***In Vitro* Antibacterial, Antioxidant and Mechanism of Antifungal Activities of Selected Indian Traditional Plants against Human Oral Microbes**

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Jayaraman *et al.*: Antimicrobial Activity of Traditional Plants against Oral Pathogens

Morus alba, *Asparagus racemosus* and *Murraya koenigii* are commonly used in Indian traditional medicines for the various ailments. The plant extracts were analysed for antibacterial, antifungal and antioxidant activities and their mechanism of action towards human oral fungal pathogens. In the present study, methanol extracts were obtained using a soxhlet apparatus and microbes were obtained from microbial type culture collection and gene bank, Pune. The total phenol and flavonoid were found to be high in *Murraya koenigii* (2.77 mg gallic acid equivalents/g) and *Morus alba* (160 µg Quercetin equivalent/mg) respectively. The maximum radical scavenging activity was shown by *Murraya koenigii* in 2,2-diphenylpicrylhydrazyl (71 %) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (82.57 %) methods. The maximum antibacterial and antifungal activities were observed in *Asparagus racemosus* towards *Lactobacillus acidophilus* (23 mm) and *Candida krusei* (10 mm) respectively. The minimum inhibitory concentration was also determined. The target of antifungal activity of *Asparagus racemosus* towards *Candida krusei* was not cell wall whereas targets the cell membrane which is confirmed by sorbitol assay, transmission electron microscopy and flow cytometry. This research can be used as a base to find the active components responsible for antibacterial, antifungal and antioxidant activities.

Key words: *Asparagus racemosus*, electron microscope, flow cytometry, *Morus alba*, *Murraya koenigii*

Federation Dentaire Internationale (FDI) World Dental Federation stated oral health as multifaceted problem which involves the ability to talk, smile, taste, chew and swallow without any pain, discomfort and disease of the craniofacial complex^[1]. There has been no improvement in oral health over the past 25 y. Since 1990, the burden of untreated oral diseases has increased dramatically worldwide because of population growth and aging^[2]. Dental plaque is the most commonly occurring microbial disease in humans. Dental plaque is considered as a polymicrobial biofilm integrated into an extracellular matrix^[3].

Chlorhexidine is an international drug used to treat oral plaques at 0.2 % concentration. However, it has been reported for the adverse side effects such as staining of teeth, dorsum of tongue, alteration of taste and super gingival calculus formation^[4]. Reducing costs and side effects compared to conventional medicines has led to the use of medicinal plants in the form of mouth rinses

to treat oral diseases considered as promising position on health care over past few years^[5]. A large number of herbal extracts have been shown to inhibit the oral pathogens and the formation of dental biofilms by minimizing adhering microbial pathogens on the surface of the tooth which is a primary event in development of tooth decay and dental plaque^[6-8].

Morus alba (*M. alba*) is an Indian tradition plant whose parts such as leaves, stems, roots and fruits are used in traditional medicine. The leaves consist of compounds like rutin and quercetin. These natural bioactive compounds have proven biological activities to exhibit excellent pharmacological effects

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against numerous diseases. These include diuretic, antioxidant, hypotensive, hypoglycemic, antidiabetic, anticholesterol and antimicrobial properties^[9,10]. In recent times, most of the research is focused on evaluating the antimicrobial activities of various parts of the plant, many research also focuses on utilization of these medicinal plants in treatment of variety of ailments^[11-13]. There are very few literatures based on extract of *M. alba* against oral microorganisms.

Asparagus racemosus (*A. racemosus*), is from the Liliaceae family and its common name is Shatavari. This plant is considered to be an important monocot medicinal plant found in tropical and subtropical countries like India^[14]. The roots of this plant is said to be tonic, galactagogue and diuretic. The plant also contains antiseptic and anthelmintic properties^[15]. *Murraya koenigii* (*M. koenigii*) is one of the herbal plants, originates in India typically called curry leaves or karipatta. Due to the presence of numerous phytochemicals, curry leaves exhibit properties like antioxidant, antimicrobial, anticancerous, antidiabetic, antiinflammatory etc.,^[16] It was found that extracts of *M. koenigii* inhibit certain dental caries and periodontal pathogens^[17].

