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Original Article

Amino acid profiling, viscosity and antibacterial activity of *acacia* gums from different locations in India

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ABSTRACT

Viscosity of *acacia* gums (also known as gum *arabic*) *viz. Acacia senegal*, *Acacia nilotica*, collected from different places, and that procured from Merck India Ltd. and Himedia, was determined using Digital Brookfield Viscometer. All the *A. nilotica* gum exudates showed Newtonian behavior with viscosity being shear rate independent and concentration dependent. Five percent solution of *A. senegal*, Sudan (ICAR-CAZRI, Jodhpur) displayed fairly high cP value (509.45) and exhibited non-Newtonian shear thinning / pseudoplastic characteristics. In the amino acid analysis of gum samples by reversed phase-high performance liquid chromatography, glycine was found to be present only in *A. senegal*, Sudan (ICAR-CAZRI, Jodhpur) and that too in a very high quantity (388.67 mg/g of gum) in comparison to other gum samples analyzed during the study. Proline was also on the higher side (31.31mg/g of gum). There appears to be some correlation between viscosity and the glycine as well as proline amino acid contents. Only two *A. nilotica* gum samples possess antimicrobial activity may be due to the presence of higher tannin content in them.

Key words: *Acacia nilotica*, *Acacia senegal*, amino acids, viscosity, antibacterial activity.

INTRODUCTION

Gums of plant origin are complex polysaccharides. Some of the polysaccharides have molecular weights in the range of 9.5 million. Gum exudated by different plants differs from each other due to their unique combination of sugars, the monosaccharide units. The most common sugars in plant gums are mannose, galactose, rhamnose, glucose, fructose, xylose, arabinose while the sugar acids are galacturonic acid and glucuronic acid [1]. Some of the sugars are linked by 1,4 bonds, while others by 1,6 bonds. Plant gums are natural polysaccharides with varying degree of viscosity, even at smaller concentrations. Their protein content influences their behavior. The amino acid composition thus can be a critical attribute for the chemical characterization of the polysaccharide gums. Amino acid contributes directly in the solubility and the functional properties of the gums. The chain length or degree of polymerization (DP) of the gum influences its viscosity and hydration rate. In comparison to small molecules, larger molecules tend to produce higher viscosities and take longer to hydrate. Highly branched molecules in the gum take up lesser space than a straight one with the same molecular weight, and, therefore, provides less viscosity like gum *arabic*. As a hydrocolloid molecule becomes longer, it sweeps out a much greater volume as it randomly tumbles in solution, leading to increased collisions with its neighboring molecules, resulting in increase in viscosity. The high viscosity experience at higher concentrations may be due to increase in the strength of molecules-molecules interaction and the corresponding reduction in molecule-solvent interaction. Thus, gelation concentration though varies with different gums but, invariably, viscosity of fully hydrated gum dispersions is concentration-dependent. Anderson *et al*. have reported that the amino acid composition of the proteinaceous component of gum *arabic (Acacia senegal* (L.) Willd. vary considerably, particularly in respect of serine and proline amino acids [2].

Infact, the surface activity, foaming and emulsifying properties of gum *arabic* are attributed to the protein molecules

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covalently linked to the polysaccharide and low viscosity [3, 4]. The antibacterial activity of *A. nilotica* gum, using freshly isolated reference strain of *Actinobacillus actinomycetemcomitans, Capnoc ytophaga spp., Porphyromonas valisgingi* has been reported by Clark *et al*. [5]. Antibacterial potentials of various solvent extracts of *A. nilotica* Linn. leaves, phytochemicals and seed-pot extracts have been reported by a number of researchers [6-9] . Recently Singh *et al*. and Dubey *et al*. have reported the antimicrobial activity of a number of gums and oleo gum-resins [10-12].

The present study reports the evaluation of amino acids, viscosity and antibacterial activity of *acacia* gums collected from different places.

