (brown & white) were tested against *Candida albicans* through agar well diffusion method (Perez *et al.*, 1990). The effect of combination of plant extracts and honey was calculated using the following formula ((Mujeeb 2010).

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

The same procedure was followed to find the calculated zone size for the combination of three.

Calculated zone size = sum of zone size of two plant extracts and honey / 3

If,

1. The observed zone size is equal to the calculated zone size- Additive
2. The observed zone size is greater than the calculated zone size – Synergistic.
3. The observed zone size is less than the calculated zone size- Antagonistic

Minimum inhibitory concentration

The minimal inhibitory concentration of chewing sticks was determined based on the modified microdilution method (Kouitcheu 2007) in 96 multi well microtiter plates. The dry extract was initially dissolved in Tween 80 (100µl) and then in PPG1%. 100 µL of PPG1% was loaded into the wells in Column 1 &12 and 50µl of PPG1% was loaded into wells in column 4-10. 100 µl of PPG1% with known concentration of test material (Fluconazole and plant extract) were loaded into column 2 and 3. 50 µl from the wells in column 3 was transferred to wells in column 4 and carried out subsequently and a concentration range (200 to 1.562 µg /ml) was obtained. 10 µl of the microbial suspension was added to all the wells in columns 3-10 & 12. Final volume was made to 200µl with PPG1%. Each plate had a set of controls: Column 2 with Fluconazole positive control. 200µl of DMSO was added to column 11. The test was done in triplicate and placed in an incubator set at 25^0C. The colour change was then assessed visually. Any colour change from red to yellow or colourless was recorded as positive. The lowest concentration at which no colour change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial and fungal strains.

Statistical analysis

The results were analyzed using SPSS 16.0 version.

RESULTS

Generally local communities worldwide are extremely knowledgeable about local plants and natural resources, on which they are so immediately and intimately dependent for healthy social life.

The ethnobotanical survey conducted in Jimma from October 2006 to November 2007 in selected Kebeles of Jimma - Ethiopia, identified a few valuable traditions viz., the use of chewing sticks for oral hygiene, cinnamon infusion in tea and dry bread with honey. According to this study report, *Clausena anisata, Clematis simensis, Clerodendrum myricoides, Juniperus procera, Justicia schimperana, Olea europaea, Phoenix reclinata, Rubus apetalus, Sida rhombifolia, Stereospermum kunthianaum* and *Vernonia amygdalina* are currently in use as chewing sticks. Nearly 50% of the inhabitants were chewing stick users.

Antimicrobial property of chewing sticks

Out of eleven chewing sticks tested, seven chewing sticks namely, *C.anisata, C.simensis, J.procera, J.schimperana, O.europaea, P.reclinata* and *R.apetalus* were able to inhibit *C.albicans* (Clinical isolate). The ZOI varied from 6.33 to 15.66. Among which highest ZOI was produced by *O.europaea* and highly significant ($P > 0.05$), whereas, the growth of *C.albicans* was not inhibited by *C.myricoides, S.kunthianum, S.rhombifolia* and *V.amygdalina*. Similar result was obtained for the referral strain of *Candida albicans.* Comparison between clinical and referral strain was done by Annova followed by Duncan's post hoc test. The results indicated that the difference was not significant at $P=0.05$. Hence the referral strain was used in further investigations. The minimum inhibitory concentration (MIC) varied from 12.5 to 100µg/ml for the chewing sticks (Table 1).

Table. 1: Antimicrobial activity of chewing sticks against *C.albicans*.

S.No.	Botanical name of the chewing sticks	Extract / Zone of inhibition in mm				
		Candida albicans (Clinical isolate)	P= 0.05	*Candida albicans* (Referral strain) ATCC 10231	P= 0.05	Remarks
1.	*Clausenia anisata*	06.66±o.57	0.492	06.66± 0.57	0.492	-
2.	*Clematis simensis*	06.33±0.57	0.492	06.33±0.57	0.492	-
3.	*Cleodendrum myricoides*	-	-	-	-	-
4.	*Juniperus procera*	10.30± 0.57	0.466	10.30± 0.57	0.466	++
5.	*Justicia schimperiana*	07.66± 0.57	0.152	07.66± 0.57	0.152	++
6.	*Olea europea*	15.66± 0.57	1.000	15.66± 0.57	1.000	+++
7.	*Phoenix reclinata*	10.00±1.00	0.466	10.00±1.00	0.466	++
8.	*Rubus apitalus*	08.33±0.57	0.152	08.33±0.57	0.152	++
9.	*Sida rhombifolia*	-	-	-	-	-
10.	*Stereospermum kunthianum*	-	-	-	-	-
11.	*Vernonia amygdalina*	-	-	-	-	-