Medicinal plants are more suitable alternate drug as well as low cost with no side effects when compared to synthetic medicine. The herbal based oral care products are helped to prevent the toxicity and tooth staining. Hence it is necessary to identify the herbal medicine to cure or reduce the symptoms of oral infections. This study is attentive to identify the traditional remedy for oral microbial infections. Therefore our research is focused with the objectives of *in vitro* antimicrobial activities of *M. alba*, *A. racemosus* and *M. koenigii* and antifungal mechanism of action towards human oral pathogens.

MATERIALS AND METHODS

Collection of plant materials:

The medicinal plants (*M. alba*, *A. racemosus* and *M. koenigii*) were collected from various parts of Tamilnadu, India. The plant identification was authenticated by plant taxonomist Dr. M. Kumar from Madras Christian College, Chennai, Tamil Nadu, India. A voucher specimen were prepared and deposited in Postharvest Research Laboratory, SRM Institute of Science and Technology, Kattankulathur, Tamilnadu, India with voucher No: PRLH10, PRLH11 and PRLH12 respectively.

Preparation of plant extract:

The collected plant materials were shade dried and ground to a coarse powder. 20 g of powdered material was weighed and extracted with methanol in a soxhlet apparatus. Extracts were concentrated by using rotary evaporator (IKA, RV 10 digital V) and used for further studies.

Qualitative analysis of phytochemicals:

The presence of various phytochemicals such as steroids, saponins, terpenoids, glycosides, tannins and flavanoids were preliminary screened by the standard methods^[18-21].

Total phenol determination:

Total phenol was quantitatively determined by Folin-Ciocalteu colorimetric method. 200 µl of plant extract was taken in a screw tube and 1 ml of Folin-Ciocalteu reagent (1:1 with water) and 1 ml of sodium carbonate (7.5 %) were added. The contents were thoroughly mixed using vortex. After 2 h of incubation, the mixture was tested for phenolic content at 726 nm in Ultraviolet-Visible (UV-Vis) spectrophotometer (Beckman, USA) and expressed in Gallic Acid Equivalents (GAE) in mg/g of plant extract^[22].

Total flavonoid determination:

Total flavonoid content was determined using aluminium chloride colorimetric method. Plant extract was dissolved in methanol in the ratio 1:10 (w/v) and then mixed with a mixture containing 0.1 ml of 10 % aluminium chloride hexahydrate, 0.1 ml of 1 M potassium acetate and 2.8 ml of deionized water. After 40 min of incubation, the mixtures were analysis at 415 nm in UV-Vis spectrophotometer (Beckman, USA) using quercetin was used as the standard. The result was expressed in µg Quercetin Equivalents (QE) per mg of dry extracts^[22].

ANTIOXIDANT ACTIVITY

2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity:

Antioxidant activity of the plant extracts was determined using DPPH radical scavenging activity. The plant extracts were diluted in the concentrations ranging from 100-500 µg/ml. The 50 µl of sample was taken in a 96 well micro titer plate (triplicates). Afterwards 100 µl of 0.1 mM DPPH was added and incubated for 30 min. The absorbance was measured at 515 nm.

The 50 µl of methanol in 100 µl of DPPH was used as control and 100 µl of 95 % methanol was used as the blank. Different concentrations of ascorbic acid (100-500 µg/ml) were used as standard. Free radical scavenging activity is inversely proportional to the absorbance measured^[23].

The ability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = [\text{OD control} - \text{OD sample} / \text{OD control}] \times 100$$

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) free radical scavenging activity:

The free radical scavenging capacity of plant extract was determined by ABTS radical cation decolorization assay using 0.7 mm ABTS. ABTS stock solution in the presence of 2.45 mm potassium persulphate produces ABTS⁺ (Cation). This was kept in a dark room for 12-16 h before use. The ABTS⁺ was diluted in 70 % ethanol and adjusted to an absorbance of 0.700 (±0.02) at 734 nm. This reading was taken as blank (A_0). The 20 µl of test sample and 2 ml of ABTS⁺ solution were added and the absorbance was recorded exactly after 6 min from initial mixing (A_t). Concordant absorbance values were taken^[24].