MATERIALS AND METHODS

In the present communication, the amino acid profiling, viscosity and antibacterial activity of *A. nilotica* gum samples collected from Sitapur (Uttar Pradesh) (AN, SR), falling in Upper Gangetic plain region; Amritsar (Punjab) (AN, AR), falling in Trans Gangetic plain region; Bilaspur (Chhattisgarh) (AN, BR), Ranchi (Jharkhand) (AN, RI), Gondia (Maharashtra) (AN, GA), falling in Eastern plateau and hills region and Bundi (Rajasthan) (AN, BI), falling in Western dry region were evaluated. *A. senegal* gum samples, numbering two, from ICAR-CAZRI, Jodhpur (Rajasthan) and ICAR-IINRG Farm, Ranchi (Jharkhand) as also gum *acacia*, procured from Merck India Ltd. and Himedia, also were studied. For the separation and quantification of amino acids of gum samples collected from different places by reversed phase-high performance liquid chromatography (RP-HPLC), their derivatization was carried out using phenyl isothiocyanate (PITC) adopting standard procedure. PITC reacts with both primary and secondary amino acids, and the phenylthiocarbamyl (PTC) amino acid derivatives formed are stable enough for automated analysis without on-line derivatization [13,14]. Normally, reversed phase chromatography is not well suited for separating highly hydrophilic substances such as amino acids. However, because pre-column derivatization derivatizes samples before they are introduced to the column, amino acids can be modified with highly hydrophobic functional groups to enable reversed phase chromatography. Since reversed phase methods provide excellent separation, it allows extremely high throughout analysis, depending upon how parameter settings are configured.

Chemicals: All the chemicals, reagents and solvents used in the present study were of analytical grade and procured from Rankem, New Delhi and Merck India Ltd., Mumbai, India. L-amino acids reference standard kit (SRL), phenyl isothiocyanate (Sigma), HPLC grade acetonitrile and water were used for amino acid analysis by RP-HPLC. After manual cleaning and sorting all gum samples were fine powdered and passed through 0.4 mm mesh sieve and after purification [15] packed in air tight containers for further analysis.

Viscosity measurement: A digital Brookfield viscometer (Model: LVDV II+Pro; Spindle number, LV-2/ LV-4; Temp., 27.5 ^{0}C) was used for the determination of viscosity of gum samples. The instrument measures the torque required to rotate an immersed spindle in a fluid. Solutions of different concentrations of the gum exudates were prepared in distilled water, appropriate enough to immerse the spindle groove in the fluid. Different rotation speed (10, 20, 50 and 100 rpm) were set to determine its effect on the viscosity values as the drag force is known to alter with changes on the spindle size and rotational speed. Three measurements were taken for each concentration and at each rotational speed.

Amino acid profiling: Derivatization of standard amino acids and gum samples was carried out as per method followed by Elkin and Wasynczuk with slight modifications [16]. In 20 µl solution of standard amino acids (1 mM), 200 µl of coupling reagent (triethyl amine: acetonitrile: water; 5:2.5:1.5) was added and the samples left for one hour shaking intermittently. Twenty µl of PITC was added to this solution, mixed thoroughly and kept for 30 min. Derivatized amino acids were extracted with 0.5 ml of 0.1 M acetate buffer (pH 5.6) and 2.5 ml of methylene dichloride. After centrifugation clear aqueous top layer solution was collected using micropipette and stored for analysis on RP-HPLC. Derivatization of gum samples was carried out with 0.1% solution in distilled water and the rest of the procedure was the same as for standard amino acids. The resultant PTC derivatives were separated and quantified by RP-HPLC (Shimadzu, Kyoto, Japan).

Antibacterial activity: Gum samples were screened for antimicrobial activity in their saturated aqueous solutions and ethanolic extracts using four antimicrobial-drug-sensitive reference strains (*Streptococcus milleri* SM-22; *Bacillus mycoides* B29-19-1; *E. coli* E-382; *Salmonella abortusequi* E-155). Antimicrobial activity screening was done of saturated aqueous solutions and ethanolic extracts of gums, agar well diffusion assay was used. Dried ethanolic extracts were dissolved in dimethyl sulfoxide to 1% strength for testing. From ethanolic extract, 6 mm discs were made to contain

2.0 mg extract / disk. Antimicrobial activity was also tested against 97 clinical and environmental isolates belonging to 6 genera of Gram positive (34) bacteria (GPB) and 19 genera of Gram negative (63) bacteria (GNB). Ciprofloxacin disks (10 µg) were used as control antimicrobial disks.

