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ORIGINAL RESEARCH

Phytochemical analysis and antimicrobial activity of herbal plants against oral microorganisms

Pratibha Yusuf, Priyangana Bora, Shyamalima Baruah, Sonia Angeline M*

Department of Life Sciences,
Kristu Jayanti College, Autonomous, Bengaluru, Karnataka- 560077, India.

Abstract

With about 700 different kinds of bacteria, the mouth cavity has the second most diverse and massive microbiota in the body, behind the gut. It supports a wide variety of microorganisms, including viruses, bacteria, fungus, and protozoa. Certain kinds of oral microbes are implicated in the development of numerous infections in other areas of the body as well as the pathophysiology of infectious disorders of the mouth, jaw, and face. Herbal plants show long lasting antibacterial and antifungal activity against human pathogens, which are essential in the treatment of dental disorders. Herbal mouthwashes are in high demand, because they have less side effects and do not disturb the normal microflora. This study includes testing the efficacy of antibacterial and phytochemical properties of herbal extracts by using the extracts of leaves of plants namely Cymbopogon citratus (Lemongrass), Bryophylum pinnatum (cathedral bells), and seeds of Croton tiglium (Jayapala) that acts against the oral pathogens. Further the anti-microbial activity of the leaf and seed extracts was analysed using agar well diffusion method. The organisms used for analysis are Staphylococcus aureus and Bacillus subtilis which are gram positive bacteria. These herbal extracts are found sensitive to the oral microorganism.

Keywords

Herbal extracts, oral microorganism, Cymbopogon citratus, Bryophyllum pinnatum, Croton tiglium, Antibacterial

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Author for Correspondence
sonia.m@kristujayanti.com

1. Introduction:

Dental issues like tooth decay, gum disease, and tooth loss can significantly affect a person's overall health. Some oral microbes play a crucial role in pathogenesis, becoming infectious initially *(Naiktari, et al., 2014).* Antiseptic solutions, used as mouthwashes, are one of the most effective ways to reduce the number of microorganisms in the mouth (*Bagchi et al., 2015).* They can be used on their own, before surgery and, in certain situations, after surgery and during the wound-healing phase. They are frequently used in conjunction with other hygiene instructions. Mouthwashes are utilised extensively in various sectors of dentistry and a wide range are now available (*Nazreen and Gayathri, 2016).* Mouthwashes contain chemicals that can fight against organisms found in saliva and on the mouth's surface (*Manipal et al., 2016).* It appears that deaths from overconsumption or adverse effects of alcohol-containing mouthwashes are uncommon; nonetheless, their use by youngsters frequently results in hazardous side effects and, on occasion, non-lethal but poisonous reactions *(Minhas, 2019).* Previous research has shown that the economic burden of oral disorders is a significant factor, accounting for up to 10% of public health expenditure in affluent nations for curative dental care *(Irani, 2017).* Herbs are thought to be more effective than chemicals *(Assous et al., 2013).* Herbal plants exhibit long-term antibacterial and antifungal activity against human infections, which is critical in the treatment of dental problems *(Nam – Hui et al., 2013).* Herbal mouthwashes are in high demand, because they have less side effects and do not disturb the normal microflora *(Ganjewala and Luthra, 2009).* This study examines the efficacy of antimicrobial and phytochemical analyses of herbal plants using leaf extracts from three different plants: *Cymbopogon citratus, Bryophyllum pinnatum, and Croton tiglium,*

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which act against the oral pathogens *Staphylococcus aureus and Bacillus subtilus.* Furthermore, the antimicrobial activity of the leaf extracts was determined using the Agar well diffusion method *(Aldhaher and Zainab, 2013).* Oral health issues, including dental caries and periodontal disease, are serious health concerns around the world. Oral health has a global impact on many people's quality of life as well as their poor health, which is linked to a variety of disorders. Oral disorders and microbiome have a strong correlation *(Nisha et al., 2022).* In this study, the herbal plants *Cymbopogon citatus, Bryophyllum pinnatum*, and *Croton tiglium* were chosen, and the leaf extract of *Cymbopogon citatus, Bryophyllum pinnatum* (*Okwu DE and Nnamdi, 2011)*, and seed extract of *Croton tiglium* was prepared for analysis. The *Croton tiglium* seed extract was used in the herbal extracts to check its efficacy. In addition to fatty acids, *C. tigiium* seeds oil is said to include crotonic acid and phorbol esters *(Hu et al., 2010).* A special detoxification method called 'Sodhana' is used to both purify and detoxify various medications used in Ayurvedic medicine, as well as to increase the therapeutic benefits of these substances and lessen their toxic contents and effects. The toxicity of *C. tiglium* seeds is reduced by a decrease in the concentration of the toxic components following purification. Hence TLC was performed to compare the unpurified and purified forms of *C. tiglium* seeds. These extracts were investigated to determine whether they inhibited the growth of bacteria such as *Staphylococcus aureus* and *Bacillus subtiiis (Taye et al., 2011).* The sample extract of leaf and seed was chosen to identify the efficacy of these herbal extracts and to study if they can be prepared into a formulation for mouthwashes. The agar well diffusion method was used to confirm the extracts' antibacterial efficacy against *Staphylococcus aureus* and *Bacillus subtilus.* The presence of alkaloids, flavonoids, sugars, phenols, and glycosides were identified by phytochemical study.