The percentage inhibition (PI) of absorbance at 734 nm was calculated using the formula:

$$\text{PI} = [\text{AC}_{(0)} - \text{AA}_{(t)} / \text{AC}_{(0)}] \times 100$$

Where, $\text{AC}_{(0)}$ is the absorbance of the control at $t=0$ min; $\text{AA}_{(t)}$ is the absorbance of the antioxidant at $t=6$ min

ANTIMICROBIAL ACTIVITY

Microbial strains:

Oral pathogenic bacteria such as *Staphylococcus aureus* (*S. aureus*) (Microbial Type Culture Collection & Gene Bank (MTCC) 7443), *S. mutans* (MTCC 497), *S. mitis* (MTCC 2695), *S. oralis* (MTCC 2696), *Lactobacillus acidophilus* (*L. acidophilus*) (MTCC 10307) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC 7453) and fungal species such as *Candida parapsilosis* (*C. parapsilosis*) (MTCC 6510), *C. krusei* (MTCC 9215) and *C. tropicalis* (MTCC 184) were obtained from MTCC, Chandigarh, India.

Disc diffusion method:

The antibacterial and antifungal activity of plant extracts were determined by disc diffusion method. The strains were cultured in Muller-Hinton (MH) broth to

logarithmic phase and were adjusted to 0.5 McFarland's using the same broth. The test strains were spread on the Muller-Hinton agar plates at 37° for 24-48 h. A sterile disc of 100 µg/ml and 250 µg/ml concentration was used to test the antibacterial and antifungal activities respectively. Plant extracts dissolved in Dimethyl Sulphoxide (DMSO) was added to sterile discs and impregnated onto agar plate with specific pathogenic strain. The 30 µg/ml of chloramphenicol, 0.5 mg/ml of fluconazole and 100 % DMSO were used as the positive and negative control respectively. The zone of inhibition was checked after 24-48 h of incubation^[25,26].

Determination of Minimum Inhibitory Concentration (MIC):

The MIC of extracts was determined using serial dilution method. The extracts were diluted in a two-fold. 2 ml of MH broth along with plant extract was taken in tube 1. This served as the stock for the further dilution. 1 ml of this stock was added to the 2nd tube and 1 ml of MH broth was added to it. Tubes 2-10 were diluted using the same procedure. 1 ml of inoculums (test strains cultured in MH broth) was added to the tubes and was incubated at 37° for 24-48 h. The MH broth was used as the negative control. All the tubes were made in triplicates^[27].

Sorbitol binding assay:

The effect of plant extracts on fungal cell wall was determined using sorbitol assay by the method of Frost *et al.* The 96 well plates containing test compounds with and without 0.1 M sorbitol in adjacent rows were prepared. The 100 µl of fungal suspension was added to each well. The readings were taken after 2nd and 6th d of incubation and MIC was calculated^[28].

Scanning electron microscopy (SEM):

Samples for SEM analysis were prepared according to Figueras and Guarro, method with slight modifications. The 100 µl of cells was inoculated in with Sabouraud's dextrose broth at the MIC concentration of plant extracts and was incubated at 37° for 48 h at 120 rpm. It was then centrifuged to obtain cell pellets. The pellets along with untreated controls were fixed with 3 % glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) at 4° for 48 h. It was then dehydrated for 20 min using 30 %, 50 %, 70 %, 95 % ethanol and absolute ethanol for 45 min. The samples were dried using liquid carbon dioxide (CO₂) at critical point and was mounted onto standard ½ inch Cambridge SEM tubes and coated with

Gold-Palladium electroplating (60 s, 1.8 mA, 2.4 kv) in a Polaron SEM coating system sputter coater. The samples were examined in a LEO435 VP SEM at 15kv to study the changes in cell morphology^[29].

Transmission Electron Microscopy (TEM):

Structural changes imparted by test compounds on fungal cell wall were evaluated using TEM. The 100 µl of cell suspension (0.5 McFarland) was added to 10 ml of Sabouraud's dextrose broth treated with MIC concentration of plant extracts and incubated at 37° for 48 h at 120 rpm. Untreated samples were used as a control. The obtained cell pellets were fixed using 2.5 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 24 h at room temperature. This was post fixed using 1 % osmium tetroxide in cacodylate buffer. Samples were dehydrated in acetone and embedded in epon. Ultra-thin sections were stained with 12.5 % alcoholic uranyl acetate and viewed under Morgagni 268D transmission electron microscope at 80 kv. The difference in the ultra-structure of treated and untreated samples were analysed^[29].