RESULTS AND DISCUSSION

The physico-chemical characterization of seventeen *A. nilotica* gum samples collected from ten Indian States covering five agro-climatic zones has already been reported elsewhere [17]. The resultant PTC derivatives of gum samples were separated and quantified on RP-HPLC using gradient elution (buffer A, 0.1 M sodium acetate, pH 5.6; buffer B, 60% acetonitrile in water) which showed remarkable variations in their amino acid content (mg/g of gum). In the gum samples of *A. nilotica*, glycine (Gly) was not found. Amongst *A. nilotica* gum samples, the highest total amino acid content (371.08 mg/g of gum) was found in the sample collected from Sitapur (Uttar Pradesh) (AN, SR) and the lowest (73.63 mg/g of gum) in Bilaspur (Chhattisgarh) (AN, BR) sample. In the gum samples of *A. senegal*, Sudan (ICAR-CAZRI, Jodhpur), histidine (His) was not found while it was highest (137.72 mg/g of gum) in the sample from *A. senegal*, ICAR-IINRG Farm (Ranchi). The data thus clearly indicates that the gum sample of *A. senegal* from Sudan (ICAR-CAZRI, Jodhpur) (EC Number, 01332/84; origin of provenance, North Kodrofan province of Sudan) only contains Gly (388.67 mg/g of gum), which was absent in all other gum samples of *A. nilotica* and *A. senegal* collected from different places in India during the present study. Similarly, *A. senegal* sample from Sudan (ICAR-CAZRI, Jodhpur) also showed the presence of the highest amino acid content (502.78 mg/g of gum) (AS 3) (Table 1; Fig. 1a and 1b).

All the *A. nilotica* gum exudates showed Newtonian behavior with viscosity being shear rate independent and concentration dependent, as viscosity of 40 percent solution of gum exudates from Bilaspur (Chhattisgarh) displayed highest cP value (284.9) in comparison to 10 percent solution (48.8) using spindle number LV-2 at 27.5 ^{0}C. Increase in viscosity with concentration may be due to increase in number of high molecular weight polymeric chains of the gums per unit volume and increased interaction between these polymeric chains in aqueous solution, responsible for increase in cohesive density and, therefore, greater resistance to flow [18]. There was also a gradual decrease in viscosity of gum exudates as temperature was increased. *A. senegal* gum exudates and gum *acacia* from Merck and Himedia displayed low viscosity. On the contrary, 5 percent solution of *A. senegal*, Sudan (ICAR-CAZRI, Jodhpur) displayed fairly high cP value (509.45) and exhibited non-Newtonian shear thinning / pseudoplastic characteristics (Fig. 2).

There appears to be some correlation between viscosity and the glycine as well as proline amino acid contents. Glycine was found only in *A. senegal*, Sudan (ICAR-CAZRI, Jodhpur) gum exudates and in a very high quantity (388.67 mg/g of gum) in comparison to other gum samples collected and analyzed during the study. Similarly, proline amino acid was also present on the higher side (Table 1). This finding corroborates that the presence of higher percentage of glycine, proline and arginine amino acids in gums enhances their viscosity as in case of *guar* gum (*Cyamopsis tetragonolobus*) which possesses these amino acids and also very high viscosity [19]. None of the two GNB reference strains was sensitive to any of the gum samples while both of the GPB reference strains were sensitive to ethanolic extract of *A. nilotica* gum samples collected from Gondia (Maharashtra) and Bilaspur (Chhattisgarh). None of the four reference strain was sensitive to saturated aqueous solution of the gum samples. Out of the 101 strains, a total of 50 strains (49.5%) were sensitive to both the gum samples and 88 (87.1%) to ciprofloxacin disks. Of the 36 GPB tested, 26 were sensitive to both the gum samples and 28 to ciprofloxacin disks. Out of 65 GNB tested, only 24 were sensitive to both the gum samples and 60 to ciprofloxacin discs. Ciprofloxacin resistance was more common in GPB than GNB (p 0.04). Minimum inhibitory concentration (MIC) for sensitive strains ranged between 100 µg to 1250 µg/ml for GNBs and 100 µg to 640 µg for GPBs. All resistant strains had MIC >2mg/ ml (Fig. 3a and 3b).

Though only two *A. nilotica* gum samples possess antimicrobial activity, yet it has wide spectrum. The presence of antimicrobial potential in only two *A. nilotica* gum samples may be due to the presence of higher tannin content in them. Identification of active antimicrobial ingredient in gum samples needs further study.

