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Antibacterial and qualitative phytochemical analysis of *Giloy* extract for application of herbal finish on cotton fabric

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The current study was planned to prepare antibacterial finish for grey cotton fabric using *Giloy* stem extract for healthcare applications. The selected grey cotton fabric was pretreated prior to application of the extract. For the extraction of the herb, maceration process was employed and the solution prepared was further subjected to soxhlet extraction to congeal the extract. *Giloy* extract was assessed for its phytochemical and antibacterial properties, to find out the bioactive components responsible for such activity. The application of the extract was carried out on pretreated cotton fabric using exhaust and pad dry cure methods (5 g/L). The treated fabric was assessed for its efficacy against the selected bacterium (*Pseudomonas aeruginosa*) using AATCC-100 Test method employing Agar well diffusion method. It is discernible from the results of the study that there was good antibacterial activity of *Giloy* stem extract treated fabric against *Pseudomonas aeruginosa* which was determined on the basis of zone of inhibition. It was also found that the finish nearly retained upto ten wash cycles and can be used for medical purposes.

Keywords: Antibacterial activity, Cotton fabric, Eco-friendly, *Giloy* stem, Soxhlet extraction
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The protective aspects of clothing and textiles have been the biggest ground for innovative developments. Along with a focus on protective and functional aspects of textiles, hygiene has also acquired importance in recent years. Though, there have been many developments in making the textile material more functional and productive, a continuous effort is being made at improving hygiene using eco-friendly methods. The increasing expansion of technical textiles and their applications has created numerous opportunities for the use of innovations finishes[1]. Microbial infestation has urged to develop resistant textiles. To control the growth of a microorganism is a tedious task where washing can only arrest the microbe but not destroy it completely[2]. In order to be free from microbes many chemical finishes are being used to impart bacterial resistance to fabric but have their own health and environmental hazards. Recently, the use of plants and it's derivatives have taken control in the preparation of antimicrobial finishes that are cost effective.

Various medicinal plants have been reported to serve as a natural agent for antibacterial finishing of natural fabrics. Among these, *Giloy* or *Guduchi* (*Tinospora cordifolia*) part of the family Menispermaceae is one of the most traditional and effective herb used for curing ailments and strengthening immune system in ancient medical system as a medicine since time immemorial[3]. *T. cordifolia* is a highly valued plant in medicine, whose uses and application regarding human benefits has been praised indefinable in various Vedic and Ayurvedic scriptures and its practices have also been highlighted[4]. Amongst the most versatile rejuvenative herbs, it mainly belongs to the tropical deciduous forests of south Indian peninsular plains.

Tinospora cordifolia is one of the most essential plants used in indigenous system of medicine and has innumerable references of its uses in traditional medicine[5]. The plant is native to India and maintains a unique place in Ayurvedic medicine as a strong adaptogen and aphrodisiac and is also known as *rasayana*[6]. It is also known as amrita, or "nectar of life," because it is effective at boosting the body's immune system and balancing the activities of many organs[7]. It has been reported that *Giloy* exhibits medicinal properties like anti-diabetic, anti-periodic,

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anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities[8].

The extract of different parts of the plant contains glucosides, alkaloids, a glycoside-giloin, gilosterol, alkaloid tinosporin, a non-glucoside-gelinie, tinosporic acid, tinosporol, berberine, tinosporidine, cordifol, columbin, chasmanthin, palmatine, isocolumbin, tembetarine, syringing, ecdysterone, cordioside, tinocordifolin, tinocordifolioside and cordifolioside, etc.[9]. It also contains carotenoids, terpenoids, flavonoids, polyphenols, tannins, saponins, pigments, enzymes and minerals. Phytochemicals are the major active principle components for many plant based drugs and are known to possess antimicrobial and antioxidant activity. *Giloy* has inhibitory effect on pathogenic bacterium such as *Staphylococcus epidermis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas saeruginosa*, *Enterobacter aerogenes* and mild effect on *Salmonella typi*, *Salmonella typhimurium*, *Proteus vulgaris*[10]. Similarly, *Rosachinensis* has been reported to possess antibacterial activity against disease causing bacterium *S. aureus* and *E. coli* due to the presence of bioactive compounds[11].

Antimicrobial textile products add to the quality of textiles as demand for fragrant, skin friendly and high performance fabrics goes on. The natural fibers such as cotton, jute, linen are highly susceptible to microbial contamination owing to their porous and moist nature. Cotton is the most widely used and preferred natural fabric owing to its properties and functionality. It is one of the most comfortable and functional fabric due to which cotton was selected for the present study. For sustainability, natural fabrics should have high levels of endurance and durability and by adding antimicrobial characteristics to the fabric, the fabric's functionality can be increased, resulting in new end uses. The use of non-toxic and eco-friendly antimicrobial textiles has become a matter of significant importance because of increased awareness about hazardous effects of synthetic antimicrobials. Individuals and the general public gain from the use of such products. Herbal finishes minimize the use of lethal chemicals which are harmful, thereby reducing textile pollution[12]. Therefore, the current study was designed to evaluate the qualitative photochemical properties and antibacterial activity of *Giloy* stem extract and its efficacy as a textile material.

Methodology

Collection of raw materials

Grey cotton fabric with 53.6×41.6 ends and picks per inch, 116 g/m^2 density was purchased from the local market of Hisar on the basis of visual properties and other physical and chemical tests. The collected samples were analyzed by physical, burning and solubility tests to determine that the samples collected were hundred per cent pure cotton. Among the hundred per cent pure cotton fabrics, medium weight grey cotton fabric was selected. Similarly, fresh green stems of *Giloy* herb were procured from Campus of CCSHAU, Hisar, which were further propagated in Hostel Garden so as to obtain ample amount of the powder required for the study. Other chemicals such as H_2SO_4 (98%), NaOH (98%), Acetic acid (98%), Soda ash and Sodium Sulphite were purchased from Merck, India.

Extraction of *Giloy* stem

Giloy stems were shade dried and grinded to form a fine powder. For extraction process, maceration and Soxhlet method were employed using aqueous solvent. Aqueous medium was used so that the bio-active components could be extracted completely. Similar extraction procedure was adopted by Saklani *et al.*[13] for the leaves of *Paeonia emodi* in order to assess the antimicrobial activity and for phytochemical analysis. For the same, concentration of the extract (150 g/L) and extraction time (36 h) were optimized on the basis of higher yield percentage keeping other variables constant were mixed in an MLR ratio of 1:40.

Maceration

The solution was kept in a shaking incubator for 36 h. The solution was then filtered using a muslin cloth and the filtrate obtained was further transferred to a round bottom flask for soxhlet extraction. Maceration was employed so that maximum active constituents could be dissociated in the solution.

Soxhlet extraction

The round bottom flask was kept on a heating mangle for evaporating extra water. The dried extract thus obtained was weighted and used for further application.

Qualitative phytochemical screening

The phytochemical analysis of *Giloy* stem extract was done in order to identify the bioactive components responsible for providing antibacterial properties to the plant. The analysis of alkaloids, tannins, flavanoids, saponins and phenols was done using standard test procedures given by[14].

Application of finish

Giloy stem finish was applied on cotton fabric after optimizing treatment pH and temperature on the basis of reduction percentage of bacterial count. On the basis of various trials the optimized conditions were pH- 5.5 and temperature- 60°C at an MLR of 1:20. Finish was applied using Exhaust and Pad dry cure methods.

Exhaust method

Considering the weight of the sample, quantity of *Giloy* stem extract and citric acid as cross linking agent was calculated. *Giloy* stem extract in concentration of 5 g/L was set in a bath with different optimized variables (pH and treatment temperature). The MLR was kept at a ratio 1: 20. The fabric samples were entered to the antibacterial bath and kept for 30 min. A post finish was given with citric acid (8% on the weight of the fabric) at room temperature. After the treatment, the samples were washed with cold water and then shade dried[15].

Pad dry cure method

On the basis of weight of the sample, quantity of the extract and citric acid was calculated. The fabric samples were immersed in a bath for 30 min where the MLR was taken as 1:20 and variables like treatment pH and treatment temperature were optimized. The fabric samples were placed in a trough (liquor space in mangle) containing the solution of *Giloy* stem extract (5 g/L concentration) for 5 min and passed through the extract solution. Further, the samples were passed amid two rollers of pneumatic padding mangle with a pressure of 2.5 psi and uniformly squeezed. The samples were again dipped in the extract solution and passed between the rollers of the padding mangle to give a wet pickup or maximum take up (two dip two nips operation). The finished samples were further sent for subsequent drying and curing operations. The samples were dried at 80°C for 3 min and cured at 120°C for 2 min on a lab model curing mangle. A post treatment was given to the finished samples with 8% citric acid as a fixing agent. The samples were then again padded on a two-roller pneumatic padding mangle at a pressure of 2.5 psi, dried and cured at 120°C[2].

Antibacterial assessment

To analyze the antibacterial efficacy of the finished fabric (AATCC-100 Test method) Agar well diffusion method was employed[16]. An LB (Luria Bertani) medium and broth was prepared where the broth was inoculated with the bacterium after autoclave and kept in an incubator. Prior to the growth of the bacterium, it was spread on LB plates and allowed to grow. After the growth of bacterium a uniform well was created using a sterilized cork borer and the extract was poured in the well for testing its antibacterial activity. It was determined by observing the zone of inhibition. For the analysis of finished samples an LB medium and broth was inoculated with bacterium along with the finished samples and control (broth without the samples) was autoclaved for 20 min at 121°C and 15 lbs pressure. It was then inoculated with the bacterial cultures (*Pseudomonas aeruginosa*) and kept in a shaking incubator for 24 h. A series of 8 test tubes were taken containing 9 mL distilled water, where 1 mL of the inoculum was transferred aseptically from the first test tube to the other. Serial dilution was done until its reduced dilution was 10^{-8} dilutions. The dilutions 10^{-5} and 10^{-8} were used to determine the bacterial count. After that 100 mL inoculum was taken from the broth and spread over the LB plates and incubator at 30±2°C for 24-48 h and the bacteria was allowed to grow. The bacterial colonies were counted and total colony forming units were calculated by using following formula:

$$\text{CFU} = \frac{\text{No. of colonies}}{\text{Amount plated (ml)}} \times \text{dilution factor}^{(17)}$$

The bacterial count or colonies formed were compared for the control and finished samples and the efficacy of the extract was evaluated.

Results and Discussion

The present study was undertaken to prepare and apply *Giloy* stem extract as an antibacterial finish on cotton fabric and to assess its phytochemicals and antibacterial activity. The efficacy of the finish with respect to antibacterial activity was studied by using standard methods. The results are presented as follows:

Qualitative phytochemical screening

The phytochemical analysis of *Giloy* stem extract was done in order to identify the bioactive components responsible for providing antibacterial properties to the plant. The analysis of alkaloids, tannins, flavanoids, saponins and phenols was done using standard test procedures given by Saidulu *et al.*[14].

i. ***Detection of alkaloids:*** For the detection of alkaloids, Wagner's test was employed. The extract was dissolved in dilute HCL and filtered. To the

Table 1 — Antibacterial activity of *Giloy* stem extract against *Pseudomonas aeruginosa*

Giloy stem extract concentration		1 mg/ mL	3 mg/ mL	5 mg/ mL
Zone of inhibition (mm)*	*Pseudomonas aeruginosa*	8.2	11.9	19.00
Controlled (sterilized distilled aqueous)			No inhibition	

*No activity (-mm), weak (<6mm), moderate (7-12 mm) and strong activity (>12)

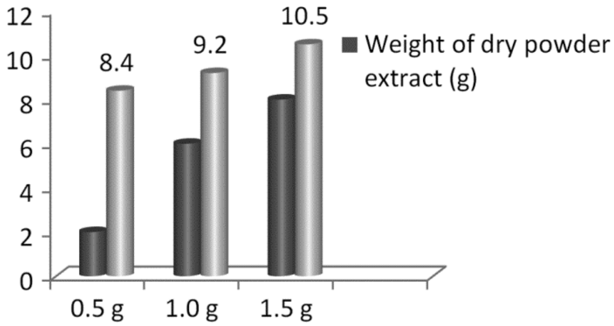

Fig. 1 — