Pak. J. Agri. Sci., Vol. 54(2), 423-430; 2017
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/17.6385
http: //www.pakjas.com.pk

EVALUATION OF ANTIMICROBIAL POTENTIAL OF *Acacia nilotica* (KIKAR) AGAINST ORAL PATHOGENS ASSOCIATED WITH CARIES AND PERIODONTITIS

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Search for cheap, easily acessable, safe and alternative treatment options is one of the evolving fields in oral health care at present. *Acacia nilotica* is a plant species that is almost ubiquitously found in diverse parts of the world. The present study investigates the antimicrobial potentialof different solvent extracts (methanol, acetone and aqueous) against *Streptococcus mitis*, *Streptococcus mutans* and *Prevotellaintermedia.* The cytotoxicity of these extracts was evaluated on Vero cells to determine cellular cytotoxicity fifty (CC_{50}). These extracts were subjected to high performance liquid chromatography (HPLC) to determine phenolic acids having antibacterial effects. The outcomes of current study revealed that the methanol extract of *A. nilotica* showed significantly higher antimicrobial activity in terms ofzone of inhibition (18.00±1.00mm, 20.00±1.15mm and 16.67±0.67mm) and minimum inhibitory concentration (MIC) (0.3125, 0.3125 and 0.15625 mg/ml) followed by acetone and aqueous extracts against *S. mutans*, *S. mitis* and *P. intermedia* respectively. The methanol extract showed higher numbers and quantity of phenolic acids as compared to other solvents. CC_{50} of the aqueous extract was significantly higher than the other solvents which means it is safest among all extracts for therapeutic purpose.
Keywords: Kikar, phenolic acids, oral pathogens, antimicrobial effects, cytotoxicity

INTRODUCTION

A Healthy mouth is a exclusive and invaluable asset. It plays an important role in overall health and quality of life. It may be considered as a basic human right (Sgan-Cohen *et al*., 2013). Oral health is often affected by numerous oral infections mostly periodontitis and dental decay, and rarely by oral cancer, lesions of HIV, orofacial pain and clefts (Jin *et al*., 2016). It is now acknowledged that oral diseases are global epidemic and a leading public health complication (Beaglehole and Bonita, 2009). Biofilm is composed of bacterial colonies encased in a matrix. It can grow on living and non living surfaces, ranging from a wooden plankto various human tissues (Huang *et al*., 2011). Dental plaque is one of the most studied forms/type of bacterial biofilm which is also called oral biofilm. Eradication of bacterial plaque biofilms is still a challenge for pharmaceutical industry and the medical field. The oral microbiota is considered to be complex and very diverse consisting of upto 700 different species(Vasudevan,2017). Oral cavity has a dynamic environment which enhances the growth of bacterial species by providing a warm and humid environment as well as neutral pH (Marsh *et al*., 2011).

Data from numerous clinical studies suggests that bacterial community within the oral cavity is an important etiologic factor for two main oral infections i.e; caries and periodontitis (Nishihara and Koseki, 2004).

Tooth decay is a chronic process which progresses gradually in most of the cases (Selwitz*et al*.,2007). It is a common disease among the children globally (Kim *et al*., 2017). Globally around 36 percent of the total population, which is estimated to be around 2.43 billion has dental caries in the secondary dentition. In about 620 million people or 9 percent of the population, it affects the deciduous dentition (Yadav and Prakash, 2016). Dental caries, is a multifaceted pathological process, and various environmental, microbial, genetic, behavioral and immunological factors are responsible for its onset and progression (Selwitz*et al*., 2007). Endogenous bacteria residing in the dental biofilm, i.e. dental plaque, ferment dietary carbohydrates and produce acidic by-products as a result of the metabolic process. The acids cause the pH in plaque to drop below a critical level, resulting in the demineralisation of tooth substance (Featherstone, 2004). Mutans streptococci are considered major pathogens in the initiation of caries due to their acidogenic and aciduric properties and their ability to adhere to the surface of tooth and coaggregate other bacteria (Thenisch *et al*., 2006).

The global burden of periodontal disease is distressing about 743 million people internationally and is considered as a major cause of tooth loss in the elderly (Lang *et al*., 2009). Gingival inflammation, a prerequisite for the onset of periodontitis, is also related to tooth mobility and tooth loss (Woelber*et al*., 2016). Periodontal diseases are mixed flora

and multifactorial diseases. Numerous host factors determine the individual susceptibility to disease (Popova *et al*., 2013). It is the need of the hour to emphasize that periodontal infections seem to originatefrom comparatively limited number of periodontal pathogens in composite dental biofilm (Haffajee and Socransky, 2005). Many different types of bacteria reside in the gingival crevice and are also present in variety of periodontal infections (Newman *et al*., 2006).Plaque may be divided into supragingival and subgingival portions. Gram positive bacteria predominate in the supragingival part and may include *Streptococcus mutans*, *Streptococcusmitis*, *Streptococcus salivarius*, *Streptococcussanguinus* and *lactobacilli*. Subgingival plaque contain primarily Gram negative anaerobes such as *Porphyromon asgingivalis*, *Actinobacillus actinomycetem comitans*, *Fusobacterium nucleatum*, *Prevotella intermedia* and oral spirochetes such as *Treponema denticola* (Aas*et al*., 2005; Paster*et al*., 2001). Periodontitis increases inflammation and inflammatory products elsewhere in the body. This inflammation may adversely impact individual's managing conditions such as coronary artery disease (CAD) (Schenkein and Loos, 2013), cerebral vascular disease (CVD) (Pradeep *et al*., 2010a) and type 2 diabetes (T2D) (Engebreston and Kocher, 2013).

Plants produce primary as well as secondary metabolites which have beneficial long-term health effect so these are used effectively to treat diseases. Specifically, it is the secondary metabolites that exert therapeutic actions in humans. It has been reported that more than one third of the total plant species, at one time or another, are used medicinally due to the amount and type of secondary metabolite they contain (Agbor and Naidoo, 2015).In developing countries globally, the rise in incidence of oral diseases, bacterial resistance to many antibiotics and other therapeutic agents, financial constraints and secondary bacterial infection in immune-compromised individuals highlighted the need for alternative and preventive measures, which are safe, effective, easily available and economical. (Palombo, 2011). *Acacia* is an important member of plant family Leguminosae, under kingdom Plantae, designated first by Linnaeus in 1773. About 1380 species of *Acacia*are found worldwide, approximately two-third of them are found in Australia and the remaining are scattered throughout tropical and subtropical regions of the world (Seigler,2003). It has been found that it possesses antibacterial, antiviral, anti inflammatory, antihypertensive, vasoconstrictor actions, antispasmodic activities and elicitanti oxidant and cytotoxic activity (Gilani *et al*. 1999; Malviya*et al*. 2011).

The present study evaluates the antibacterial activity of different solvent extracts of local *A. nilotica*plant present in and around Faisalabad city against the oral microorganisms associated with dental caries, periodontitis and determination of its phenolic acids through High performance liquid chromatography (HPLC). The cytotoxicity of these extracts was evaluated to assess the CC_{50} of these extracts.

MATERIALS AND METHODS

***Procurement of Medicinal plants*:** *Acacia nilotica* (kikar) was obtained from local source. The specimen was further identified and authenticated from Department of Botany, University of Agriculture, Faisalabad, Pakistan.

***Sample collection and extraction*:** The twigs of *A. nilotica* were ambient dried and sample was ground by using agrinding mill. The plant sample (20g) was extracted in three different solvents (deionized water 200 ml, 70% methanol; water 70: 30, v/v and 70% acetone: water 70: 30 v/v (Sultana *et al*., 2007). After extraction, the extract was passed through Whatman No. 1 filter paper and the filtrate obtained was concentrated in vaccum using rotary evaporator that then frozen and freeze-dried for further use.

***Bacterial Strains*:** Three bacterial strains*S.mutans* (ATCC 25175), *S. mitis* (ATCC 6249) and *P. intermedia* (ATCC 25611) were obtained from Institute of Microbiology, University of Agriculture, Faisalabad. *S. mutans* and *S. mitis*were grown on mitissalivarius agar (Himedia, India) in aerobic condition at 37^{o}C for 24 hrs. *P. intermedia* was grown on anaerobic basal agar (Himedia, India). The inoculated media was immediately incubated in an anaerobic environment for 72 hrs.