CONCLUSION

The amino acid analysis of gum samples by RP-HPLC showed remarkably striking variations. Glycine was found to be present only in gum sample of *A. senegal* from Sudan (ICAR-CAZRI, Jodhpur) in a very high quantity as also higher quantity of proline amino acid which may be responsible for its enhanced viscosity in comparison to the rest of the gum samples collected and analyzed during the present study.

All the *A. nilotica*, *A. senegal* gum exudates and the gum *acacia* procured from Merck India Ltd. and Himedia showed Newtonian behavior with viscosity being shear rate independent and concentration dependent except *A. senegal* from Sudan (ICAR-CAZRI, Jodhpur) which displayed non Newtonian shear thinning / pseudo plastic characteristics. Only two *A. nilotica* gum samples possess antimicrobial activity may be due to the presence of higher tannin content in them.

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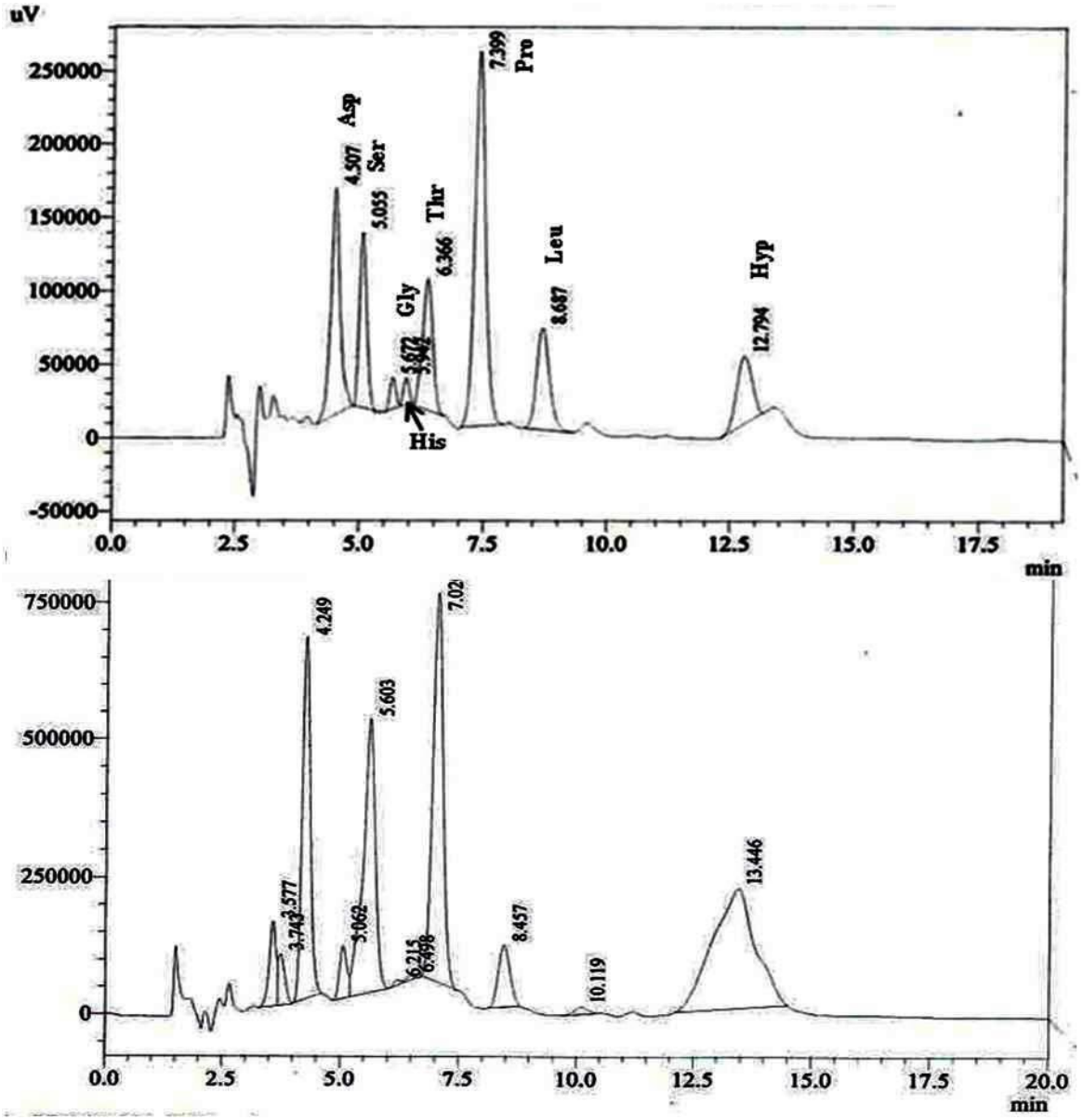

