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Evaluation of Antimicrobial Activity and Phytochemical Screening of Twenty Medicinal Plants against Oral Microbes

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Abstract

The present study was carried out to evaluate the phytochemical and antimicrobial activity of twenty medicinal plants against five microbial strains causing oral infections. The phytochemical analysis carried out revealed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, reducing sugar and steroids in most of the medicinal plants. The antimicrobial activity of ethanolic extract of medicinal plants were evaluated using well diffusion method against Streptococcus mutans, Enterococcus faecalis, Lactobacillus acidophilus ,Candida albicans and Candida tropicalis. Ethanolic extracts of Aloe barbadensis, Calendulla officinalis Cinnamum zeylanicum and Tinospora coridfolia, were not effective against Streptococcus mutans and Enterococcus faecalis respectively. However, Azadirachta indica, Centella asiatica, Lannea coromandelica, Rosa centifolia, Zingiber officinale were showing week and the extract of Allium sativum, Citrus limon, Citrus sinesis, Curcuma longa, Emblica officinalis ,Glycyrrhiza glabra, Juglans regia, Ocimum sanctum, Piper nigrum and Psidium guajava displaying strong antimicrobial activity against most of the test species. The ethanol extracts of Syzygium aromaticum showing strong antimicrobial activity against all test species. The results provide justification for the use of the medicinal plants to treat various oral infections.

Keywords: *Medicinal plants, Oral infections, Antimicrobial activity, Phytochemical activity, Well diffusion method, Ethanol extract, Streptococcus mutans, Enterococcus faecalis, Lactobacillus acidophilus ,Candida albicans, Candida tropicalis*

Introduction

Medicinal plants represent a rich source of antimicrobial and phytochemical agents. Plants are used medicinally in different countries of world and as source of many powerful drugs. A wide range of medicinal plant parts is used for extraction of raw drugs as they posses varied medicinal properties[1]. Plants are believed to be important source of new phytochemical with potential therapeutic effects. Traditional medicinal plants should be able to play a greater role in the modern primary oral healthcare system of many countries. Many studies have been reported that the plants are as good as the conventional ones (in controlling oral microbial load) [2,3]. The use of medicinal plant-derived tooth brushes, is a common traditional oral care practice in many parts of the world. Within a community, chewing-stick plants are often specific, but tend to vary from one culture to the next [4]. As far back as the 1970s, it was suggested that, regular use of the African chewing-stick acting as an antimicrobial agent, may control the formation and activity of dental caries and therefore reduces the incidence of gingivitis and possibly tooth decay [5].

Many microbes produce oral diseases which are manifested in or about the dental caries. Some of these diseases are of specific nature and are produced by contribution to the problem of caries etiology [6]. The highest dental plaque susceptibility is in the age group of 20-45 years, the females are more susceptible to caries as compared to males [7].

Dental decay is a chemical microbial process consisting of two stages- (i) the decalcification of enamel or its total destruction (ii) the decalcification of dentine (dissolution of the softened residue). The cariogenic *Streptococcus* species are critical to the development of pathogenic plaque. A large number of *Streptococcus* [8] and *Lactobacillus* species are involved in tooth decay and periodontal diseases [9]. Root canal infections can be caused by a combination of microbes- *Enterococcus faecalis* has been frequently isolated from infected canal and persistent infections in post-endodontic treatment [10] . *Enterococcus faecalis* have the ability to penetrate into the dentinal tubules and survive in the root canals without other bacterial support [11].

Enterococcus faecalis has also been detected in 50% of cases with chronic apical periodontitis [12]. *Lactobacillus*

acidophilus [13], *Candida albicans, Candida tropicalis*[14] etc are some other microbial species that knowingly cause several oral diseases, such as dental caries, endodontic infections, periodontal diseases and oral Candidiasis.

Several antibiotics, such as chlorhexidine, ampicillin and antiseptics have been very effective in preventing dental caries [15]. However, various adverse effects such as- increasing of calculus formation, tooth and restoration staining, disarrangements of the oral, intestinal flora and diarrhea has been associated with the use of these drugs [16]. Viridans group *Streptococci* including *Streptococci mitis, Streptococci sanguis* and *Streptococci mutans*, the most representative human cariogenic microbe are moderately resistant to antibiotics [17]. These drawbacks justify further research for natural antimicrobial agents that are safe for the host or specific for oral micro flora. The natural phytochemicals could offer an effective alternative to antibiotics and drugs, hence represent a promising approach in prevention and therapeutic strategies for endodontic infections, dental caries and other oral infections. Resistance development against antibiotics, antiseptics and drugs is also a growing cause which has limited the preventive measures. Therefore there is a continuing need to search for new antimicrobial agents [18]. The use of medicinal plant products has been one of the most useful and successful strategies for the discovery of new antimicrobial agents [19]. Medicinal plants have been used for several of years in folk medicines and they are believed to be the new source of drugs [20].

In this work twenty medicinal plants of different families were selected to assess for their antimicrobial and phytochemical activities. Some of the plants are well known for their uses as traditional medicines.

Material and Methods

Collection and Identification of Medicinal plants

The twenty medicinal plants listed in table 1 were collected from Himachal Pradesh and Uttarakhand, India. The plant materials were authenticated by botanist Dr. A. S. Sandhu, National institute of pharmaceutical education and research (NIPER), Mohali, India.