2. Materials and Methods:

2.1. Collection of the Leaf Samples

The leaf samples of *Cymbopogon citratus* (Lemongrass), *Bryophyllum pinnatum* (Patharchatta plant) and seeds of *Croton tiglium* (Jamalgota) were purchased from local market, Bangalore.

2.2. Preparation of Leaf Extracts

The leaves were washed with double distilled water and then dried in a hot air oven. The *Cymbopogon citratus* was cleaned with double distilled water before being cut into small pieces and dried in a hot air oven at 60°C for 3 to 4 days *(Ademuyiwa and Grace, 2015)*, while the *Bryophyllum pinnatum* leaves were dried in a hot air oven at 50°C for two days. The dried leaves were ground to a fine powder under sterilised conditions. The leaf extracts were preserved in containers for future use. In the case of *Croton tiglium*, seeds were steeped in water overnight, then the outer cover (testa) and cotyledon were removed using a knife. The resultant endosperm was milled into a fine powder. To produce a pottali, the coarse powder was wrapped in cotton fabric. An iron pot with cow's milk inside of it was used to hang pottali so that it was completely submerged but not touching the bottom of the pot. To facilitate the sodhana process (detoxification technique), a hot plate was used to continuously heat the vessel at 120°C for 3 hours *(Pal et al., 2014)*. New milk was added to the vessel at regular intervals during the sodhana process in order to maintain a steady milk level. The pottali was taken out of the milk after three hours, cleaned three times in hot water, and then dried for two hours on aluminium foil in a hot air oven that was preheated to 60°C. The entire process was repeated three times, according to ayurvedic traditions. *(Sastri, 2010)*. The leaf and seed extracts were prepared by dissolving 0.1 g of extract with 1 mL of ethanol as solvent.

2.3 Antibacterial Activity

Agar well diffusion method was used for antibacterial activity. Different concentrations of ethanolic extract of leaves and seed (5, 10, 15, 20 µg/ ml) were prepared and 20µL of each dilution was impregnated into the well on the nutrient agar plates. Antibiotic penicillin discs were used as positive control and ethanol served as negative control. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone around the wells and disc. Anti-microbial activity was expressed as the mean zone of inhibition diameters (mm) produced by the leaf and seed extracts *(Suharitha et al., 2021)*. The inhibitory zone was then assessed for *Staphylococcus aureus* and, *Bacillus subtilis*. The tests were performed in triplicates.

2.4. Phytochemical Analysis

The following methods were used for qualitative phytochemical analysis of herbal leaf extracts of *Cymbopogon citratus*, *Bryophyllum pinnatum* and seed extract of *Croton tiglium (Senguttuvan et al., 2014).*

2.4.1. Test for Carbohydrates

The herbal extracts about 2ml were combined with 1ml of Molisch's reagent, and then a few drops of concentrated sulfuric acid were added. The presence of carbohydrates was indicated by a purple coloration.

2.4.2. Test for Flavonoids

Ferric chloride test: Distilled water is added to a little amount of the extracts. The extract solution was mixed with a drop of ferric chloride, and the result was seen.

NaOH test: After dissolving a small amount of the extract in a 10% aqueous NaOH solution, diluted HCL was added, and the mixture was observed.

2.4.3. Test for Alkaloids

On the water bath, 0.1g of the extract was mixed with 10ml of 1% aqueous hydrochloric acid before being filtered. 3ml of the filtrate was divided into three.

A few drops of freshly made Dragendoff's reagent were added to the first 1ml filtrate. Next 1ml of Meyer's reagent was added to the second filtrate. Following which 1ml of Wagner's reagent was added to the third filtrate and was observed.