Fig. 1a: HPLC chromatogram of standard amino acids and *Acacia senegal* (Sudan, ICAR-CAZRI, Jodhpur)

Table 1: Amino acid content (mg/g of gum) in the *A. nilotica* and .*A. senegal* gum samples collected from different places in India

Amino acids	AN, BR	AN, GA	AN, RI	AN, AR	AN, SR	AN, BI	AS1	AS2	AS3	GAM
Asp	1.44	5.47	9.30	5.51	5.24	5.55	6.40	5.43	52.95	6.34
Ser	16.28	30.66	26.46	37.04	37.91	49.45	75.94	32.11	8.66	66.53
Gly	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	388.67	0.00
His	21.46	40.05	32.73	49.20	192.80	95.20	137.72	72.15	0.00	108.75
Thr	4.36	26.73	22.07	25.29	65.75	63.80	96.55	40.12	0.64	83.28
Pro	0.21	0.27	0.20	0.20	0.28	0.30	0.25	0.21	31.31	0.38
Leu	29.88	55.18	38.14	75.99	69.11	86.73	120.61	69.76	20.57	110.33
Total	73.63	158.37	128.89	193.24	371.08	301.03	437.48	219.78	502.78	375.62

AN, *A. nilotica* (BR, Bilaspur; GA, Gondia; RI, Ranchi; AR, Amritsar; SR, Sitapur; BI, Bundi).
AS1, *A. senegal* (ICAR-IINRG Farm, Ranchi); AS2, *A. senegal* (ICAR-CAZRI, Jodhpur); AS3, *A. senegal*, Sudan (ICAR-CAZRI, Jodhpur); GAM, Gum *acacia* (Merck India Ltd.).
Asp, Aspartic acid; Ser, Serine; Gly, Glycine; His, Histidine; Thr, Threonine; Pro, Proline; Leu, Leucine.