Table 1 List of Medicinal Plants Used in the Study

Sr. No.	Botanical name of Medicinal Plants	Common name	Family	Part Used	Herbarium/ Museum No.
1.	*Allium sativum*	Garlic	Liliaceae	Bulb	NIP-NPM-CD-160
2.	*Aloe barbadensis*	Aloe vera	Liliaceae	Leaves	NIP-H-206
3.	*Azadirachta indica*	Neem	Meliaceae	Leave	NIP-H-207
4.	*Calendulla officinalis*	Pot marigold	Asteraceae	Flower	NIP-H-208
5.	*Centella asiatica*	Brahmi	Mackinlayaceae	Leave	NIP-H-209
6.	*Cinnamum zeylanicum*	Dalchini	Lauraceae	Bark	NIP-H-210
7.	*Citrus limon*	Lemon	Rutaceae	Fruit peel	NIP-H-211
8.	*Citrus sinesis*	Orange	Rutaceae	Fruit peel	NIP-H-212
9.	*Curcuma longa*	Turmeric, haldi	Zingiberaceae	Root	NIP-NPM-CD-161
10.	*Emblica officinalis*	Amla	Phyllanthaceae	Fruit	NIP-H-213
11.	*Glycyrrhiza glabra*	Mulethi	Leguminosae	Root	NIP-NPM-CD-162
12.	*Juglans regia*	Walnut	Juglandaceae	Bark	NIP-H-214
13.	*Lannea coromandelica*	Jhingangummi	Anacardiaceae	Twig	NIP-H-215
14.	*Ocimum sanctum*	Tulsi	Lamiaceae	Leave	NIP-H-218
15.	*Piper nigrum*	Black pepper	Piperaceae	Dried Berries	NIP-NPM-CD-163
16.	*Psidium guajava*	Guava	Myrtaceae	Twig	NIP-H-219
17.	*Rosa centifolia*	Red Rose	Rosaceae	Flower	NIP-H-220
18.	*Syzygium aromaticum*	Laung, clove	Myrtaceae	Flower buds	NIP-NPM-CD-164
19.	*Tinospora coridfolia*	Giloy	Meninspermaceae	Stem	NIP-H-221
20.	*Zingiber officinale*	Ginger	Zingiberaceae	Rhizome	NIP-NPM-CD-166

Preparation of extracts

Dried powdered parts of plants material (100gm) of table no. 1, were extracted using ethanol (500ml) separately by soaking it for 48hrs at room temperature. The solvents were removed under reduced pressure to obtain crude extracts of ethanol.

Qualitative Analysis of Phytochemicals

The extracts prepared for the study were subjected to phytochemical screening by using different reagents for identifying the presence of various phytoconstituents viz., reducing sugar, steroid, flavonoids, alkaloids, tannins and terpenoids in various extracts of medicinal plants. The phytoconstituents were tested as per the standard method [21, 22].

Test for Alkaloids

0.5 g of the extract was diluted to 10 mL with acid alcohol, boiled and filtered. 2 mL of dilute ammonia was added to 5 mL of the filtrate, followed by the addition of 5 mL of chloroform. The mixture was shaken gently to

extract the alkaloidal base, and the chloroform layer was extracted with 10 mL of acetic acid. The chloroform layer was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for Cardiac Glycosides

0.5 g of extract was diluted to 5 mL in water, and 2 mL of glacial acetic acid containing one drop of ferric chloride solution was added to it. 1 mLof concentrated sulphuric acid was added to form a layer, and the colour at the interphase was recorded. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for Terpenoids

2 mL of chloroform was added to 0.5 g of the extract. Concentrated H_2SO_4 (3 mL) was carefully added to form a layer, and the solution was observed for a reddish brown coloration at the interface, which indicated the presence of terpenoids.

Test for Steroids

Extracts were separately evaporated on water bath and residue was formed. A few mg of residue was taken in 2ml of chloroform. To this 2ml of concentratedH_2SO_4 was added by the side of the testy tube. The test tube was shaken for few minutes. A red color developed in the chloroform layer and lower layer of acid gave greenish yellow fluorescence. This colorization and fluorescence is due to presence of steroids.

Test for Flavonoids

Three methods were used to test for flavonoids. (i) Dilute ammonia (5 mL) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 mL) was then added. A yellow coloration that disappeared on standing indicated the presence of flavonoids. (ii) A few drops of 1% aluminum solution were added to a portion of the filtrate. A yellow coloration indicated the presence of flavonoids. (iii) A portion of the extract was heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered, and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow coloration indicated the presence of flavonoids.

Test for Tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added, and the solution was observed for brownish green or a blue-black coloration.

Test for Reducing Sugars

The ethanol extract (0.5 g in 5 mL of water) was added to boiling Fehling's solution (A and B) in a test tube. The solution was observed for a colour reaction (a purple ring at the junction of two liquids).

Test for Saponins

5 mL of distilled water was added to 0.5 g of extract in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The froth was mixed with three drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion.

Preparation and Standardization of Microbial Inoculum

All the microbial strains used in the antibacterial and antifungal bioassay were procured from Institute of Microbial Technology (IMTECH), Chandigarh, India *Enterococcus faecalis* (MTCC 439), *Streptococcus mutans* (MTCC 890),*Lactobacillus acidophilus* (MTCC 10307), *Candida tropicalis* (MTCC 184) and *Candida albicans* (MTCC 854).The microorganisms were sub cultured on the culture media recommended for different microorganisms such as Lactobacillus MRS agar (*Lactobacillus acidophilus*), Brain heart infusion agar (*Streptococcus mutans* and *Enterococcus faecalis*), Sabouraud's Dextrose Agar (*Candida tropicalis* and *Candida albicans*) and incubated at 37°C. Turbidity produced was adjusted to match 0.5 McFarland standard (10^8 cfu/ml) which was further adjusted 10 cfu/ml [23].

Antimicrobial activity

The antibacterial and antifungal activity of different medicinal plant extracts were evaluated by using the agar well diffusion technique. The 20 ml of sterilized agar's (Lactobacillus MRS Agar, Brain Heart Infusion Agar, Sabouraud's dextrose agar) were poured into sterile petriplate, after solidification, 100 µl of microbial inoculums were swabbed on the respective plates. The wells were punched over the agar plates using sterile gel puncher. The punched agars were filled with 100µl of plant extracts. 2% Chlorhexidine was taken as standard reference. The plates were incubated at 37ºC for 24 hours. After incubation, zone of inhibition for extracts were measured in millimeters using veneer calipers.

Statistical Analysis

The results of inhibitory zones were subjected to statistical analysis. All the experiments were performed in triplicates. The values of growth inhibitory zones

expressed in mean ± SD (standard deviation) of three triplicates.

Results & Discussion

The ethanol extracts of twenty medicinal plants were tested against the pathogenic oral microbes' viz., *Streptococcus mutans* a most common bacteria, which virulent strains can cause dental plaque and caries; *Enterococcus faecalis* associated with various periradicular diseases including- primary endodontic infections, persistent infections and asymptomatic chronic periradicular, *Lactobacillus acidophilus, Candida albicans* and *Candida tropicalis* etc are some other pathogenic microbial species that knowingly cause several oral diseases, such as endodontic infections, dental caries, periodontal diseases and oral thrush.