2.4.4. Test for Phenols

The test was performed with 2ml of ferric chloride solution, added to 2ml of the plant extract. Appearance of bluish-black colour indicated the presence of phenols.

2.4.5. Test for Glycosides

When 0.5 mL of crude extract was mixed with 1 mL of distilled water and NaOH, a yellowish colour was formed, signifying the presence of glycosides *(Angeline et al., 2021).*

2.5. Thin Layer-Chromatography

TLC strips coated with silica gel were used for thin-layer chromatography (TLC) as the solid phase. A 9:1 solvent mixture of methanol and chloroform serves as the mobile phase. A start point situated just above the bottom of the TLC strip; a small amount of a compound is placed. After that, the same is developed for 15 minutes in a developing chamber with a shallow solvent pool that is slightly lower than the level at which the sample was applied. Each component either stays with the solid phase or dissolves in the solvent and moves up the strip as the solvent is pulled up by capillary action. After drying, the TLC strips were seen in either regular daylight or ultraviolet light *(Hahn-Deinstrop, 2006).* The retention factor (Rf) is calculated using the below formula.

Retention factor (Rf) = Distance moved by a solute (compound) / Distance moved by a solvent.

3. Results & Discussion

3.1. Sample collection

Figure 1: *Leaf Samples: (A) Cymbopogon citratus; (B) Croton tiglium; (C) Bryophyllum pinnatum*

The leaf samples were collected from the local market in Bangalore. It was washed and dried for further use *(Figure 1).*

3.2. Phytochemical Analysis

The phytochemical screening of the *Cymbopogon citratus, Croton tiglium* and *Bryophyllum pinnatum* extracts was carried out using standard techniques for the plant secondary metabolites. The tests proved the presence of alkaloids in *C. citratus, C. tiglium* and was absent in *B. pinnatum*, flavonoids were present in *C. citratus, C. tiglium* and absent in *B. pinnatum*. Molisch test showed carbohydrates were present in *C. citratus, B. pinnatum* and absent in *C. tiglium* whereas Fehling's test showed the presence of carbohydrates in all the three leaf extracts. Phenols were present in *C. citratus, B. pinnatum* and absent in *C. tiglium*. Glycosides were found only in *C. tiglium.* Presence of these phytochemicals proves that the selected herbal sources have potential therapeutic role *(Table 1).*

3.3..Antibacterial Activity

The herbal leaf extracts of the plants exhibited difference of inhibition activity against the bacterial species and the zone of inhibition results were expressed in terms of the diameter (mm) *(Table 2, Table 3),* antibiotic Penicillin was used as positive control and ethanol was used as negative control. The results clearly showed the antibacterial activity of *Cymbopogon citratus, Croton tiglium* and *Bryophyllum pinnatum* against *Staphylococcus aureus (Figure 2)*, the highest zone of inhibition of 20mm was observed with 20µg/ml extract of *B. pinnatum*. The antibacterial activity against *Bacillus subtiiis* revealed the highest zone of inhibition of 16mm with 20µg/ml extract of *B. pinnatum (Figure 3)*. These results prove that *Bryophyllum pinnatum* exhibited better antimicrobial activity compared to *Cymbopogon citratus, and Croton tiglium.*

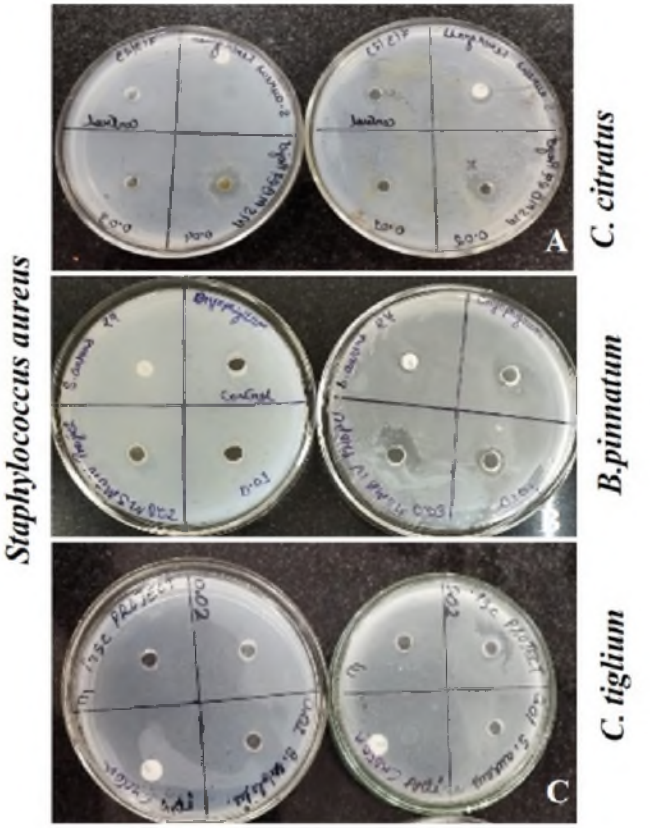

Figure 2: *Antibacterial activity of leaf extracts (A) C. citratus (B) B. pinnatum (C) C. tiglium against Staphylococcus aureus*

