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Medicinal Evaluation of Common Plants against Mouth Microflora

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Abstract
Herbal remedy is one, in which the main therapeutic activity rests on plant metabolites (active principle), which it contains. The effectiveness of plant barks on mouth microbes using all available plants (total 13) in the garden of Divisional Public School, Rawalpindi was tested. The bark samples were dried, grinded and centrifuged to collect clear supernatant in sterilized tubes. Five discs made of Whatmann paper were dipped in each freshly extracted bark extract to be used for its antimicrobial activity against mouth microbes. Mouth microflora was cultured in nutrient agar and incubated overnight at 37^{o}C. Then five pre-soaked discs from each aqueous extract were placed at equal distance from each other in pre-incubated agar plate containing mouth microflora. The plates were incubated overnight at 37^{o}C and next day measured the zones of inhibition. The barks of some these plants showed significant antimicrobial activities against mouth microflora. *Callistiman lanceolatus* (bottle brush) showed highest level of activity and its zone of inhibition was quite clear and large (8 mm), followed by *Zizypus jujuba* (bairy) showing clear zones of inhibition (6 mm), *Syzigiun cuminii* (Jaman) and *Eriobotrya japonica* (Lokat) (4mm) each, and *Mangifera indica* (Aam) and *Eucalptus alba* (Safaida) were positive with 2 mm zone of inhibition. The least activity was observed in the case of *Melia azedarch* (Adraik) -a very common plant in this region, with maximum activity 1 mm. The aqueous extracts from remaining six plant species showed no activity. Two positive controls were also used, one was broad-spectrum antibiotic (Ampicillin) and other was common toothpaste (Colgate). Plant barks are good source of valuable therapeutic agents as they have active components that can kill/retard the activity of microflora of our mouth cavity.
Keywords: Herbal remedy, therapeutic activity, antimicrobial activity, zones of inhibition, Divisional Public School, Rawalpindi.

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INTRODUCTION

Medicinal plants are being used in various parts of the world. Herbal remedy is one, in which the main therapeutic activity rests on plant metabolites (active principle), which it contains. A variety of medicinal plant parts is used for extraction of raw drugs as they have diverse medicinal properties (Srivastava *et al.,* 2006; Al- Mahweety, 2016a; Al- Mahweety, 2016b). In Asian countries, including Korea, China, and Japan, infectious diseases are cured by traditional herbal medicines since ancient times (Wong *et al.,* 2010). However, in Pakistan, there are 36,881 registered Tabibs and 539 registered Vaids (Omar, 1990). There are an estimated 1500 species of indigenous plants claimed to possess medicinal properties. Out of these, about 300 are used in the preparation of recipes dispensed by the traditional practitioners (Hakims) who provide health coverage to 75% of the population residing in villages and rural areas (Hussain, 1993).

Dental diseases and related gum diseases is a growing health problem throughout the world especially in our country due to change in life style, dietary habits, tobacco use, excessive use of sugar and other sugar products (Petersen *et al.,* 2005). Miswak is one of the great Sunnah of our Holy Prophet (sullullaho alaihay wasalam) and importance of miswak is well known by mankind since pre-historic times. The idea of this study is based on, whether the bark of tree has some active component(s) that can control the problem causing microbes in the mouth cavity or not. If yes, than which bark would contain the large amount of active component(s), because, it is a experimentally proved that bark of the plant is a storage organ of the plant, which may carry some components that could be really effective in controlling the disease causing

agents of class mammalia and very especially against mouth microbes (Asghar *et al*., 2003). Screening for herbal medicines effective against mouth microflora is the prerequisite in the identification of natural phyto-chemicals that could be used as antimicrobial substances (Wong *et al.,* 2010). Therefore, we have tested the effectiveness of plant barks on mouth microbes using all available plants (total 13) in the garden of Divisional Public School, Rawalpindi.

MATERIALS AND METHODS

Selection of plants

A total of 13 plants were selected from the garden of Divisional Public School, Rawalpindi (Table 1).