Phytochemical constituents such as- alkaloids, tannins, phenols flavonoids, reducing sugar, saponins, and several other compounds are secondary metabolites of plants that serve as a defense mechanism against many microorganisms [24]. In the present study, we studied twenty medicinal plant extracts revealed the presence of medicinally active constituents. The phytochemical activity and antimicrobial constituents' activity of the selected plants investigated are summarized in Table 2 and 3.

Table 2 Phytochemical activity of ethanolic extract medicinal plants

Sr. No	Ethanolic extract of Medicinal Plants	Alkaloids	Glycosides	Terpenoids	Steroids	Flavonoids	Tannins	Reducing Sugars	Saponin
1.	*Allium sativum*	+	+	+	+	+	-	+	-
2.	*Aloe barbadensis*	+	-	+	+	+	+	+	-
3.	*Azadirachta indica*	+	+	+	+	+	-	+	+
4.	*Calendulla officinalis*	+	+	+	+	+	-	-	+
5.	*Centella asiatica*	+	+	+	+	+	+	+	-
6.	*Cinnamum zeylanicum*	+	+	+	+	+	+	+	+
7.	*Citrus limon*	+	-	+	+	+	+	+	-
8.	*Citrus sinesis*	+	-	+	+	+	+	+	+
9.	*Curcuma longa*	+	-	-	+	-	+	+	-
10.	*Emblica officinalis*	+	+	-	-	+	+	+	+
11.	*Glycyrrhiza glabra*	-	-	-	+	+	-	-	+
12.	*Juglans regia*	+	+	+	-	+	+	-	+
13.	*Lannea coromandelica*	–	–	+	–	+	+	-	-
14.	*Ocimum sanctum*	+	+	+	+	+	+	+	+
15.	*Piper nigrum*	+	+	+	+	+	+	-	+
16	*Psidium guajava*	+	+	+	+	-	+	+	+
17.	*Rosa centifolia*	+	+	+	-	+	+	+	+
18.	*Syzygium aromaticum*	+	+	-	+	+	+	+	+
19.	*Tinospora coridfolia*	+	+	+	+	+	+	+	+
20.	*Zingiber officinale*	+	+	+	-	+	+	+	+

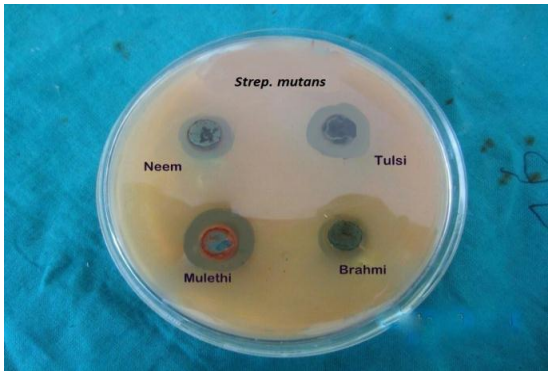
The most important of these bioactive include - alkaloids, steroids saponins, tannins, terpenoids and flavonoids etc [25] . Phytochemical screening of ethanolic extract of twenty medicinal plants studied showed that most of the ethanolic extracts have the tannins, steroids, saponins and flavonoids. The most of phytochemicals were found in *Cinnamum zeylanicum, Ocimum sanctum* and *Tinospora coridfolia.* The minimum numbers of secondary metabolites were observed in *Glycyrrhiza glabra* and *Curcuma longa.* The saponins are used as intracellular histochemical dyes to allow antibody access to intracellular proteins and as mild detergents. In medicine, it is used as antioxidant, anti- cancer, anti inflammatory agents, hypercholesterolemia, hyperglycemia, central nervous system activities [26] and for weight loss. It is also known for its antifungal properties [27]. In this study, the plants having saponins are *Azadirachta indica, Piper nigrum, Syzygium aromaticum, Cinnamum zeylanicum, Glycyrrhiza glabra, Ocimum sanctum, Tinospora coridfolia* and *Zingiber officinale.* Plant steroids are well known for their insecticidal, anti- microbial and cardiotonic activities. They are also used in herbal medicine, nutrition, and cosmetics [28]. In the present study, steroids are present in all extracts except *Zingiber officinale.* Human physiological activities such as:- host-mediated tumor activity, stimulation of phagocytic cells and a wide range of anti-microbial actions have been assigned to tannins. One of their biochemical actions is too complex with proteins such as hydrogen bonding and hydrophobic effects as well as by covalent bond formation [29]. Thus, their mode of action may be related to their ability to inactivate several enzymes, microbial adhesions and cell

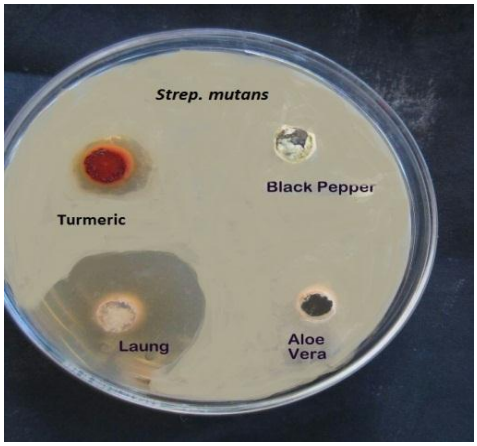
envelope transport proteins [30]. That's why these medicinal plants contain, tannins showed good antibacterial activity. In this work, except *Curcuma longa* and *Psidium guajava* flavonoids were present in all of the medicinal plants. Any studies have reported that, flavonoids being phenolic compounds are free radical scavengers and water soluble antioxidants, which are capable of preventing oxidative cell damage and shows strong anticancer activity [31]. Alkaloids have been studied for many biological activities including - Antidiabetic, Cytotoxic, Antiprotozoal [32] and anti-inflammatory properties. In the present work all ethanolic extract of twenty medicinal plants have alkaloid except *Glycyrrhiza glabra*.