Propidium iodide influx analysis:

Propidium iodide influx analysis was done according to Cho *et al.* method. *C. parapsilosis* cells (2×10⁶ cells/ml) in log phase were inoculated in yeast extract peptone dextrose medium. The plant extracts were added and incubated for 2 h, 28° at 140 rpm. The cells were harvested, resuspended in Phosphate Buffered Saline (PBS) and treated with propidium iodide (9 µm, final concentration) for 5 min. Flow cytometry was performed via Fluorescence-Activated Cell Sorting (FACS) Caliber flow cytometry^[30].

Flow cytometric analysis:

Morphological changes imparted by plant extracts onto *Candida* cells were determined by flow cytometry by illuminating the cells at 480 nm using an argon ionic laser, Forward Light Scatter (FSC) and Side Scatter (SSC).

Statistical analysis:

Results were expressed as mean±Standard Deviation (SD) for triplicates. All the data were statistically evaluated with Statistical Package for the Social Sciences (SPSS)/19.0 software.

RESULTS AND DISCUSSION

The analysis of various phytochemicals such as steroids,

saponins, terpenoids, glycosides, tannins, phenols and flavonoids were carried out in *M. alba*, *A. racemosus* and *M. koenigii*. The result showed the presence of all phytochemicals except steroids in *A. racemosus* and terpenoids in *M. koenigii* (Table 1).

The phenol and flavonoid content was tested using Folin-phenol reagent and aluminium chloride respectively. The result of total phenol and flavonoid were expressed in GAE/g and QE/mg respectively (Table 2).

Among the sample tested *M. koenigii* showed significantly ($p < 0.05$) higher phenolic content followed by *M. alba*. The *M. alba* showed higher flavonoid content followed by *M. koenigii*. The phenol and the flavonoid contents of *M. koenigii* leaves were found to be 2.77 mg/g and 66 µg/mg respectively. The above result correlates with Thangavel *et al.* and Pratima *et al.*^[31,32]. Phenolic content of the plant is directly proportional to the antioxidant activity^[33] as it absorbs the free radicals by decomposing peroxide^[34,35]. Our results confirmed the above said statement.

The presence of phytochemicals like phenols and flavonoids makes this plant a potent antioxidant^[36] and the methanolic extract of *M. koenigii* can scavenge cationic and neutral free radicals due to the presence of these phytochemicals^[37].

Our result also confirmed that *M. koenigii* possess high phenol content also showed high radical scavenging activity (71 %). The *M. alba* and *A. racemosus* showed 49 % and 23 % of radical scavenging activity respectively. Zhishen *et al.* reported that mulberry leaves showed the superoxide ion scavenging activity by 46.5 % at 5 µg/ml concentration^[38]. *M. koenigii* leaves extract showed 96 % radical scavenging activity at 0.1 mg/ml^[39]. Benzene extract of *M. koenigii* showed the highest activity of 88.3 %^[40].

Oxidation of ABTS with potassium per sulphate generates ABTS free radicals and it is based on the antioxidant capacity of the unknown compounds to reduce free radical. The scavenging activity of *M. koenigii* was significantly higher (82.57 %) than the other two plants extracts (*M. alba* (60.4 %) and *A. racemosus* (26.57 %)). The standard vitamin E showed 99.6 % scavenging activity. *M. koenigii* showed 100 % radical scavenging activity at 10 µg/ml^[41] and the antioxidant activity of three different varieties of *M. koenigii* were 12-75 % at 40 µg/ml and the high antioxidant activity is due to the presence of high phenolic content in *M. koenigii*^[42].

The extracts of *M. alba*, *A. racemosus* and *M. koenigii*

(100 µg/ml) and chloramphenicol (30 µg/ml) showed significant antibacterial activity for most of the bacteria tested except *Streptococcus mitis* (*S. mitis* and *S. mutans*) are the most common bacteria in cariogenicity of human teeth and they possess moderate resistance to antibiotics^[43]. Our results also confirmed that *S. mitis* are resistant to all extracts tested and *S. mutans* susceptible only to *M. koenigii* (18 mm) (Table 3).