Table 1. List of Medicinal plants used in the study

Sr. No.	Botanical names	Local names	Family	Part used
1.	*Callistiman citrinus*	Bottle brush	Myrtaceae	Bark
2.	*Zizypus jujuba*	Bairy	Rhamnaceae	Bark
3.	*Eriobotrya japonica*	Lokat	Rosaceae	Bark
4.	*Syzigiun cuminii*	Jaman	Myrtaceae	Bark
5.	*Eucalptus globules*	Safaida	Myrtaceae	Bark
6.	*Mangifera indica*	Aam	Anacardiaceae	Bark
7.	*Melia azedarach*	Adhraik	Meliaceae	Bark
8.	*Musa paradisiaca*	Qaila	Musaceae	Bark
9.	*Morus alba*	Shehtoot (Safaid)	Moraceae	Bark
10.	*Citrus limon*	Leemon	Rutaceae	Bark
11.	*Pongamia pinnata*	Sukh chain	Fabaceae	Bark
12.	*Psidium guajava*	Amrood	Myrtaceae	Bark
13.	*Oxalis coroniculata*	Khatti Booti	Oxalidaceae	Bark

Extraction of plant materials

The barks were removed from plants with the help of scalpel- at least six inches. The barks were dried in shady sunlight for two days and then in oven at 50^{o}C for further two days. Weighed out one gram of each dried sample and minced. The minced material was ground in electric grinder to get fine powder. Each powdered sample (0.5 gram) was suspended in two ml of freshly prepared, cold normal saline solution and the suspension was vigorously mixed by using vortex. The material was further processed for freeze-thaw three times and then centrifuged at 1000 xg to collect the clear supernatant in autoclaved tubes. Discs were made by using Watman filter paper with the help of common paper borer, autoclaved and finally five discs were dipped in each freshly extracted bark extract to be used for its antimicrobial activity against mouth microbes.

Culturing of microbes

Autoclaved nutrient agar medium was dispensed in autoclaved petri dishes @ 25 ml per plate. A sip of freshly prepared normal saline was taken in mouth, properly swirled it and after holding in mouth for one minute, the same half milliliter solution was applied on nutrient agar plates. The culture was then incubated at 37^{o}C for 1 hour.

Antimicrobial activity

Antimicrobial activity was determined by agar disc diffusion method. Five pre-soaked discs from each aqueous extract were placed at equal distance from each other in pre-incubated agar plate containing mouth microflora. Ampicillin and Colgate were used as positive control. The plates were incubated overnight at 37^{o}C and next day measured the zones of inhibition.

RESULTS

The results of this study indicate that some of the plant's barks have considerable amount of activity against mouth microflora (Figure 1).

Callistiman lanceolatus (bottle brush) showed highest level of activity and its zone of inhibition was quite clear and large (8 mm) (Figure 2), followed by *Zizypus jujuba* (bairy) (6 mm) (Figure 3), *Syzigiun cuminii* (Jaman) (Figure 2) and *Eriobotrya japonica* (Lokat) having free zone of 4 mm (Figure 4) each and third in the rank of antimicrobial activities. *Mangifera indica* (Aam) (Figure 4) and *Eucalptus alba* (Safaida) (Figure 3) were also positive with 2 mm activity on their plates. The least activity was observed in the case of *Melia azedarch* (Adraik) -a very common plant in this region (Figure 4), with maximum activity 1 mm (Table 2).

The aqueous extracts from remaining six plant species, *Psidium guajava* (Amrood), *Citrus limon* (Leemon), *Musa paradisiacal* (Qaila), *Pongamia pinnata* (Sukh chain), *Morus alba* (Shehtoot), and *Oxalis coroniculata* (Khatti), have shown no activity (Figures 2, 3, 4 and 5). Two positive controls were also used, one is broad-spectrum antibiotic (Ampicillin) and other is common toothpaste (Colgate) (Figure 6). All measured values of free zones are the average values of quarduplicates (Table 2).

Table 2. Zones of inhibition (in mm) by pre-soaked discs from different plant extracts against mouth microbes

Sr. No.	Botanical names	Common names	Zone of inhibition (mm)
1.	*Callistiman citrinus*	Bottle brush	08
2.	*Zizypus jujuba*	Bairy	06
3.	*Eriobotrya japonica*	Lokat	04
4.	*Syzigiun cuminii*	Jaman	04
5.	*Eucalptus globules*	Safaida	02
6.	*Mangifera indica*	Aam	02
7.	*Melia azedarach*	Adhraik	01
8.	*Musa paradisiaca*	Qaila	00
9.	*Morus alba*	Shehtoot (Safaid)	00
10.	*Citrus limon*	Leemon	00
11.	*Pongamia pinnata*	Sukh chain	00
12.	*Psidium guajava*	Amrood	00
13.	*Oxalis coroniculata*	Khatti Booti	00
14.	Positive control	Colgate Tooth paste	08
15.	Positive control	Ampicillin	10