***Inoculum Preparation*:** The suspension of bacteria was adjusted through a spectrophotometer to 0.5 McFarland density.

***Agar Well diffusion method*:** All the extracts of *Acacia nilotica*were evaluated for their antimicrobial activity by agar well diffusion method with slight modification (Owhe-Ureghe*et al.*, 2010). This assay for each bacterial strain was performed on Mueller Hinton agar (Himedia, India) and anaerobic basal agar for facultative anaerobes and obligate anaerobes respectively to determine the antibacterial activity of plant extracts. Approximately 50 µL of the extract solution was poured into each well which filled them to fullness. These plates were incubated aerobically and anaerobically at 37^{o}C for 24 and 48 hours for facultative anaerobes and obligate anaerobes respectively. The diameter of zones of inhibition was measured in millimeters which represents the antimicrobial potential of the respective extract. Each extract was evaluated in triplicate to know the mean value.

***Determination of minimum inhibitory concentration (MIC)*:** Microdilution method, using 96 multi-well microtiter plates, was adopted with little modification to determine the MIC of plant extracts (Chaieb*et al*., 2013). Dry extract of plant was initially dissolved in 100 µl of dimethyl sulfoxide (DMSO). Then serial twofold dilutions of extract were prepared in Mueller Hinton broth and anaerobic basal broth. The standardized inoculum (10 µl) of each bacterial strain was added to wells containing serially diluted plant extracts and

incubated at 37°C for 24 h. P-iodonitrotetrazolium chloride (INT) was added to the wells (40 µl of 0.2 mg/ml) as an indicator of bacterial growth, and incubated at 37°C for 30 min (Buwa and van Staden, 2006). The MIC was taken as the lowest concentration of extract which did not show any growth.

***Minimum bactericidal concentration (MBC)*:** MBC values were determined by taking 10 µL of each well medium with no visible growth and inoculated on respective agar plates. After 24 h of incubation at 37°C, MBC was defined as the lowest concentration at which 99% of the tested bacteria were killed.

***Cytotoxicity assay (MTT)*:** The clorimetric MTT assay was executed in 96-well plates. Vero cells at a concentration of 5×10^4 cells were seeded in 96-well plate with 100 µl suspensions of cells in each well. When the cells anchored to the plate, the media was removed from the plate. A fresh media without serum was added containing various concentration of test compounds. Duplicate wells were set for each sample. The cells with test compounds were incubated for 48 hours. Subsequently, the cells were processed within MTT (Liu *et al*, 2003). Following incubation at 37°C for 48 hours, 20 µl of 5 g/l of MTT was added to each well and the cells were incubated for another 4 hours. The absorbance of the culture at a wavelength of 490 NM was measured. Control cells were cultured in the same way in conditions without drugs.

Percentage of inhibition for each set of data was calculated by using the given formula.

$$\text{Inhibition } \% = \left[100 - \left(\frac{\text{ODt}}{\text{ODs}}\right) \times 100\right]\%$$

ODt and ODs indicates the optical density of the test substances and the solvent control, respectively. The concentration of 50% cellular Cytotoxicity (CC_{50}) of test substances was calculated.

***HPLC analysis of plants extracts*:** Plants extracts were subjected to HPLC according to the procedure with little modification (San and Yildirim, 2010). For the removal of any bubble present in mobile phase, mobile phase was first passed through the degasser (DGU-12A, Schimadzu, Japan). Retention time and peak areas of samples were compared with the standards for both quantitative and qualitative analysis. Appropriate mathematical calculations were employed for estimation of amounts of different phenolic acids present in the plants extracts.

***Statistical analysis*:** Data was analyzed by using computer software, Statistical Package for Social Sciences Version 17 (SPSS Inc. Chicago, U.S.A).