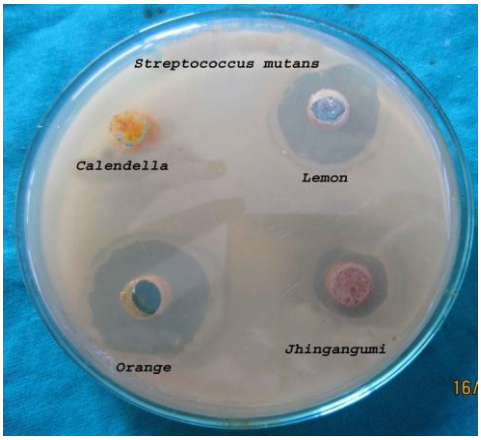
Antibacterial and antifungal activities of the twenty medicinal plants extracts investigated are summarized in Table 3 and figure 1.1, 1.2, 1.3, 1.4, 1.5. Twenty medicinal plants tested for antimicrobial activity, all ethanolic extracts showed antimicrobial activity by inhibiting one or more test species. The zone of inhibition by the test oral species against twenty medicinal plants extract shows that ethanolic extracts of *Calendulla officinalis* and *Cinnamum zeylanicum* were not effective against *Enterococcus faecalis, Streptococcus mutans* and *Candida tropicalis* respectively. However, *Azadirachta indica, Centella asiatica, Lannea coromandelica, Rosa centifolia* and *Zingiber officinale* were showing week. However, the ethanolic extracts of *Allium sativum, Citrus limon, Citrus sinesis, Curcuma longa, Emblica officinalis , Glycyrrhiza glabra, Juglans regia, Ocimum sanctum, Piper nigrum* and *Psidium guajava* were more potent plants than other plants screened for antimicrobial activity against standard strains of the microorganisms. *S. aromaticum* was the most potent plant than the other medicinal plants screened against clinically isolated bacterial strains, for *Streptococcus mutans, Candida albicans* and *Candida tropicalis* zone of inhibition of *S. aromaticum* was higher than Chlorhexidine. This study gives an indication of the efficacy of the plants obtained from the traditional healers.

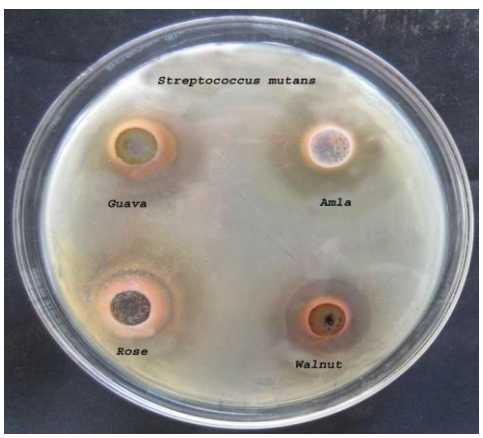
Table 3 Antimicrobial activity of Medicinal Plants expressed in Mean ± SD (standard deviation)

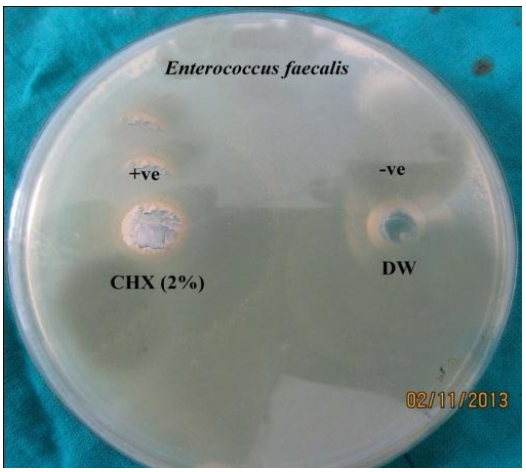
Sr no.	Medicinal Plants	*S. mutans*	*E. faecalis*	*L. acidophilus*	*C. albicans*	*C.tropicalis*
		Zone of Inhibition in Millimeters				
1.	Chlorhexidine (+ ve control)	30.3 ± 2.0	30 ± 3	25 ± 1	20 ± 2.4	19 ± 1
2.	Distil water (-ve control)	-	-	-	-	-
3.	*Allium sativum*	25 ± 1.1	27 ± 2	18 ± 1	18 ± 3	15 ± 2
4.	*Aloe barbadensis*	-	15 ± 1	15 ± 2	20 ± 1	15 ± 1
5.	*Azadirachta indica*	17.6 ± 2.0	17.3 ± 1.5	22.3 ± 2.08	20.3 ± 3.05	19.3 ± 0.5
6.	*Calendulla officinalis*	-	-	12.3 ± 1.5	18 ± 2	-
7.	*Centella asiatica*	15 ± 1	10 ± 2	15 ± 3	15 ± 4	14.6 ± 1.1
8.	*Cinnamum zeylanicum*	-	-	19.3 ± 2.0	25 ± 1	26.3 ± 1.5
9.	*Citrus limon*	19.3 ± 1.1	14.3 ± 1.5	30.3 ± 0.5	20.3 ± 2.5	20 ± 1
10.	*Citrus sinesis*	20 ± 2	-	28.3 ± 1.5	18.6 ± 0.5	20 ± 2
11.	*Curcuma longa*	18 ± 2	21 ± 2	17 ± 2	21 ± 2	22.3 ± 2.08
12.	*Emblica officinalis*	24.6 ± 0.5	22.6 ± 1.5	26.6 ± 1.5	18.6 ± 1.5	22.3 ± 2.08
13.	*Glycyrrhiza glabra*	20 ± 2	25 ± 3	19.3 ± 1.5	17 ± 2	18 ± 1
14.	*Juglans regia*	19.6 ± 1.5	20 ± 2.6	19 ± 3	20.6 ± 1.1	19.6 ± 2.5
15.	*Lannea coromandelica*	16.3 ± 1.5	12.3 ± 1.1	18.3 ± 1.5	15.3 ± 1.1	-
16.	*Ocimum sanctum*	20 ± 2	22 ± 2	17.6 ± 2.0	17 ± 2	16 ± 2
17.	*Piper nigrum*	-	20 ± 4	18 ± 1	25 ± 4	20 ± 2
18.	*Psidium guajava*	20.6 ± 1.5	19.6 ± 0.5	20.6 ± 1.5	18.6 ± 0.5	20 ± 1
19.	*Rosa centifolia*	15 ± 1	-	11 ± 2	16.3 ± 1.5	12 ± 3
20.	*Syzygium aromaticum*	32.3 ± 0.5	25 ± 1	21 ± 1	30.6 ± 2.08	25 ± 2
21.	*Tinospora coridfolia*	-	11 ± 2	14.3 ± 0.5	14 ± 3	12 ± 3
22.	*Zingiber officinale*	18 ± 2	20 ± 1	15.3 ± 1.1	15 ± 2	14 ± 2