Table 1: *Phytochemical Analysis of Leaf Extracts: C. citratus, B. pinnatum, C.tiglium.*

Phytochemicals	**Tests**	***C. citratus***	***C. tiglium***	***B. pinnatum***
Alkaloids	Dragendoff's	+	+	-
	Meyer's	-	-	-
	Wagener's	+	+	-
Flavonoids	$FeCl_2$	+	+	-
	NaOH	+	-	-
Carbohydrates	Molisch	+	-	+
	Fehling's	+	+	+
Phenols	$FeCl_2$	+	-	+
Glycosides	$FeCl_2$	-	+	-

Table 2: *Zone of Inhibition for Staphylococcus aureus*

Concentration of extract µg/ml	**Leaf extracts action on *Staphylococcus aureus* (Zone of inhibition)**		
	C. citratus (mm)	*B. pinnatum (mm)*	*C. tiglium (mm)*
5	12	10	10
10	15	13	11
15	10	13	13
20	15	20	14
Penicillin	11	11	12
Ethanol	00	00	00

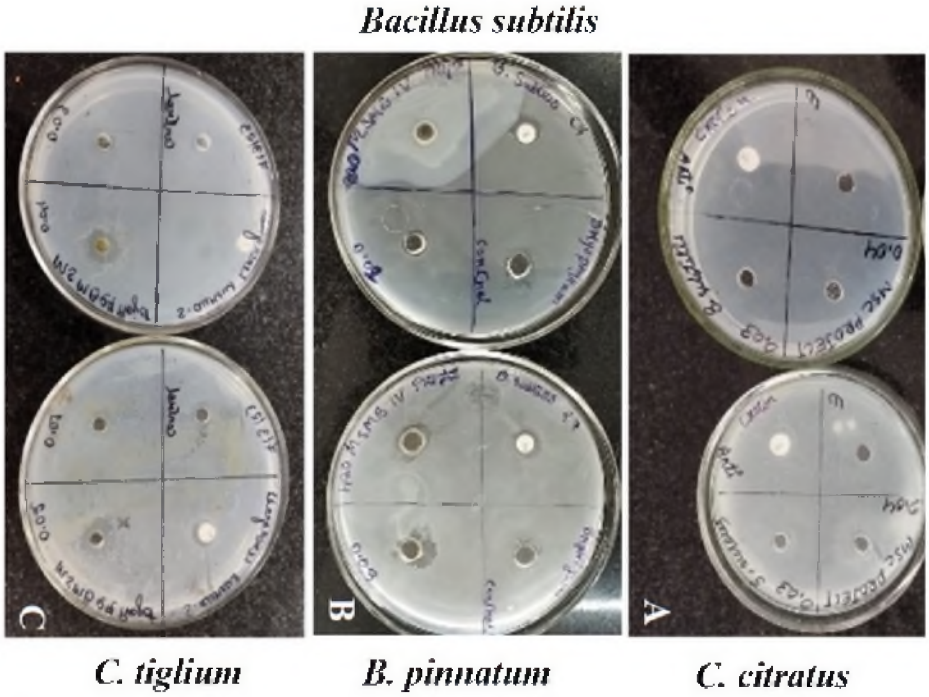

Figure 3: *Antibacterial activity of leaf extracts (A) C. citratus (B) B. pinnatum (C) C.tiglium against Bacillus subtilis*

Table 3: *Zone of Inhibition for Bacillus subtilis*

Concentration of extract µg/ml	**Leaf Extracts Action on *Bacillus subtilis* (Zone of Inhibition)**		
	C.citratus (mm)	*B. pinnatum (mm)*	*C.tiglium (mm)*
5	05	10	11
10	06	10	15
15	07	14	12
20	07	16	14
Penicillin	11	11	12
Ethanol	00	00	00

3.3. TLC analysis of Croton tiglium

TLC was performed to confirm that the purified form of *Croton tiglium* had lesser toxicity when compared to the unpurified form. UV analysis was done using transilluminator and the Rf value was calculated to confirm the reduction in toxicity *(Figure 4)*. The TLC strips were analysed and the retention factor was recorded. Retention factor of purified sample was 0.875 and that of unpurified sample was 0.98 *(Figure 5)*.