Each value = Mean ± SD Antimicrobial activity- + = Low ++ = Medium +++ = High
Ethanol – Negative control Fluconazole – Positive control

Table. 2: Antimicrobial activity of chewing sticks with brown and white honey against *C.albicans*.

S.No.	Botanical Name of the chewing sticks	Antimicrobial activity of chewing sticks with Brown honey				Antimicrobial activity of chewing sticks with White honey			
		Calculated ZOI in mm	Observed ZOI in mm	P = 0.05	Remarks	Calculated ZOI in mm	Observed ZOI in mm	P = 0.05	Remarks
1	*Clausenia anisata*	3.33 ± 3.67	-	-	Antagonistic	3.33 ± 3.67	8.66 ± 0.57	1.000	**Synergistic**
2	*Clematis simensis*	3.17 ± 3.48	-	-	Antagonistic	3.17 ± 3.48	6.30 ± 0.57	0.306	**Synergistic**
3	*Cleodendrum myricoides*	-	14.00 ± 1.00	1.000	**Synergistic**	-	-	-	-
4	*Juniperus procera*	5.17 ± 5.67	-	-	Antagonistic	5.17 ± 5.67	-	-	Antagonistic
5	*Justicia schimperiana*	3.63 ± 4.21	-	-	Antagonistic	3.63 ± 4.21	-	-	Antagonistic
6	*Olea europea*	7.83 ± 8.58	11.66 ± 0.57	1.000	**Synergistic**	7.83 ± 8.58	10.33 ± 0.57	1.000	**Synergistic**
7	*Phoenix reclinata*	5.00 ± 5.51	-	-	Antagonistic	5.00 ± 5.51	-	-	Antagonistic
8	*Rubus apitalus*	4.17 ± 4.57	7.00 ± 0.00	1.000	**Synergistic**	4.17 ± 4.57	6.66 ± 0.57	0.306	**Synergistic**
9	*Sida rhombifolia*	3.33 ± 3.67	-	-	Antagonistic	3.33 ± 3.67	-	-	Antagonistic
10	*Stereospermum kunthianum*	-	-	-	-	-	-	-	-
11	*Vernonia amygdalina*	-	-	-	-	-	7.66 ± 0.57	1.000	**Synergistic**

Each value = Mean ± SD Ethanol served as negative control +ve control - Fluconazole 10 mg /ml
Brown and white honey did not had any effect against *C.albicans* at 100 mg/ml

Table. 3: Effect of Chewing sticks in combination with cinnamon against *Candida albicans*.

S.No.	Botanical Name of the chewing sticks	Antimicrobial activity of chewing sticks (ZOI in mm)	Antimicrobial activity of chewing sticks & Cinnamon			
			Calculated (ZOI in mm)	Observed (ZOI in mm)	P = 0.05	Remarks
1	*Clausenia anisata*	06.66 ± 0.57	17.00 ± 11.33	14.33 ± 0.57	1.000	Antagonistic
2	*Clematis simensis*	06.33 ± 0.57	16.83 ± 11.51	31.66 ± 1.52	0.102	Synergistic
3	*Cleodendrum myricoides*	-	13.61 ± 14.97	07.66 ± 0.57	0.575	Antagonistic
4	*Juniperus procera*	10.30 ± 0.57	18.83 ± 09.32	08.00 ± 1.00	0.575	Antagonistic
5	*Justicia schimperiana*	07.66 ± 0.57	17.50 ± 10.78	29.66 ± 0.57	0.102	Synergistic
6	*Olea europea*	15.66 ± 0.57	21.50 ± 06.41	25.66 ± 0.57	1.000	Synergistic
7	*Phoenix reclinata*	10.00 ± 1.00	18.67 ± 09.52	-	-	Antagonistic
8	*Rubus apitalus*	08.33 ± 0.57	17.83 ± 10.42	19.66 ± 0.57	0.102	Synergistic
9	*Sida rhombifolia*	06.66 ± 0.57	17.00 ± 11.33	12.66 ± 0.57	1.000	Antagonistic
10	*Stereospermum kunthianum*	-	13.67 ± 14.97	20.66 ± 0.57	0.102	Synergistic
11	*Vernonia amygdalina*	-	13.67 ± 14.97	23.00 ± 1.00	1.000	Synergistic