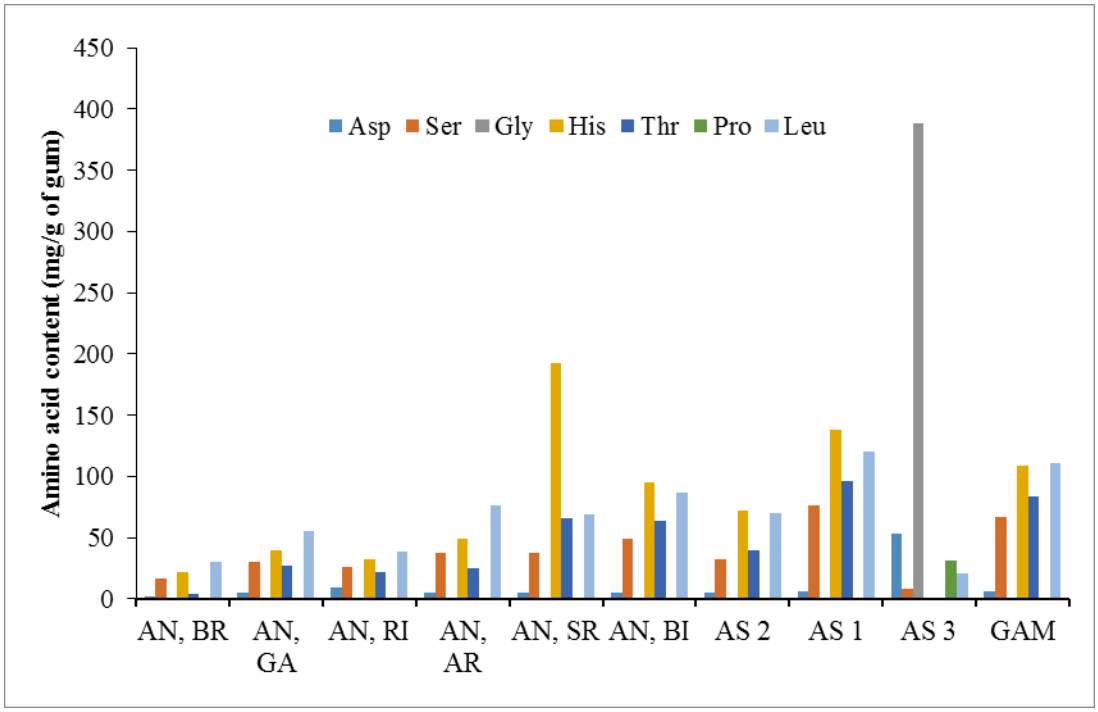

Fig. 1b: Amino acid content in gum samples collected from different places

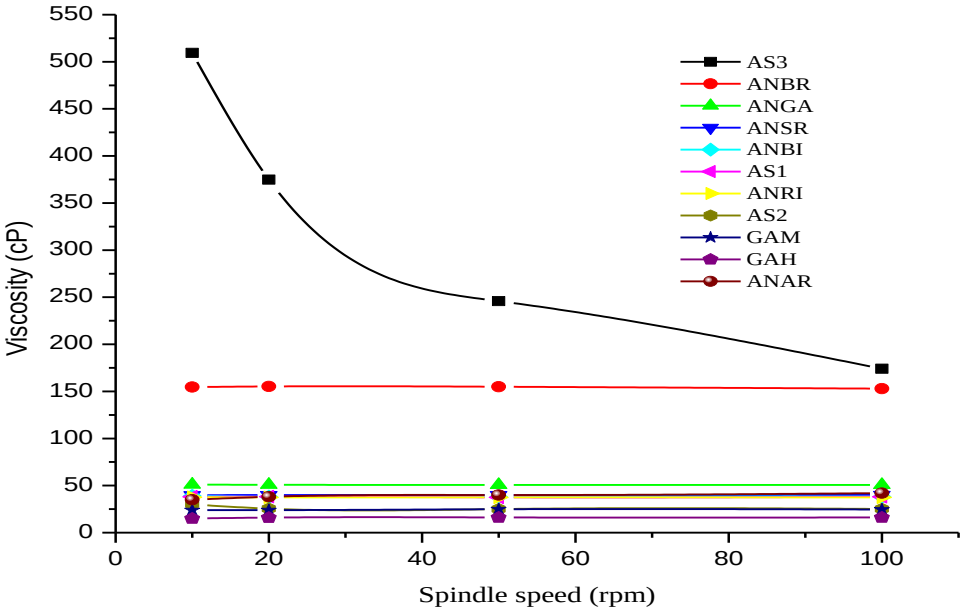

Fig. 2: Viscosity of *acacia* gums collected from different places

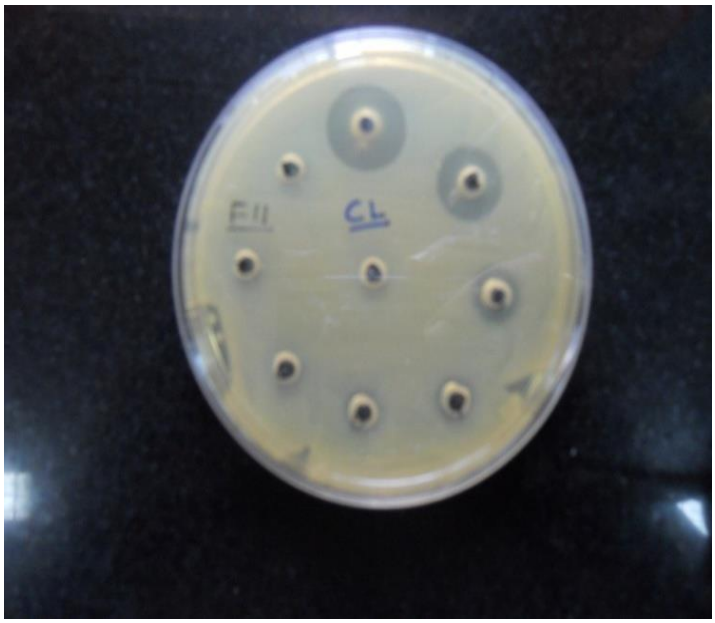

Fig. 3a: Determination of MIC of ethanolic extract of *A. nilotica* gum for a *Escherichia coli* strain (MIC of the strain was 1250 µg), the centre well is control negative filled with only DMSO while periphery wells contain the extract in DMSO (50 µl) [Clock wise 5 mg, 2.5 mg, 1.25 mg, 640 µg, 500 µg, 400 µg, 200 µg, 100 µg].