The methanolic extracts of *M. alba* and *A. racemosus* showed maximum antibacterial activity (23 mm) against *S. oralis* and *L. acidophilus* respectively (Table 3). These results coincide with Aditya *et al.*^[44] and Chaitali *et al.*^[45]. Baskaran *et al.*^[46] concluded antibacterial activity of *M. koenigii* against *S. aureus* (15 mm) and *P. aeruginosa* (8 mm). The antibacterial activity of mulberry was due to the presence of high concentration of phenolic acids^[47].

The fungal species showed the resistance towards all the extracts tested except *C. krusei* sensitive to *A. racemosus*

TABLE 1: QUALITATIVE PHYTOCHEMICAL SCREENING OF *M. alba*, *A. racemosus* AND *M. koenigii*

Phytochemicals	<i>M. alba</i>	<i>A. racemosus</i>	<i>M. koenigii</i>
Steroids	+	-	+
Saponins	+	+	+
Terpenoids	+	+	-
Glycosides	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+
Phenols	+	+	+

Note: (+) Present; (-) Absent

TABLE 2: TOTAL PHENOL AND FLAVONOID ESTIMATION OF *M. alba*, *A. racemosus* AND *M. koenigii*

Methanolic extract	Total phenolic content (mg GAE/g)	Flavonoid content (µg QE/mg)
<i>M. alba</i>	1.50±0.05 ^b	160±2.00 ^c
<i>A. racemosus</i>	0.28±0.01 ^a	3.5±0.50 ^a
<i>M. koenigii</i>	2.77±0.03 ^c	66±1.00 ^b

Note: Data are represented as mean±SD, with experiments carried out in triplicates. Values with various superscripts denote significant difference among different plant extracts (p<0.05)

TABLE 3: ANTIBACTERIAL ACTIVITIES OF *M. alba*, *A. racemosus* AND *M. koenigii*

Microorganism	<i>M. alba</i>	<i>A. racemosus</i>	<i>M. koenigii</i>	Chloramphenicol
<i>S. aureus</i>	19±2.08	17±2.08	12±1.52	24±0.15
<i>S. mitis</i>	-	-	-	15±0.28
<i>S. oralis</i>	23±2.64	15±2.51	-	19±0.8
<i>S. mutans</i>	-	-	18±1.52	22±0.57
<i>L. acidophilus</i>	-	23±3.60	-	9±1.75
<i>P. aeruginosa</i>	20±2.08	-	10±1.52	20±0.52

Note: Values are mean inhibition zone (mm)±SD of triplicates, plant extracts-100 µg/ml; chloramphenicol-30 µg/ml; (-) no inhibition

(10±0.55 mm) and at 250 µg/ml concentrations (Table 4) and MIC was 62.5 mg/ml. Our results were similar with Uma *et al.*^[48], *C. krusei* showed sensitivity towards the methanol extract of *A. racemosus*. The MIC for antibacterial activity ranges from 0.5-6.25 µg/ml (Table 5).

The assay was performed to determine the action of plant extracts on fungal cell wall. The MIC (62.5 mg/ml) of *A. racemosus* towards *C. krusei* was similar in both presence and absence of sorbitol (0.8 M) clearly indicates the target of inhibition of plant extract towards fungi was not by cell wall synthesis.

Citral, an unsaturated monoterpene aldehyde showed the identical MIC values in both presence and absence of sorbitol^[49] showed that the target of antifungal compound is not cell wall^[50]. Our results also showed the identical MIC values both in presence and absence of sorbitol confirmed the target is not the cell wall.

SEM was performed to determine the morphological changes in *C. krusei* after treatment with the MIC concentration of *A. racemosus* (62.5 mg/ml) for 48 h (fig. 1a, fig. 1b). Untreated cells were regular with intact spherical shape and uniform central density whereas, plant extracts treated cells showed changes in morphology. The cells showed irregular shape with deformed surface and elongated. This indicates the bactericidal activity of *A. racemosus* on losing cell membrane integrity and release of cytoplasmic contents which leads to cell death.