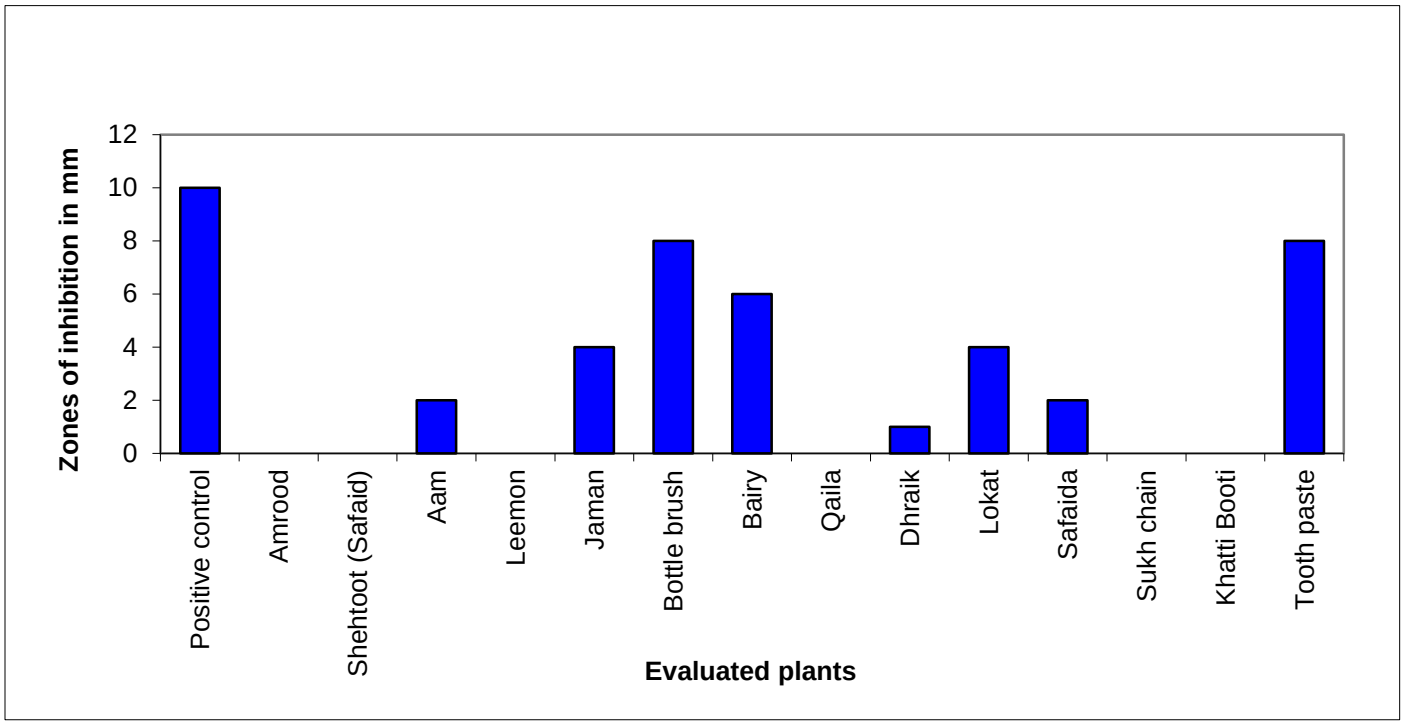

Fig. 1. Levels of antimicrobial activity from plant extracts (common names) against mouth microflora

Fig. 2. Zone of growth inhibition of mouth microflora produced by bark extracts of *Callistiman lanceolatus (bottle brush), Citrus limon (Leemon), Eugenia jambolina* (Jaman) and *Psidium guajava* (Amrood) plants.