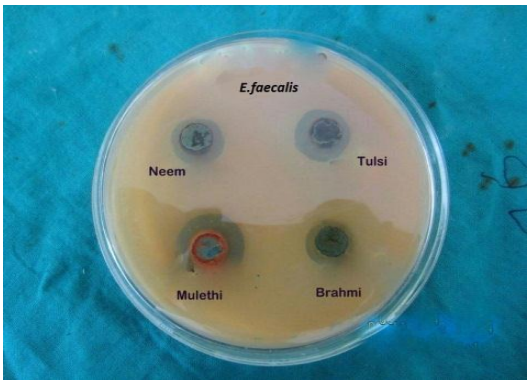


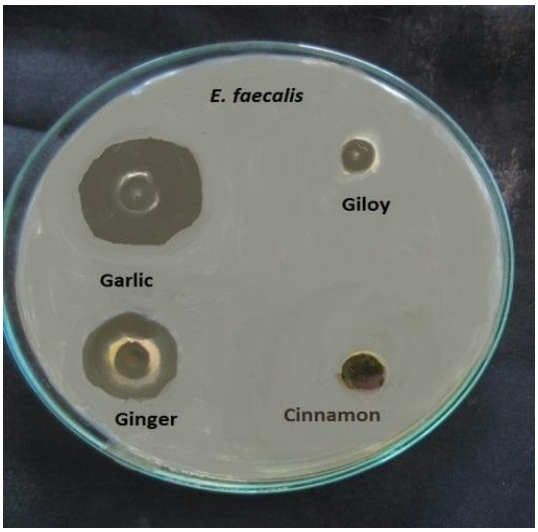












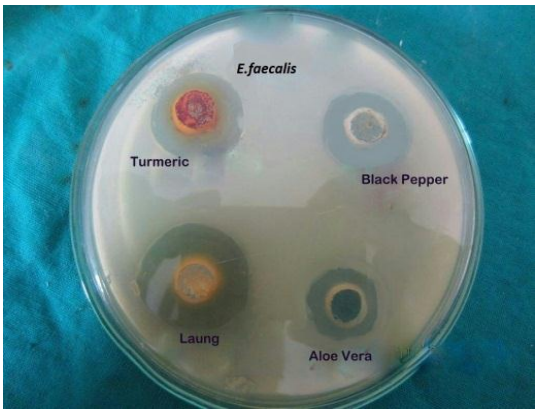
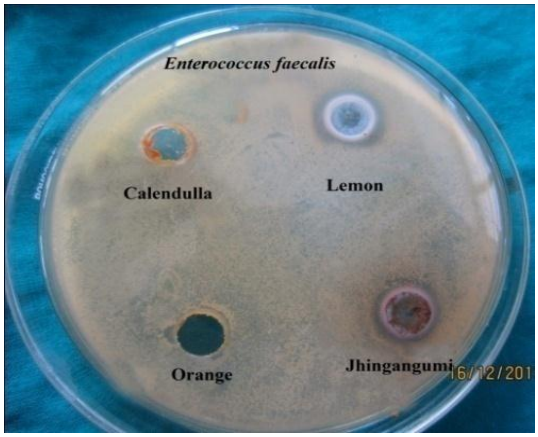


Figure 1.1 Antibacterial activities of medicinal plants against *Streptococcus mutans*



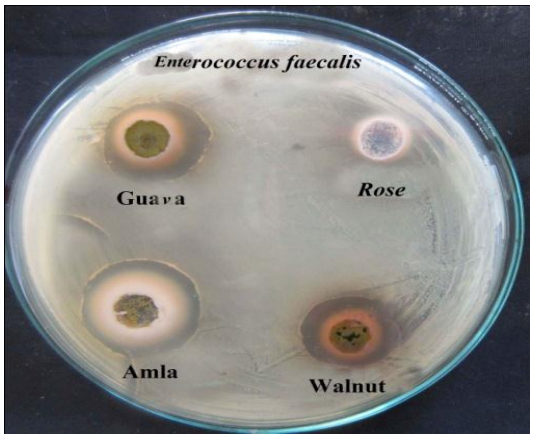
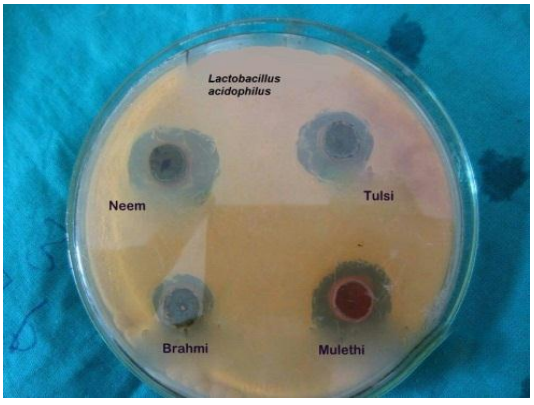
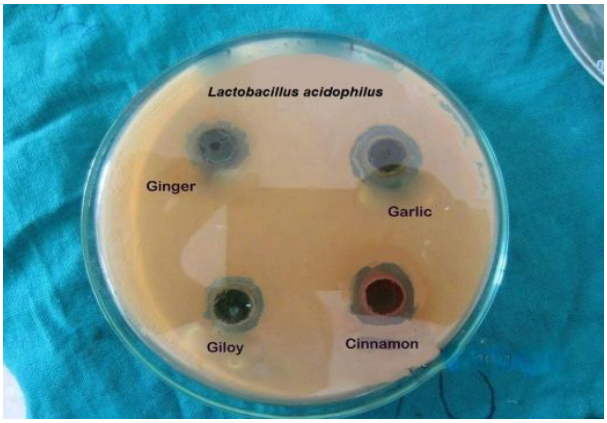
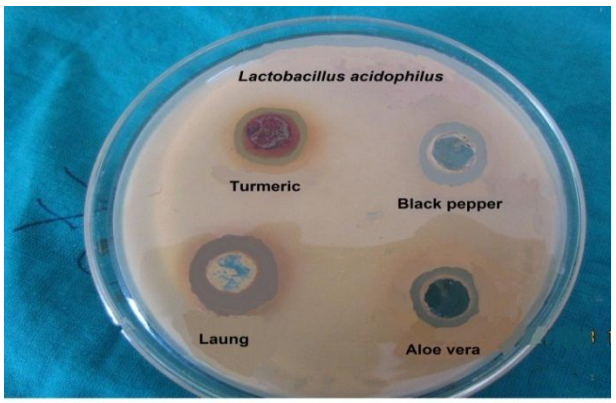
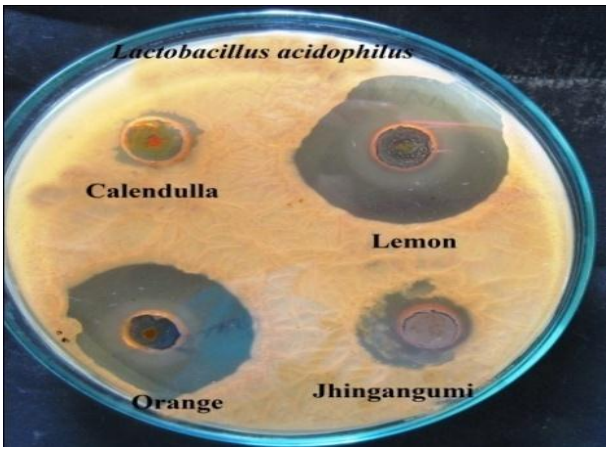