The mechanism of action of antifungal activity was analysed using TEM. The untreated cell of *C. krusei* showed a regular spherical morphology with clear cytoplasm and smooth surface (fig. 2a) whereas the plant extract treated cell showed an irregular shape and an improper cell membrane. This confirms the damage in the cell membrane which leads to the disruption in the semi permeability transport of molecules across the cell membrane. This is followed by the deterioration and reduced intracellular volume of cytoplasm and cellular constituents, results in structural disorganization and

reduced intracellular volume of cytoplasm finally causes cell death (fig. 2b-fig. 2g). This result coincides with our previous study^[51]. The ethanolic extract of whole fruit of pomegranate was tested against *C. albicans*, *C. krusei* and *C. rugosa*. The MIC was observed at the concentration of 125 µg/ml. The vacuole formation, irregular hyphae were identified in the *C. albicans* treated with pomegranate extract by TEM^[52].

Flow cytometry was performed to evaluate the effect of the drug on cell membrane. The increased fluorescence was observed due to the internalization of propidium iodide in cells with severe membrane lesions. The following graph explains the cell membrane damage in *C. krusei* by *A. racemosus* at MIC concentration

TABLE 4: ANTIFUNGAL ACTIVITIES OF *M. alba*, *A. racemosus* AND *M. koenigii*

Plant extracts	<i>C. parapsilosis</i>	<i>C. krusei</i>	<i>C. tropicalis</i>
<i>M. alba</i>	-	-	-
<i>A. racemosus</i>	-	10±0.55	-
<i>M. koenigii</i>	-	-	-
Fluconazole	19±3.21	24±1.07	13±3.5

Note: Values are mean inhibition zone (mm)±SD of three replicates, plant extracts-250 µg/ml; fluconazole-0.5 mg/ml, (-) no inhibition

TABLE 5: MIC VALUES OF *M. alba*, *A. racemosus* AND *M. koenigii* AGAINST ORAL BACTERIA

Bacteria	<i>M. alba</i>	<i>A. racemosus</i>	<i>M. koenigii</i>
<i>S. aureus</i>	1.56	3.12	3.12
<i>S. mitis</i>	-	-	-
<i>S. oralis</i>	1.56	6.25	-
<i>S. mutans</i>	-	-	3.12
<i>L. acidophilus</i>	-	6.25	-
<i>P. aeruginosa</i>	3.12	-	1.56

Note: Values are means of triplicates

(62.5 mg/ml). Morphological changes were observed on scatter gram upon treatment with test agents. The graph clearly mentioned that cell count damage is high in treated sample (orange colour) than untreated (blue). DMSO (green) was used as a negative control (fig. 3). This results were coinciding with our previous study^[51]. The present study confirms the presence of antibacterial, antifungal and antioxidant activities of the Indian traditional plants. Future detailed investigation on these plants hopefully lead to the development of medical applications.

In this study, the antimicrobial and antioxidant activities of three traditional plants which were used by the traditional practitioners for oral infections were assessed. The phenol content is found to be high in *M. koeingii* and flavonoids are high in *M. alba*. The presence of high concentrations of phenols in *M. koeingii* and flavonoids in *M. alba* makes these plants a potent antioxidant and antimicrobial respectively. Based on the available literatures, mechanism of phenol is to alter cell membrane potential which disrupts ion transport and cause cell death which was confirmed by TEM. The potent antifungal activity was observed in *A. racemosus* towards *C. krusei* by deteriorating cell membrane which results in the damage of overall morphology.

Future directions based on this research includes the identification of specific phytochemicals responsible for activity, synergistic effect of plant extracts and the possible route of administration of the specific phytochemicals for the proper release of active antimicrobial compound at the target site of infection.

Fig. 1: SEM micrographs of (a) *C. kusei* untreated cells with uniform morphology; (b) treated cells with deformed and elongated morphology

Fig. 2: (a) TEM images of untreated *C. krusei* cells with intact and uniform cell density; (b-g) TEM images of *C. krusei* with *A. racemosus* treated sample with non-uniform cell density, deteriorated cell membrane and reduced intracellular volume of cytoplasm

Fig. 3: Flow cytometric analysis of *C. krusei* treated with *A. racemosus*. Damage in treated sample (yellow) is comparatively higher than untreated sample (blue) due to the influx of propidium iodide into cells, (red) antibiotic; (yellow) plant extract; (green) DMSO; (blue) untreated

Therefore further investigations are required to identify the active compounds of the medicinal plants to develop oral care products for the oral infections.

Conflicts of interest:

The authors declared no conflicts of interest.

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