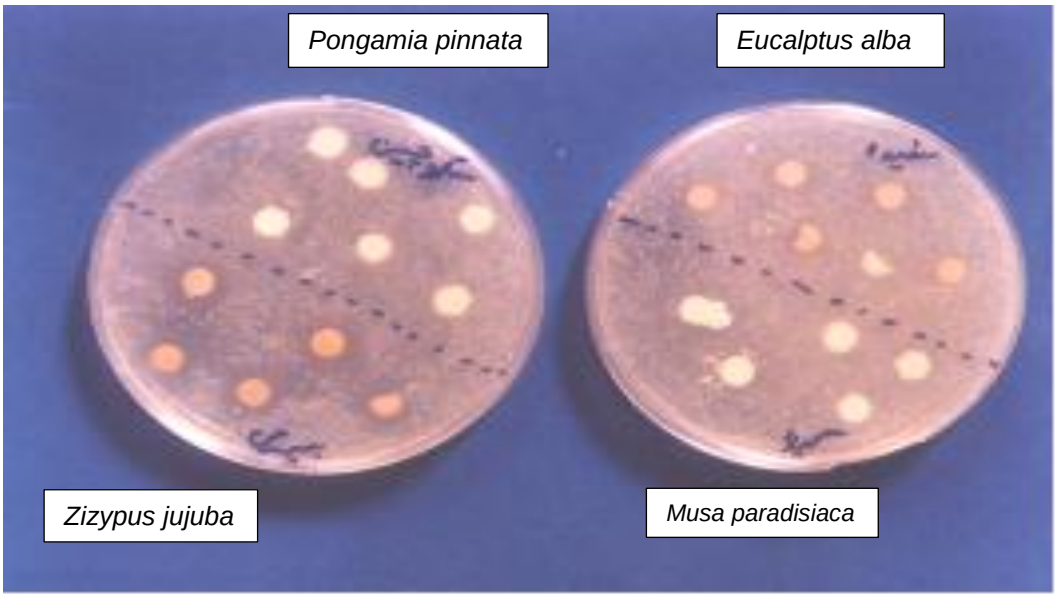

Fig. 3. Zone of growth inhibition of mouth microflora produced by bark extracts of *Pongamia pinnata* (Sukh chain), *Zizypus jujuba* (bairy), *Eucalptus alba* (Safaida) and *Musa paradisiaca* (Qaila) plants.

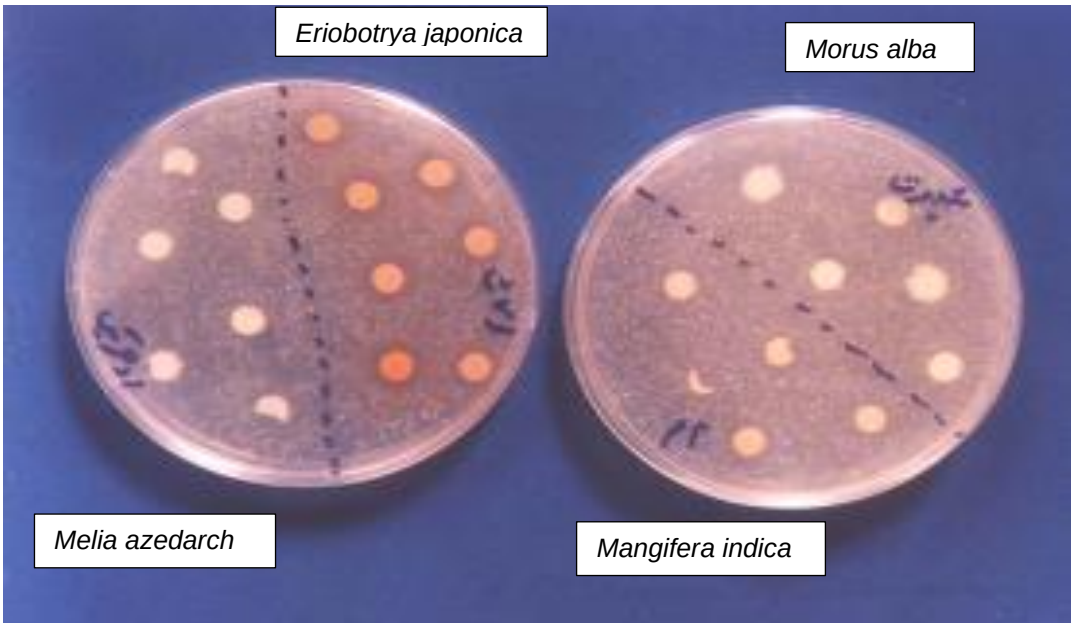

Fig. 4. Zone of growth inhibition of mouth microflora produced by bark extracts of *Melia azedarch* (Adraik), *Eriobotrya japonica* (Lokat), *Mangifera indica* (Aam) and *Morus alba* (Shehtoot) plants