Figure 1.2 Antibacterial activity of medicinal plants against *Enterococcus faecalis*









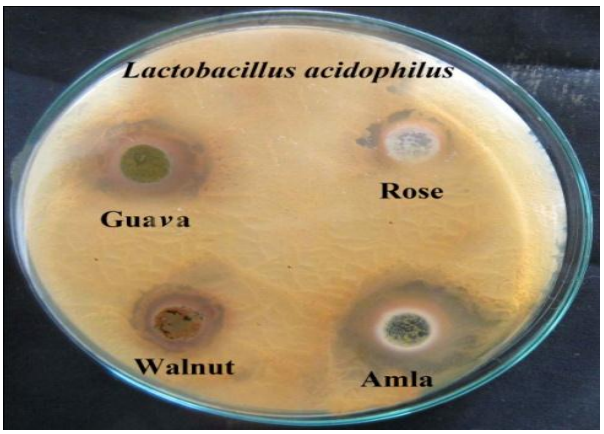
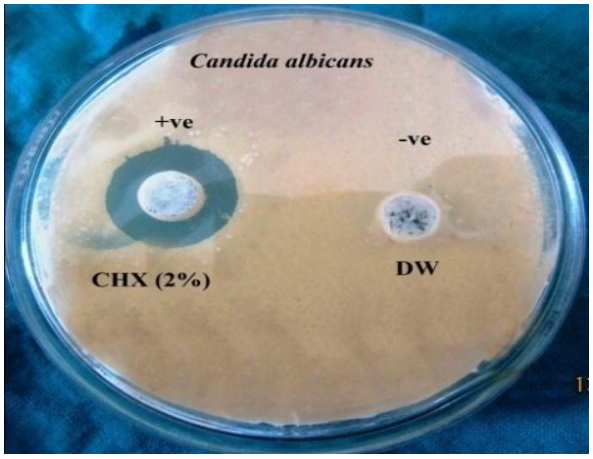
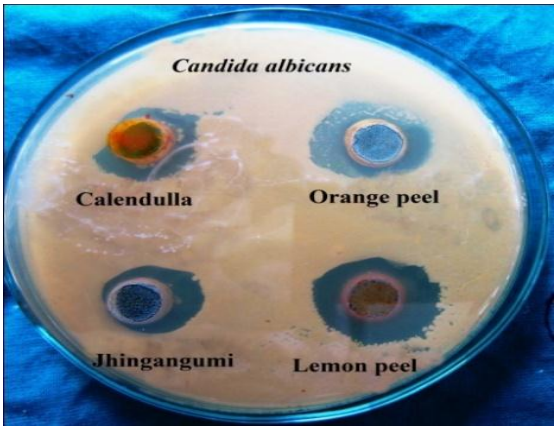
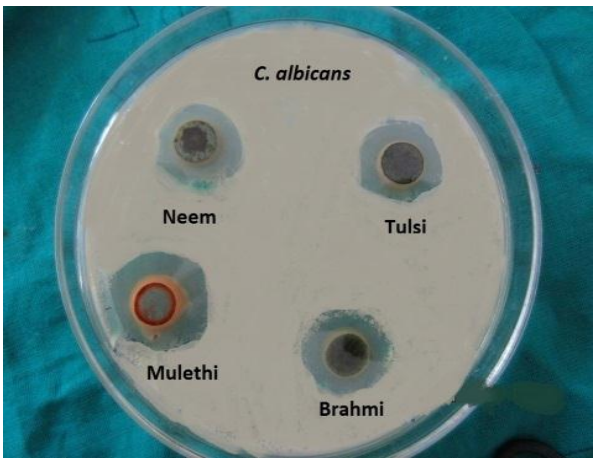


Figure 1.3 Antibacterial activities of medicinal plants against *Lactobacillus acidophilus*