RESULTS

***Antibacterial effects by well diffusion method*:** The different solvent extracts of *A. nilotica* showed varying degree of antimicrobial activity against tested microorganism. Concentration dependent sensitivity was exhibited by all the bacterial pathogens against all the extracts. The methanol extract of *A. nilotica* showed significantly higher activity against S. mitis, S. mutans and P. intermedia followed by acetone and aqueous extracts. The results of zone of inhibition against *S. mitis*, *S. mutans* and *P. intermedia*are depicted in Table 1, 2 and 3 and Fig. 1, 2 and 3 respectively by different extracts.

Table 1. Antimicrobial activity *A. nilotica* aqueous, acetone and methanol extract against *S. mitis*

Concentration mg/ml	Aqueous (zone of inhibition in mm)	Methanol (zone of inhibition in mm)	Acetone (zone of inhibition in mm)
0.625	9.00±1.15C	10.33±0.88D	9.67±0.33D
1.25	9.67±0.88C	11.33±0.67CD	10.00±0.58D
2.50	11.00±0.58BC	12.00±1.53CD	12.00±1.15CD
5.00	12.00±1.00BC	15.00±1.00BC	14.33±0.33BC
10.00	13.33±0.67B	20.00±1.15A	16.67±0.33AB
Vancomycin	17.10±0.21A	17.10±0.21AB	17.10±0.21A
PBS	0.00±0.00D	0.00±0.00E	0.00±0.00E
Overall Mean	10.30±1.12	12.25±1.36	11.40±1.22

Values are mean diameter of inhibition zones in millimeter (mm) ± S.E of three replicate; The Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05); PBS; phosphate buffer saline, vancomycin (30 µg) was used as reference antibacterial compound

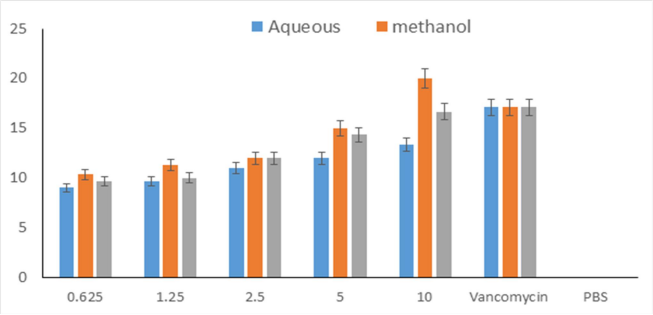

Figure 1. Graphic presentation of antimicrobial activity of *A. nilotica* aqueous, acetone and methanol extract against *S. mitis*

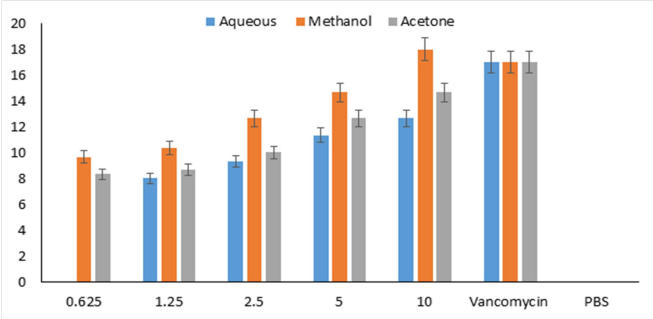

Figure 2. Graphic presentation of antimicrobial activity of *A. nilotica* aqueous, acetone and methanol extract against *S. mutans*

Table 2. Antimicrobial activity *A. nilotica* aqueous, acetone and methanol extract against *S. mutans.*

Concentration mg/ml	Aqueous (zone of inhibition in mm)	Methanol (zone of inhibition in mm)	Acetone (zone of inhibition in mm)
0.625	0±0E	9.67±1.45C	8.33±0.88D
1.25	8±0D	10.33±0.88C	8.67±1.2D
2.50	9.33±0.67CD	12.67±0.33BC	10±1CD
5.00	11.33±0.88BC	14.67±0.33AB	12.67±0.67BC
10.00	12.67±0.88B	18±1A	14.67±0.88AB
Vancomycin	17±0.58A	17±0.58A	17±0.58A
PBS	0±0E	0±0D	0±0E
Overall Mean	8.33±1.33	11.76±1.28	10.19±1.17

Values are mean diameter of inhibition zone in millimeter (mm) ± S.E of three replicate; The Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05); PBS; phosphate buffer saline, vancomycin (30 µg) was used as reference antibacterial compound.