Fig. 3b: Determination of MIC of ethanolic extract of *A. nilotica* gum for a *Staphylococcus aureus* strain (MIC of the strain was 100 µg), the centre well is control negative filled with only DMSO while periphery wells contain the extract in DMSO (50 µl) [Anti clock wise 5 mg, 2.5 mg, 1.25 mg, 640 µg, 500 µg, 400 µg, 200, 100 µg]

REFERENCES

1. Snowden MJ *et al*. Functional characteristics of gum *arabic*. Food Hydrocoll 1987; 1(4):291-300.
2. Anderson DM *et al*. The amino acid composition of the proteinaceous component of gum *arabic (Acacia senegal* (L.) Willd. Food Add Cont 1985; 2(3):159-164.
3. Phillips GO, Williams PA (Eds.). Handbook of Hydrocolloids 2000, CRC Press, ISBN: 978 1 84569 414 2, Boca Raton, Florida.
4. Randall RC *et al*. The role of the proteinaceous component on the emulsifying properties of gum *arabic*. Food Hydrocoll 1988; 2(2):131-140.
5. Clark DT *et al*. The effects of *Acacia arabica* gum on the *in vitro* growth and protease activities of periodontopathic bacteria. J Clinical Periodontol 1993; 20(4):238-243.
6. Raghavendra MP *et al*. *In vitro* evaluation of antibacterial spectrum and phytochemical analysis of *Acacia nilotica*. J Agri Technol 2006; 2(1):77-88.
7. Vijayasanthi M *et al*. Evaluation of the antibacterial potential of various solvent extracts of *Acacia nilotica* Lin. leaves. Hygeia J D Med 2012; 4(1):91-96.
8. Mbatchou VC, Moubarak AO. Antibacterial activities of seed pot extracts of *Acacia nilotica* Willd to *Artenia salina* larvae. J Applied Bio Sci 2011; 40:2738-2745.
9. Mbatchou VC *et al*. Antibacterial activity of phytochemicals from *Acacia nilotica*, *Entada africana* and *Mimosa pigra* L. on *Salmonella typhi*. J Animal and Plant Sci 2011; 10(1):1248-1258.
10. Singh BR, Siddiqui MZ. Antimicrobial activity of *Commiphora wightii* gum (guggul gum) extract against Gram positive and Gram negative bacteria. J Microbiol Antimicrobial Agents 2015; 1(2):36-39.
11. Singh BR *et al*. Antimicrobial activity of natural edible gums. World J Pharmaceu Sci 2015; 3(11):2217-2221.
12. Dubey S *et al*. Antimicrobial activity of ethanolic and aqueous extracts of common edible gums against pathogenic bacteria of animal and human health significance. Research & Reviews: J Pharma and Nanotechnol 2015; 3(3):30-36.
13. Cohen SA *et al* . PITC derivatives in amino acid analysis. Nature 1986; 320:769.
14. Heinrikson RL, Meredith SC. Amino acid analysis by reverse phase-high performance liquid chromatography: Precolumn derivatization with phenylisothiocyanate. Anal Biochem 1984; 136:65.
15. Karawya MS *et al*. Investigation of the carbohydrate content of certain mucilaginous plants. Planta Medica 1971; 20:14.
16. Elkin RG, Wasynczuk AM. Amino acid analysis of feedstuff hydrolysates by pre-column derivatization with phenylisothiocyanate and reversed phase-high performance liquid chromatgraphy. Cereal Chem 1987; 64(4):226-229.
17. Siddiqui MZ *et al*. Physico-chemical properties and protein profiling of gum exudates of *Acacia nilotica* collected from different agro-climatic zones in India. Res J Chem Environ 2015; 19(4):29-36.
18. Siddiqui MZ *et al*. Evaluation of phytochemicals, physico-chemical properties and antioxidant activity in gum exudates of *Buchanania lanzan*. Proceedings of the Nat Acad Sci, India Sect B: Biol Sci 2015 (DOI: 10.1007/s40011-015-0539-4).
19. Anderson DM *et al*. The amino acid composition of the proteinaceous component of *guar* gum (*Cyamopsis tetragonolobus*). Food Add Cont 1985; 2(4):225-230.