4. Conclusion

Oral hygiene is the process of keeping one's mouth healthy and disease-free. Oral hygiene should be performed on a regular basis to help avoid dental problems and foul breath. The most frequent types of dental illness are tooth decay and gum diseases, such as gingivitis and periodontitis.

According to the World Health Organisation (WHO), 75% of the global population relies on herbs for basic health care. WHO has advised that traditional medical systems such as Ayurveda be incorporated into the primary health care system in communities where they are recognised *(Nazreen and Gayathri, 2016)*. All of the native herbal plants are readily available in rural areas where people's socioeconomic conditions do not allow them to purchase expensive toothpaste or curative drugs *(Bagchi et al., 2015)*. The literature revealed that there are several ayurvedic medications made from herbal plants that can be utilized for both the prevention and treatment of oral illnesses. Many of the herbal plants analysed have antibacterial, anti-inflammatory, analgesic, and antiulcerogenic properties when tested using contemporary standards *(Nam – Hui et al., 2013)*.

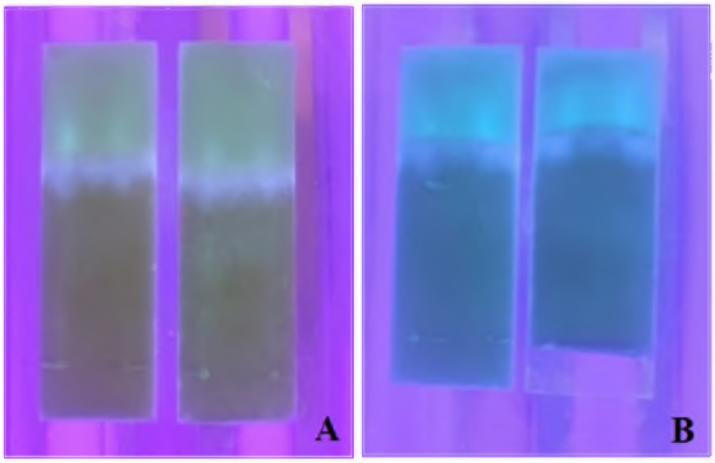

Figure 4: *UV Analysis of Croton tiglium (A) Purified (B) Unpurified*

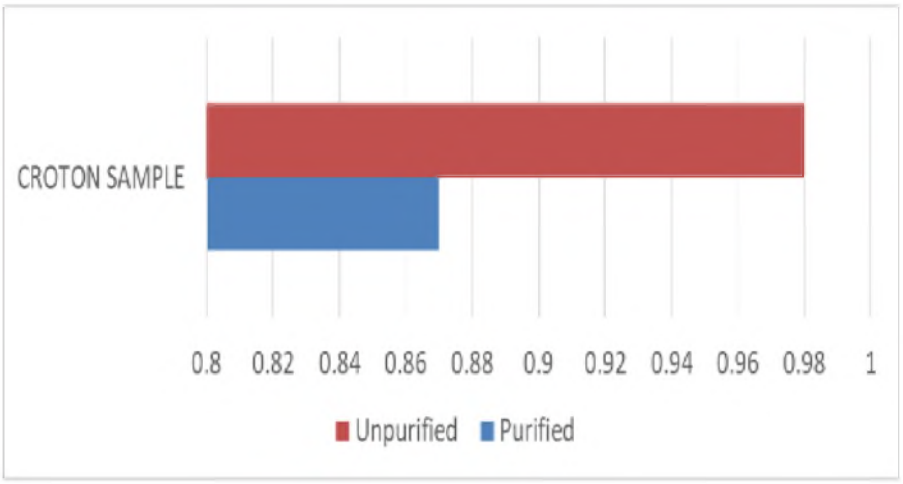

Figure 5: *Graphical Representation of TLC analysis of Croton tiglium*

The extracts of *Cymbopogon citratus*, *Bryophyllum pinnatum*, and *Croton tiglium* have all been shown to prevent disease-causing oral microbes. The term "herbal shotgun" or "synergistic multi-target effects" refers to the approach of combining several extracts *(Taye et al., 2011)*. The study evaluating the antimicrobial efficacy of a combination of these plant extracts against dental caries will aid in the development of a novel, innovative method that can simultaneously inhibit the most common oral diseases in humans while also slowing the development of drug resistance.

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