Table 3. Antimicrobial activity *A. nilotica* aqueous, acetone and methanol extract against *P. intermedia*

Concentration mg/ml	Aqueous (zone of inhibition in mm)	Acetone (zone of inhibition in mm)	Methanol (zone of inhibition in mm)
0.625	8.67±0.88D	9±0.58C	10.33±0.88E
1.25	9.67±0.88BC	10.67±0.33BC	12.33±0.67D
2.50	10±1.15ABC	12.33±0.88B	13.67±0.33CD
5.00	12±1ABC	12.67±0.33AB	16±0.58AB
10.00	13.67±0.67A	14.67±0.33A	16.67±0.67A
Clindamycin	12.67±0.33AB	12.67±0.33AB	12.67±0.33CD
PBS	0±0D	0±0D	0±0E
Overall Mean	9.52±0.98	10.29±1.02	11.67±1.17

Values are mean diameter of inhibition zone in millimeter (mm) ± S.E of three replicate; The Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05); PBS; phosphate buffer saline, clindamycin (30 µg) was used as reference antibacterial compound

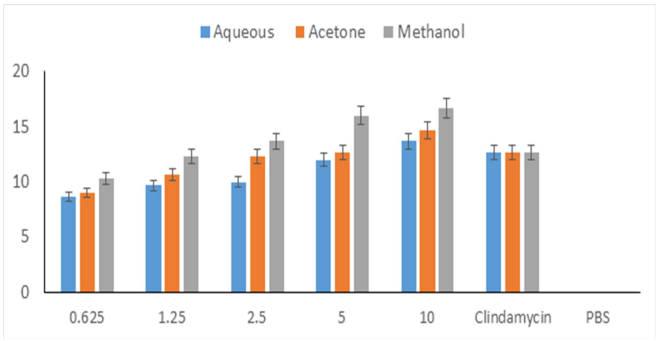

Figure 3. Graphic presentation of antimicrobial activity of *A. nilotica* aqueous, acetone and methanol extract against *P. intermedia*

***Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)*:** Three solvent extracts from *A. nilotica* were tested at various concentration and the evaluated MIC (Table 4 and Fig. 4).

Table 4. Minimum inhibitory concentration (MIC mg/ml) of different solvent extracts of *Acacia nilotica* against *P. intermedia*, *S. mitis* and *S. mutans*

Microorganism	Aqueous	Acetone	Methanol
Strepococusmitis	0.313±0.00c	0.313±0.00a	0.313±0.00b
Strepococusmutans	0.625±0.00a	0.313±00b	0.313±0.00b
Prevotellaintermedia	0.313±0.00a	0.313±0.00a	0.156±0.00b

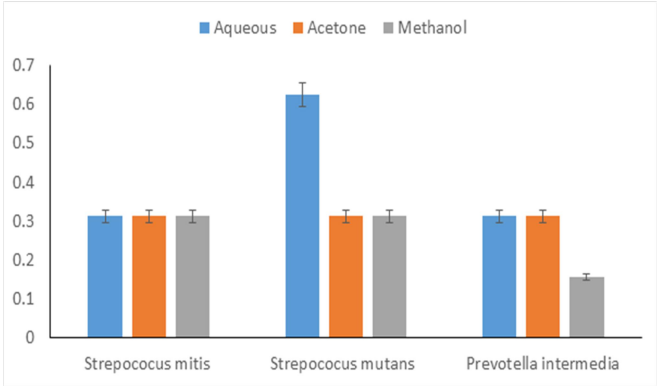

Figure 4. Graphic presentation of MIC of different extracts of *A. nilotica* against bacteria

The acetone and methanol extracts showed significantly higher activity (0.3125±00, 0.3125±00 mg/ml each) followed by aqueous extract against *S. mutans*. All three extracts showed similar activity against *S. mitis*. The methanol extract showed significantly higher activity than acetone and aqueous extracts against *P. intermedia*. The MBC values of tested microorganisms are depicted in Table 5 and Fig. 5. The MBC of acetone and methanol extract was same as MIC (0.3125 mg/ml) and MBC of aqueous extract was higher (0.625±00 mg/ml) than MIC against *S. mitis*.