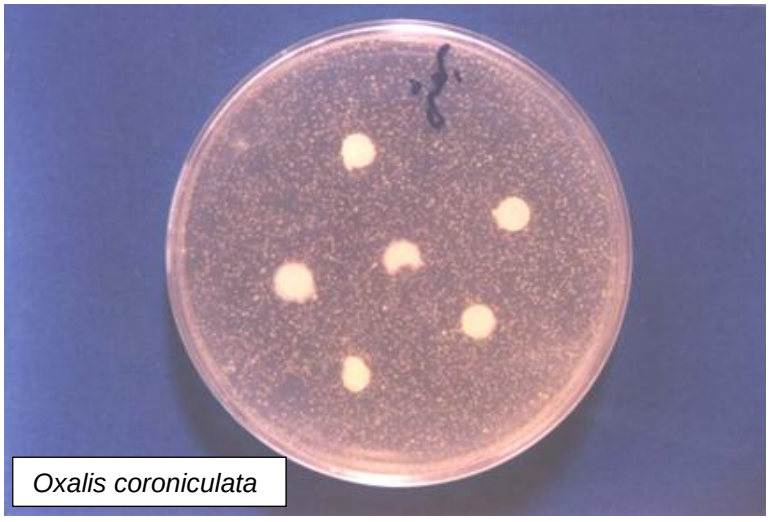

Fig. 5. Zone of growth inhibition of mouth microflora produced by bark extracts of *Oxalis coroniculata* (Khatti) plant.

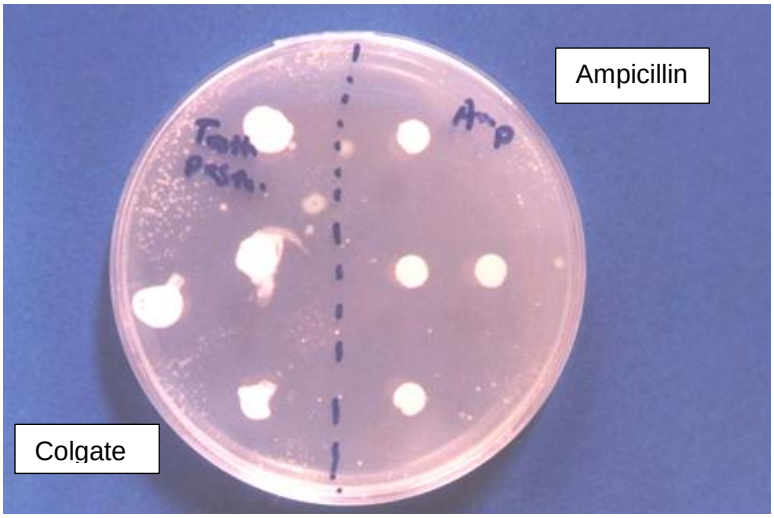

Fig. 6. Zone of growth inhibition of mouth microflora produced by Positive controls (Ampicillin and Colgate)

DISCUSSION

Antimicrobial agents of plant origin are effective in the treatment for infectious diseases due to enormous therapeutic potential. The barks of some the tested plants showed significant antimicrobial activities against mouth microflora while some plants did not show any activity. Antimicrobial effects of plants extracts are due to the presence of natural compounds (Ali *et al.,* 2017). There are several reports about the antimicrobial effect of plant extracts against oral microbes (Abu-Gharbia *et al.,* 2014; Gauniyal and Teotia, 2015; Kumar *et al.,* 2014; Devi *et al.,* 2012; Adebisi and Ojokoh, 2011; Teanpaisan *et al.,* 2017). There exists a balance in a person's oral microbial population. Opportunistic microorganisms can proliferate, disturbing the balance and enabling the initiation of disease processes. Honey can be used as an alternative for treatment of various infections, especially those caused by antibiotic resistant bacteria (Iqbal *et al.,* 2015).

Therefore, compounds possessing good antimicrobial properties may be useful in prevention and treatment of various oral diseases (Al-Hakami *et al.,* 2016). Medicinal plants can contribute hugely to the traditional medicines through providing ingredients for drug or having played central roles in the drug discoveries (Kalim *et al.,* 2016; Hussain *et al.,* 2016). The effects of extract may benefit if incorporated in tooth paste, mouth rinses and dental products to reduce dental diseases. The bark of tree has some active component(s) that can control the problem causing microbes in the mouth cavity. This study also supports the use of Miswak which is one of the great Sunnah of our Holy Prophet (P.B.U.H). Further studies are required to better evaluate the effect of these extracts.

CONCLUSION

Plant barks are good source of valuable therapeutic agents as they have active components that can kill/retard the activity of microflora of our mouth cavity. Miswak or bark can effectively wash the mouth cavity as good as the toothpaste and may give the better results because of presence of natural components.

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CONFLICT OF INTEREST

The authors verify having no interest in competition and have no conflicts of interest.

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