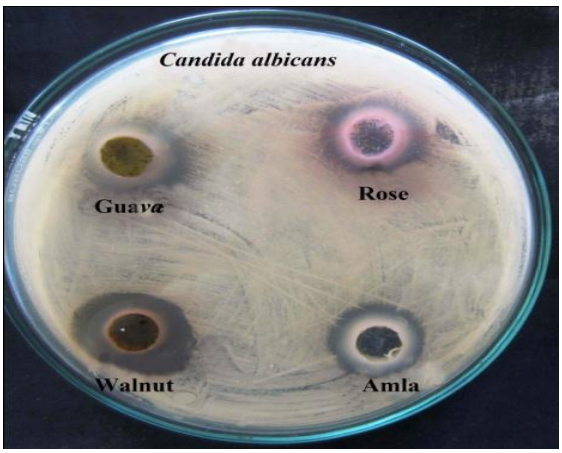
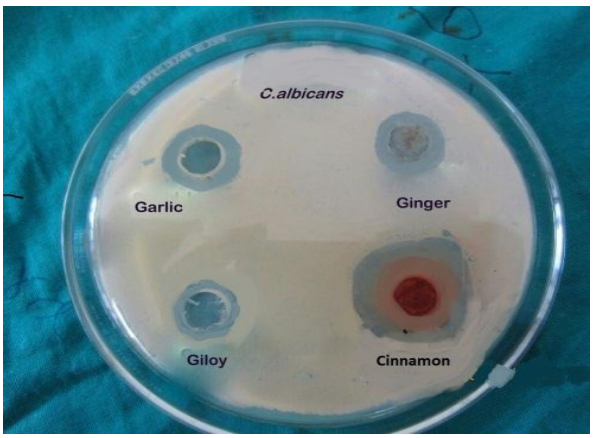
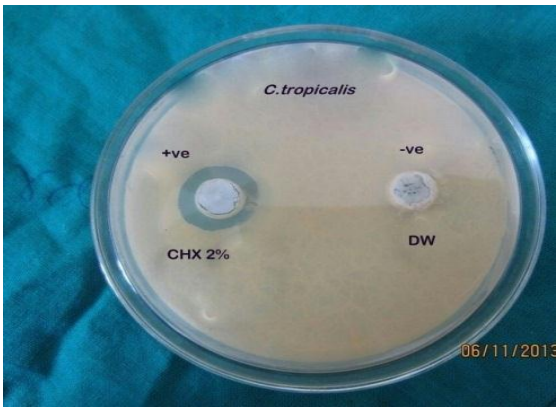
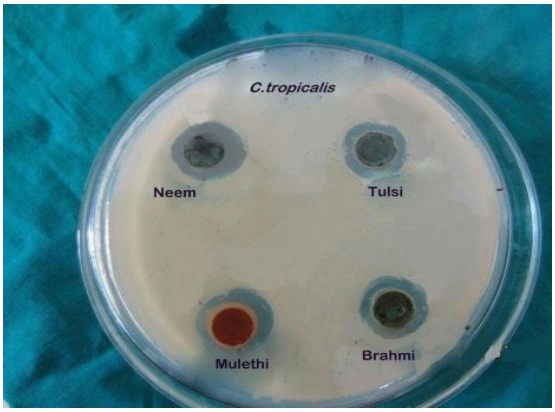


Figure 1.4 Antifungal activities of medicinal plants against *Candida albicans*







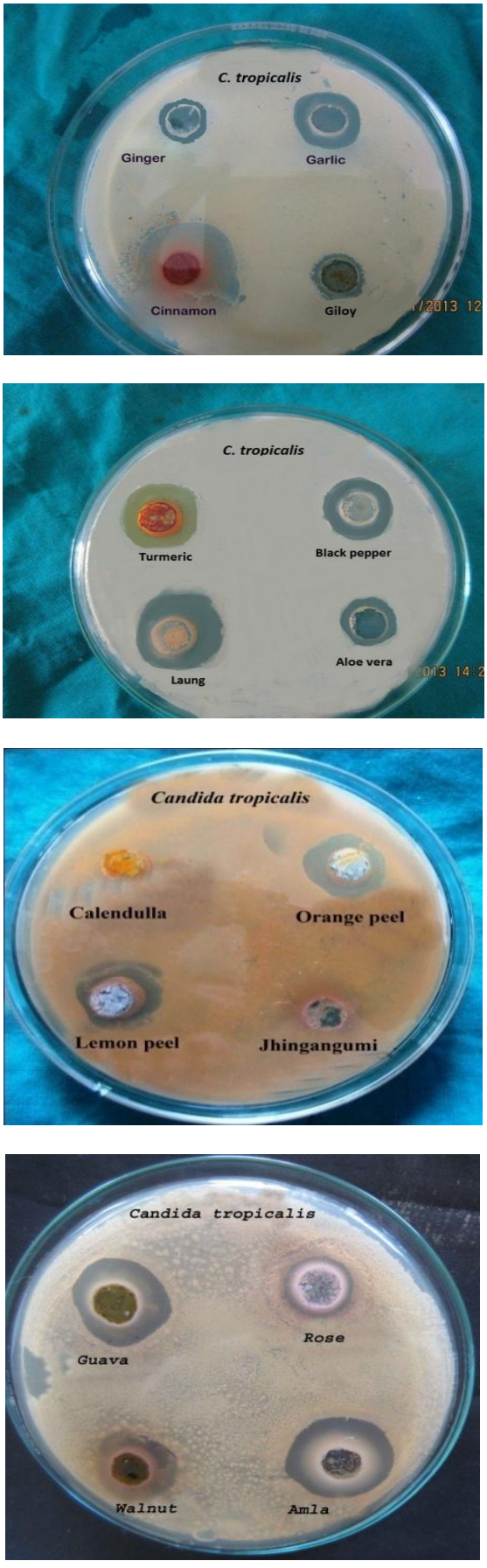


Figure 1.5 Antifungal activities of medicinal plants against *Candida tropicalis*

Conclusion

In the present work almost all twenty medicinal plants showed phytochemical, antibacterial and antifungal activity but*, Allium sativum, Citrus limon, Citrus sinesis, Curcuma longa, Emblica officinalis ,Glycyrrhiza glabra, Juglans regia, Ocimum sanctum, Piper nigrum* and *Psidium guajava* were displaying strong antimicrobial activity, against all the test species. However, *Lannea coromandelica* and *Rosa centifolia* were showing week against most of the investigated oral test microbes. The minimum numbers of phytochemicals were observed in *Curcuma longa* and *Glycyrrhiza glabra.* The maximum numbesrs of phytochemicals were observed in *Ocimum sanctum, Cinnamum zeylanicum* and *Tinospora coridfolia.* The ethanol extracts of *Syzygium aromaticum* showing strongest antimicrobial activity against all test species. This study gives an indication of the efficacy of the medicinal plants obtained from the traditional healers. The effects of extract may benefit if incorporated in tooth paste, mouth rinses and dental products to reduce plaque and dental caries. Further studies are required to better evaluate the effect of these extracts if used as endodontic irrigants. In vivo clinical testing is essential to confirm in vitro results. More research into medicinal plants in dentistry can give us simple, effective solutions to dental diseases

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References

[1]. J. Srivastava, Lambert and V. Vietmeyer, (2006), Medicinal Plants: An expanding role in development. World Bank Technical Paper. No. 320.