The MBC of aqueous, acetone and methanol extracts was higher (1.25±00, 0.625±00 and 0.625±00 mg/ml) respectively than MIC against *S. mutans*.
The MBC of aqueous, acetone and methanol extracts was higher (1.25±00, 0.625±00 and 0.3125±00 mg/ml) respectively than MIC against *P. intermedia*.

Table 5. Minimum bactericidal concentration (MBC mg/ml) of different solvent extracts of *A. nilotica* against *P. intermedia*, *S. mitis* and *S. mutans*

Microorganism	Aqueous	Acetone	Methanol
Strepococusmitis	0.625±0.00c	0.313±0.00b	0.312±0.00a
Strepococusmutans	1.250±0.00a	0.625±0.00b	0.625±0.00b
Prevotellaintermedia	1.250±0.00a	0.625±0.00c	0.312±0.00b

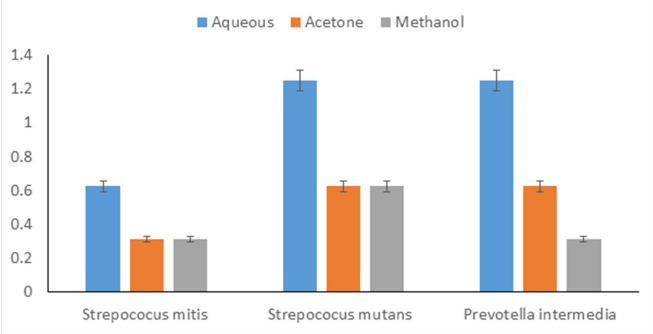

Figure 5. Graphic presentation of MBC of different extracts of *A. nilotica* against bacteria

***Cytotoxicity assay*:** All three solvent extracts of *A. nilotica* showed dose dependent cytotoxicity to Vero cells. Percentage inhibition of cells was increased with higher concentration of the extracts. The results of inhibition percentage by *A. nilotica* methanol, acetone and aqueous extracts are shown in Table 6, 7 and 8, respectively.

Table 6. Percentage inhibition of Vero cells by *A. nilotica* (methanol)

Concentration µg/ml	Absorbance	%age inhibition	t vs control
Control	0.96±0.07	-	-
78.125	0.65±0.01	32.26±4.39	7.210
156.25	0.52±0.01	45.95±2.94	15.452
312.50	0.38±0.02	59.96±2.81	21.216
625.00	0.31±0.01	67.14±2.38	28.083
1250.00	0.26±0.01	73.27±0.68	107.234

Assay were performed in triplicate Mean±SE

Table 7. Percentage inhibition of Vero cells by *A. nilotica* (acetone)

Concentration µg/ml	Absorbance	%age inhibition	t vs control
Control	0.96±0.07	-	-
78.125	0.70±0.02	27.17±3.93	6.728
156.25	0.68±0.01	28.88±3.85	7.328
312.50	0.58±0.01	39.60±3.91	9.975
625.00	0.51±0.01	46.70±2.60	17.75
1250.00	0.23±0.01	75.59±1.49	50.70

Assay were performed in triplicate Mean±SE

***Cellular Cytotoxicity fifty*:** The cellular cytotoxicity fifty (CC_{50}) was calculated through regression analysis from the inhibition dose response data. The aqueous extract showed significantly higher CC_{50} (929.65±47.48 µg/ml) followed by acetone (632.78±24.38 µg/ml) and methanol extract (278.43±29.77 µg/ml).
The results are shown in Table 9 and Figure 6.

Table 8. Percentage inhibition of Vero cells by *A. nilotica* (aqueous)

Concentration µg/ml	Absorbance	%age inhibition	t vs control
Control	0.96±0.07	-	-
78.125	0.74±0.02	23.11±2.99	7.472
156.25	0.70±0.00	26.65±4.87	5.332
312.50	0.60±0.05	36.82±9.09	3.983
625.00	0.50±0.02	47.40±4.94	9.494
1250.00	0.43±0.02	54.91±0.74	73.59

Assay were performed in triplicate Mean±SE

Table 9. CC_{50} µg/ml of different extracts of *A. nilotica*

Aqueous	929.65±47.48a
Acetone	632.78±24.38b
Methanol	278.43±29.77c

CC_{50} represent the reduction in cells number by 50% relative to control without test compound

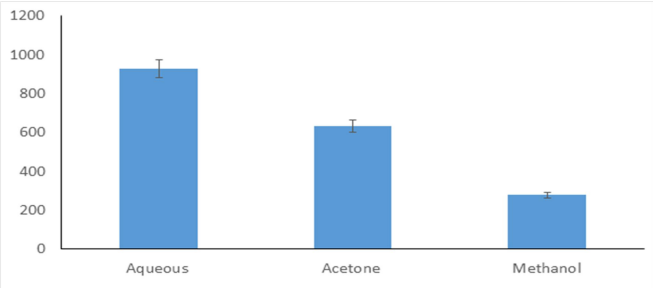

Figure 6. Graphic presentation of CC_{50} of different *A. nilotica* extracts

***HPLC analysis of A. nilotica*:** The aqueous, acetone and methanol extracts of *A. nilotica* were subjected to HPLC analysis to determine the phenolic acids in the extract. The maximum number of phenolic acids were determined in methanolic extract followed by aqueous and acetone extracts. Different concentration of phenolic acids were present in different extracts that are shown in Table 10 and Figure 7.

Table10. Phenolic of different solvents of *A. nilotica* (ppm)

Solvent	Caffeic Acid	Vanillic Acid	4-Hydroxy 3 Methoxy benzoic Acid	Syringic Acid	M-Comeric Acid	Cromato-tropic Acid	Gallic Acid	Chlorogenic Acid	P-Comeric Acid	Ferulic Acid	Quercetin
Methanol	4.10± 0.21B	7.10± 0.06A	30.30± 0.35B	4.90± 0.23A	3.13± 0.04A	27.87± 0.16A	0.00± 0.00	0.00± 0.00	0.00± 0.00B	0.00± 0.00	8.08± 0.09B
Acetone	0.00± 0.00C	0.00± 0.00B	66.30± 0.38A	0.00± 0.00B	0.00± 0.00B	0.00± 0.00C	0.00± 0.00	0.00± 0.00	5.13± 0.09A	0.00± 0.00	14.44± 0.28A
Aqueous Solution	7.96± 0.09A	0.00± 0.00B	26.14± 0.19C	0.00± 0.00B	0.00± 0.00B	17.30± 0.19B	0.00± 0.00	0.00± 0.00	0.00± 0.00B	0.00± 0.00	14.60± 0.18A

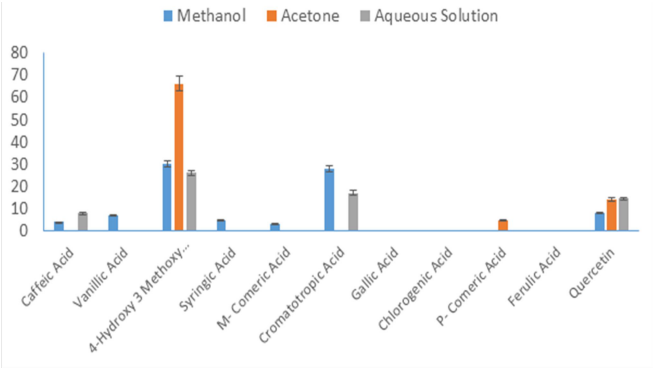

Figure 7. Graphic presentation of phenolic acids of different solvents of *A. nilotica* (ppm)

DISCUSSION

Oral diseases are still a major health problem throughout the world (Dash *et al*., 2014). Although the periodontal diseases and dental caries are major oral health problem but different lesions of oral cavity especially cancers of oral and pharyngeal tissues are also of great importance (Petersen, 2003). Oral health plays an important role in general well-being of humans. It is deeply concerned with the quality of life and has its effects on other systems of the body beyond the craniofacial complex. Oral diseases are among the most costly diseases to be treated in some countries (Sheiham, 2005).

Medication of botanical origin and other natural products are being used since long. Generally, these are effective against many ailments. Some natural products are under trial in the recent past for their effectiveness against oral pathogens (Palombo, 2011).

Phenolic products, a group of secondary metabolites of the plants are universally and more commonly distributed in various dietary products like fruits, beverages, vegetables and chocolates. Phenolic products may be subdivided into three main groups: flavonoids, phenolic acids and tannins (Borges *et al*., 2013). Acacia species were responsible for inhibition of cariogenic and periodontal pathogens (Pradeep *et al*., 2010b; Almas, 2001).

The present research was carried out to determine the antimicrobial potential of aqueous, methanol and acetone extract of *A. nilotica* against oral microorganisms associated with periodontal diseases and caries.

On the basis of agar well diffusion test, methanol extract of*A. nilotica* has significantly higher antibacterial effect against all tested microorganisms followed by acetone and aqueous extract. The MIC values were similar for all three tested solvents against *S. mitis* that was 0.3125±00 mg/ml and MBC value of aqueous extract was higher than MIC while MBC of acetone and methanol extract was same as MIC. The MIC value of methanol and acetone extract against *S. mutans* was lower (0.3125±00 mg/ml) than the aqueous extract. The MBC of all extracts was higher than MIC value against S. mutans. The methanol extract had lower MIC value (0.15625±00 mg/ml) as compared to other two extracts against P. intermedia. The MBC values of all solvents were higher than MIC.

Our findings coincide with result of (Saini *et al*., 2008) who reported that highest antibacterial activity was seen with methanolic extract of *A. nilotica*against various pathogens due to the presence of polyphenols and tannis. In another study, the aqueous isolated constituents and ethanol extracts of *A. nilotica* showed high antibacterial activity (Raghavendra*et al.*, 2006). The hydrophilic substances may easily be extracted by polar solvents like ethanol and methanol (Cos *et al*., 2006). In an otherstudy ethanolic extract of *A. nilotica* showed maximum activity against Gram negative and Gram positive bacteria (Khan *et al*., 2009) that was in accordance with our finding. In contrary to the earlier observation that Gram negative bacteria are scarcely susceptible to lower doses of plant extracts i.e 2 x 105 µg/mL (Suffredini*et al*., 2006). Our results showed activity against gram negative at dose of 0.3125±00 mg/ml. The susceptibility variation of test organism against various extracts might be influenced by the penetration of active ingredients of the extract through the bacterial cell wall and cell membrane. (Priya and Ganjewala, 2007). The HPLC analysis of *A. nilotica* showed that methanol proved to be the best solvent for the extraction of phenolic compounds followed by aqueous and acetone. The higher numbers of phenolic are shown in methanol extract of *A. nilotica* with highest concentration of benzoic acid (30.30±0.5ppm). The acetone extract of *A. nilotica* showed three phenolic

compounds with highest concentration of benzoic acid (66.30±0.54ppm) and in aqueous extract showed the presence of four compounds with higher concentration of benzoic acid (26.14±0.27ppm).

The cytotoxicity of different extracts of *A. nilotica* were observed on Vero cell to access the toxicity toward the normal cells. The % age inhibition was observed in dose depended manner. The CC_{50} of the extracts were calculated from dose response inhibition curve of cells. The higher the CC_{50} of tested compounds, highest is the safety of the compound. The aqueous extract showed significantly higher CC_{50} (929.65±47.48 µg/ml) compared to acetone (632.78±24.38 µg/ml) and methanol (278.43±29.77 µg/ml) extracts. The different solvents used for extraction behaved differently to Vero cells this was due to the phytodiversity or different methods linked with each phyto compound (Chatelain*et al*., 2011). The crude extract extracted with organic solvents were more toxic due to the presence of phenolic compounds (Kalaivani*et al*., 2011).

Conclusion: Methanol proved to be the best solvent for extraction of phenolic acids and its extract is most effective against pathogens under study. On the basis of CC_{50}, aqueous extract is the safest among the three solvent extracts to be used for therapeutic purposes. The results of present study suggest that *A. nilotica* may be used alone or in combination with other plant extracts in mouth wash or tooth paste to prevent oral diseases.

***Acknowledgement*:** The authors are grateful to Institute of Microbiology, University of Agriculture Faisalabad, for support of this work.

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