Table. 4: Antimicrobial activity of chewing sticks in combination with cinnamon and brown/white honey.

S.No.	Botanical Name of the chewing sticks	Antimicrobial activity of chewing sticks, Cinnamon & Brown honey				Antimicrobial activity of chewing sticks, Cinnamon & White honey			
		Calculated (ZOI in mm)	Observed (ZOI in mm)	P = 0.05	Remarks	Calculated (ZOI in mm)	Observed (ZOI in mm)	P = 0.05	Remarks
1	*Clausenia anisata*	11.33 ± 12.34	12.33 ± 0.57	0.139	Synergistic	11.33 ± 12.34	07.33 ± 0.57	0.150	Antagonistic
2	*Clematis simensis*	11.22 ± 12.39	08.66 ± 0.57	1.000	Antagonistic	11.22 ± 12.39	29.66 ± 0.57	1.000	Synergistic
3	*Cleodendrum myricoides*	09.11 ± 13.67	-	1.000	Antagonistic	09.11 ± 13.67	13.66 ± 0.57	0.058	Synergistic
4	*Juniperus procera*	12.56 ± 11.95	10.66 ± 0.57	1.000	Antagonistic	12.56 ± 11.95	13.00 ± 1.00	0.058	Synergistic
5	*Justicia schimperiana*	11.67 ± 12.21	32.33 ± 2.08	1.000	Synergistic	11.67 ± 12.21	29.66 ± 1.15	1.000	Synergistic
6	*Olea europea*	14.33 ± 11.88	14.06 ± 0.57	0.139	Additive	14.33 ± 11.88	15.0 ± 1.00	1.000	Synergistic
7	*Phoenix reclinata*	12.44 ± 11.99	-	-	Antagonistic	12.44 ± 11.99	-	-	Antagonistic
8	*Rubus apitalus*	11.89 ± 12.13	13.66 ± 0.57	0.139	Synergistic	11.89 ± 12.13	06.66 ± 0.57	0.150	Antagonistic
9	*Sida rhombifolia*	11.33 ± 12.34	10.66 ± 0.57	1.000	Antagonistic	11.33 ± 12.34	12.22 ± 1.15	0.058	Synergistic
10	*Stereospermum kunthianum*	09.11 ± 13.67	10.66 ± 0.57	1.000	Synergistic	09.11 ± 13.67	07.33 ± 0.57	0.150	Antagonistic
11	*Vernonia amygdalina*	09.11 ± 13.67	18.66 ± 0.57	1.000	Synergistic	09.11 ± 13.67	20.33 ± 0.57	1.000	Synergistic

Table. 5: Chewing plants which showed synergistic activity with brown/white honey, cinnamon, cinnamon and brown/white honey.

S.No.	Chewing Stick & Brown Honey	Chewing Stick & White Honey	Chewing Stick & Cinnamon	Chewing Stick, Cinnamon & Brown Honey	Chewing Stick Cinnamon & White Honey
1	*C.myricoides*	*C.anisata*	*C.simensis*	*C.anisata*	*C.simensis*
2	*O. europaea*	*C.simensis*	*J.schimperana*	*C.simensis*	*C.myricoides*
3	*R.apetalus*	*O. europaea*	*O. europaea*	*J.schimperana*	*J.procera*
4	-	*R.apetalus*	*R.apetalus*	*R.apetalus*	*J.schimperana*
5	-	*V.amygdalina*	*S.kunthianum*	*S.kunthianum*	*O. europaea*
6	-	-	*V.amygdalina*	*V.amygdalina*	*S.rhombifolia*
7	-	-	-	-	*V.amygdalina*