[2]. V. D.Weijden, C.J. Timmer, M.F. Timmerman, E. Reijerse, M.S. Mantel and U. Velder Velden, (1998), The effect of herbal extracts in an experimental mouth rinse on established plague and gingivitis, *Journal of Clinical Periodontal.,* v. 25, pp. 399-403.

[3]. B.H Mullally, J.A. James, W.A. Coulter and G.J. Linden, (1995), The efficacy of herbal based tooth paste and control of plague and gingivitis, *Journal of Clinical Periodontal*, v. 22, pp. 686-689.

[4]. C.D. Wu, I.A. Darout, N Skaug, (2001), Chewing sticks-Timeless natural toothbrush for oral cleansing, *Journal of Periodontal Research* , v. 36, pp. 275–284.

[5]. E.S. Akpata , E.O. Akinrimisi ,(1977), Antibacterial activity of extracts from some African chewing sticks" , *Oral Surgery*, *Oral Medicine*, *Oral Pathology* , v. 44, pp. 717–722.

[6]. S. Williams and K. Williams, (1994), Clinical practice of dental hygienist 7th Ed., Esther in W. Willikins, London.

[7]. P. Nigam & A B Srivastava, (1990), Betel chewing and dental decay, *Federation of operative dentistry*. 1990, v.1, No.1, pp. 36- 44.

[8]. W. Loesche, (1986), Role of *Strepococcus mutans* in human dental decay, *Microbiology Review*, v. 50, pp. 353-380.

[9]. P. Sachipach, V. Osterkwalder & B. Guggenheim, (1995), Human root caries microbiota in plaque covering sound carious and arrested carious root surfaces, *Caries Research*, v. 29, pp. 382-395.

[10]. M. Sakamoto, J.F. Siqueira, I. N. Rôças, Y. Benno, (2008), Molecular analysis of the root canal microbiota associated with endodontic treatment failures, *Oral Microbiology* and *Immunology,* v. 23, pp. 275-81

[11]. R.M. Love, (2001), *Enterococcus faecalis* – a mechanism for its role in endodontic failure, *International Endodontic Journal*. , v. 34, pp. 341-5

[12]. J.F. Siqueira, I.N. Rocas, (2005), Exploiting molecular methods to explore endodontic infection: *Endodontic microbiota*, v.31 , pp. 488-98.

[13]. P.J. Hollowa, W.J. Moore (1983), The role of sugar in the etiology of dental caries, *Journal of dental*, v. 11,No..3, pp. 189–213.

[14]. Akpan, A. Morga,(1991), Oral candidiasis-Review, *Postgraduate Medical Journal,* v. 78, pp. 455–459.

[15]. V.W.K Tsui, R.W.K Wong, A.B.M. Rabie, (2008), The inhibitory effects of narigin on the growth of periodontal pathogens in vitro, *Phytotherapy,* v. 22, pp. 401-406.

[16]. G. More, T.E. Tshikalange, N. Lall, F. Botha, J.J.M. Meyer, (2008), Antimicrobial activity of medicinal plants against oral microorganisms, *Journal of Ethnopharmacology* , v. 119, No. 3, pp. 473–477.

[17]. M. Venditti , P. Baiocchi , C .Santini , C. Brandimarte , P. Serra , G. Gentile, (1989), Antimicrobial susceptibilities of *streptococcus* species that cause septicemia in neutropenic patients, *Antimicrobial Agents Chemotherapy*, v. 33, pp. 580-2.

[18]. L. Cai , C.D. Wu, (1996), Compounds from Syzygium aromaticum possessing growth inhibitory activity against oral pathogens, *Journal of natural products*, v.59, pp. 987–90.

[19]. A. Harvey, (2000), Strategies for discovering drugs from previously unexplored natural products*, Drug Discovery Today*, v.5, pp. 294- 300.

[20]. Wings TY Loo, (2010), Evaluation of Ellagic acid on the activities of oral bacteria with the use of adenosine triphosphate (ATP) bioluminescence assay, *African Journal of Biotechnology,* v.9, pp.3938-3943.

[21]. Kokate CK, Khandelwal KR, Pawar AP, Gokhale SB, Practical Pharmacognosy 3rd ed., Nirali Prakashan, (1995), Pune, 137.

[22]. Trease and Evans, Text Book of Pharmacognosy 12th ed., ELBS Publication, (1989), pp. 49, 126, 132-137, 205, 248.

[23]. A. Odebiyi, A.E. Sofowora, (1979), Antimicrobial alkaloids from a Nigeria chewing stick *Fagara zanthozyboides", Plantamedica*, v. 40, pp. 204- 207

[24]. G.H.S. Bonjar, A.K. Nik, S .Aghighi, (2004), Antibacterial and antifungal survey in plants used in indigenous herbal medicine of south east regions of Iran, *Journal of Biological Science*, v.4, pp. 405-412.

[25]. Anpin Raja, S. Jeeva, J.W. Prakash, M. Johnson and V. Irudayaraj, (2011), Antibacterial activity of selected ethnomedicinal plants from South India, Asian Pacific Journal of Tropical Medicine, , v. 4, pp. 375- 378.

[26]. Malairajan, P., G. Geetha, S. Narasimhan and K. JessiKalaVeni, (2006), Analgesic activity of some Indian medicinal plants. *Journal of Ethnopharmacology,* v. 19, pp. 425-428.

[27]. A. Argal and A.K. Pathak, (2006), CNS activity of *Calotropis gigantea* root, *Journal of Ethnopharmacology,* v.106, pp. 142-145

[28]. R.K. Callow, (1936), The Steroids, Proc Royal Soc,London Series A. pp.157:194.

[29]. D. E. Okwu, (2004), Phytochemicals and vitamin C content of indigenous spices of Southeastern Nigeria, *Journal of Sustainable Agriculture Environment*, v. 6, pp.30-37.

[30]. L. Alan, N.D. Miller, (1996), Antioxidant flavonoids: Structure, function and clinical Usage, *Alternative Medicine Review*, v.1, pp. 103-111.

[31]. B.P. Kaufman, J.L. Cseke, S. Waber, (1999), Natural products from Plants, 1stEdition, CRC press LLC, USA. p.p, 22, 25, 19, 17,172, 242 and 200.

[32]. A. J. Akindele and O.O. Adeyemi, (2007), Anti inflammatory activity of the aqueous leaf extracts of *Byrsocarpus coccineus,* Fitoterapia, v. 78, pp.25-28.