Chewing sticks and brown honey

Expected ZOI was calculated for chewing sticks and brown honey combination. The calculated ZOI predicted susceptibility of *C.albicans* to eight chewing sticks namely *C.anisata, C.simensis, J. procera, J.schimperiana, P.reclinata, R.apetalus, S.rhombifolia* and *V. amygdalina*. Among these, only *O.europaea* and *R.apetalus* were able to inhibit the test organism and produced 11.66 and 7mm of ZOI respectively. In addition, *C.myricoides* which was originally inactive and not expected to inhibit the test organism produced synergistic effect when mixed with brown honey and the observed ZOI was 14mm (Table 2).

Chewing sticks and white honey

Similar experiment with chewing sticks and white honey, the calculated results were same as that of the previous experiment with chewing sticks and brown honey. Out of eight plants, only 5 plants namely *C.anisata, C.simensis, O.europaea R.apetalus* and *V. amygdalina* were able to inhibit the growth of *C.albicans*. Among the three plants *C.myricoides, S.rhombifolia and V. amygdalina,* which were predicted to show absence of activity, only *V.amygdalina* produced 7.66mm ZOI (Table 2).

Chewing sticks and cinnamon

Cinnamon at 100mg/ml concentration produced 12.33±0.57mm of ZOI. The results of chewing sticks and cinnamon combination showed that the calculated ZOI ranged from 13.61 to 21.50 whereas the observed results produced ZOI ranging from 8.00 to 31.66. Highest ZOI was observed with *C.simensis* and Cinnamon followed by *J.schimperiana, O.europaea, V.amygdalina, S.kunthianum and R.apetalus.* Though the activity of *C.anisata, C.myricoides* and *S.rhombifolia* has been enhanced by the combination, the observed ZOI was less than that of the expected ZOI (17mm) and hence considered antagonistic. The sensitivity of *P.reclinata* was completely nullified by the combination (Table 3).

Chewing sticks, cinnamon and brown honey

In chewing stick, cinnamon and brown honey combination, the calculated values indicated moderate to high activity. Whereas the actual observed values of ZOI indicated that only one plant (*O.europaea*) had additive effect. *C.anisata, J.schimperana, R.apetalus, S.kunthianum and V.amygdalina* showed synergistic activity.

The ZOI produced by *J.schimperana,* cinnamon and brown honey was found to be similar with that of Fluconazole the antifungal agent and the positive control used in this experiment. *C.myricoides* and *P.reclinata* did not produce any observed ZOI and *C.simensis, J. procera,* and *S.rhombifolia* also showed reduced ZOI (Table 4).

Chewing sticks, cinnamon and white honey

Similar experiment was repeated with chewing sticks, cinnamon and white honey and the observed results revealed that (*C.anisata, P.reclinata, R.apetalus* and *S.kunthianum* showed antagonistic effect and the rest were synergistic when compared with the calculated value.

The effect of Chewing stick plants either alone or in combination showed statistically significant variance at $P > 0.05$.

Synergistic activity

The overall results of the 4 experiments were combined and analyzed. It showed that the synergistic effect increased with the combinations. Maximum number of 7 plants showed synergistic effect in the combination of chewing sticks cinnamon and white honey (Table 5).

DISCUSSION

The incidence of both community acquired and nosocomial fungal infections has significantly increased over the past decades, accompanying the growing number of high risk patients particularly those with impaired immunity (Pinto *et al.*, 2009). Though antibiotic therapy continues to be the core fighter of fungal infections, malicious side effects have drawn the attention of the researchers towards herbal remedies. In addition, (Kieren, 1998) found that *C.albicans* isolates became resistant to fluconazole and Amphotericin B after 2 weeks of antifungal drug exposure. Natural therapy will be the only solution for economically underprivileged people in developing countries like Ethiopia. Astonishingly nearly 300 different species of trees and shrubs in East Africa are used in making chewing sticks (Veronica, 2010). Ethnobotanical studies in Jimma, revealed the use eleven chewing which were collected from home gardens and frequently from shops (Fig.1). Most of the inhabitants use chewing sticks after food. Thus the extract of chewing stick plants stays longer, has direct contact with all parts of the mouth and part is taken internally. Thus, use of chewing sticks after the meal sounds

scientifically rationale, in the sense the plant removes the debris as well as offers protection for prolonged period of time.

Fig. 1: Chewing sticks (market).

Most traditional recipes involve more than one plant and with or without additives. Often cinnamon is used as a stimulant to other herbs enabling the herbal remedies to work faster (http://www.wic.Herbal encyclopedia - on line). Thus, in this research cinnamon is combined with chewing sticks and the results of the research confirmed that stem bark extract of cinnamon has enhanced the activity of *C.simensis, J.schimperiana, O. europaea, R.apetalus, S.kunthianaum* and *V.amygdalina.* In the same experiment it was found that it has reduced the activity of *J.procera* & completely suppressed the activity of *P.reclinata.* The combination of cinnamon and the chewing stick plants selected in this research has not been done before.

In traditional medicine cinnamon was used for the treatment of inflammation, cough, toothache, antiseptics, expectorant and fungal infections like candidiasis. The antimicrobial properties was probably due to the major component cinnamaldehyde (Rastogi, 2002). In an experiment conducted by Shahidi, (2004) stem bark extract of *Cinnamomum zeylanicum* of Iran produced 22mm ZOI against *C.albicans* whereas in the current experiment *Cinnamomum zeylanicum* of Jimma, Ethiopia produced 27.33 mm ZOI. Apart from the curative properties, inhalation of cinnamon odor boosts the brain activity and serves as an excellent source of fiber and trace mineral manganese and calcium (Zoldz, 2004). Thus cinnamon not only offers protection against *C.albiacns*, it also helps to strengthen the teeth.

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, and hence included in the current study. Previously, Ansari, (2009) found that none of the concentrations tested (1, 25, 50 and 100%) of honey produced by African sculata – Guyana, was effective against *C.albicans*. Similar result was obtained in the current study with brown and white honey of Jimma – Ethiopia but found to enhance the antimicrobial activity of the chewing sticks. Vanessa, 2010 states that in addition to the antimicrobial property, honey also appears to stimulate lymphocytic and phagocytic activity. These are key body immune responses in the battle against infections. Fukuda, (2009) reported that oral intake of honey augmented antibody production in primary and secondary immune responses against thymus-dependent and thymus independent antigens. The therapeutic potential of uncontaminated, pure honey is grossly underutilized. It is widely available in most communities and although the mechanism of action of several of its properties remains obscure and needs further investigation, the time has now come for conventional medicine to lift the blinds off this traditional remedy and give it its due recognition (Zumla, 1989).

Justicia schimperana

In Ethiopia, fresh leaves of the plant used to cure evil eye, hepatitis B, madness (maratuu), over sweating and fever (Haile, 2008). The decoction of the dried leaves of the plant mixed with local beer (Tella) is taken as a remedy for bronchial asthma (Abebe, 2003). (Zelalem, 2009) identified that chloroform extract of *A.schimperana* has tracheal relaxant effect on guinea pigs. The unique combination of bioactive compounds present in the extract of *J. Schimperana* (Fig.2), cinnamon and brown honey might have produced a broad spectrum antifungal activity against *Candida albicans,* whereas the other chewing sticks failed to produce the same effect. Much scientific exploration is needed to strengthen the above mentioned concept. Up to our knowledge such experiment on combination of chewing sticks with cinnamon and honey was never reported before. The result of the study justifies the use of combination of plants and additives in traditional medicine.

Fig. 2: *Justicia schimperana.*

CONCLUSION

Use of chewing stick is gradually disappearing in most of the developed and developing countries due to scientific advancement and non availability of chewing sticks, Ethiopia is an

outstanding exception to it. The findings benefit not only Ethiopians and also all those who suffer from oral candidiasis across the globe.

ACKNOWLEDGEMENT

The author thanks all the Ethiopians who helped in providing first-hand information and in translation. The authors acknowledge the institution Jimma University College of Agriculture and Veterinary Medicine in permitting to conduct the research in the Pathology laboratory and in availing library and internet resources.

REFERENCES

Abebe, D., and A. Ayehu. Medicinal Plants and Enigmatic Health Practices in Northern Ethiopia. In World Health Organization, Ethiopian Ministry of Health Project No. AF/ ETH/TRM/001RB and the United Nations Development Programme.(1993).

Abebe, D., Debella, A.,and K.Urga. Medicinal plants and other useful plants of Ethiopia. Camerapix Publishers's International, Singapore: (2003) 99-101.

Endashaw and Bekele.Study on Actual situation of medicinal plants of Ethiopia. Addis ababa.(2007).

Haile, Y., Delenasaw,Y.,and D.Teketay. Ethnomedical plant knowledge and practice of the Oromo ethnic group in Southwestern Ethiopia. Journal of Ethnobiology and Ethnomedicine. 2008; 4 (11).

Jaco, H. Building a Regional Initiative for Traditional Medicine and AIDS in Eastern and Southern Africa. African health monitor. 2003; 4 (1):24.

Kieren, A.M., Lyons,C.N., Rusted,T., Bowden,R.A., and White, C.T. Rapid transient Fluconazole resistance in Cnadida albicans is associated with increased mRNA levels of CDR Antimicrob.Agents Chemother. 1998; 42: 2584-2589.

Kouitcheu, M.L., Kouam,J.,Penlap Beng,V,.Bona venture,T.,Ngadjui,Z.,Fomum,T.,and F.X.Etoa. Evaluation of antimicrobial activity of the stem bark of *Cylicodiscus ganunensis* (Mimosaceae). Afr. J. Traditional, Complimentary and Alternative Medicines. 2007; 4 (1):87-93.

McClatchey, W.C., G.B. Mahady, B.C. Bennett, L. Shiels, and V. Savo. Ethnobotany as a pharmacological research tool and recent developments in CNS-active natural products from ethnobotanical sources. Pharmacology & therapeutics. 2009; 123 (2):239-254.

Mujeeb, U.R., Shereen,G.,Ejaz,E.O.,Umed,A.S.,Irfan.A. Affectivity of Zataria multiflora Boiss Alcoholic extracts against bacteria. Lybian Agriculture research centre. 2010; 1 (3):147-152.

Pinto, E., L. Vale-Silva, C. Cavaleiro, and L. Salgueiro. Antifungal activity of the clove essential oil from Syzygium aromaticum on Candida, Aspergillus and dermatophyte species. Journal of medical microbiology. 2009; 58(11):1454.

Rastogi, R.P., and Mehrotra, B.N. Glossary of Indian Medicinal Plants. National Institute of science communication, New Delhi, India.(2002)

Runyoro, D.K.B., Ngassapa,O.D., Matee,M.I.N., Joseph,C.C., and M.J. Moshi. Medicinal plants used by Tanzanian traditional healers in the management of Candida infections. Journal of Ethnopharmacology.2006; 106:158–165.

Shahidi, B., Aghighi, S., and N.Karimi. Antimicrobial and Antifungal survey in plants used in indegenous herbal medicine of South East regions of Iran. Journal of Biological Sciences. 2004; 4 (3):405-412.

Vazquez, J.A. Therapeutic options for the management of oropharyngeal and esophageal candidiasis in HIV/AIDS patients. HIV Clinical Trials.2000; 1: 47-59.

Veronica, A. http:hubpage/The Chewing Stick - Nature's Nifty Little Toothbrush. (2010).

Wu, CD., Darout,N., and N.Skaug. Chewing sticks: Timeless natural tooth brushes for oral cleansing. J.Periodont.Res.2001; 36:275-284.

Zelalem, P., Eyasu,M and D.Asfaw. Tracheal relaxant effect of column chromatographic elutes of chloroform fraction of *Adhatoda schimperiana* leaves in Guinea pigs. Pharmacognosy magazine. 2009; 4:86-91.

Zoldz, P., Raudenbush, B., and S.Lilley. Cinnamon perks performance. Sarasota,fl: Annual meeting - Association for chemoreceptioon sciences. (2004).

Zumla, A., and A. Lulat. Honey--a remedy rediscovered. Journal of the Royal Society of Medicine. 1989; 82(7):384-385.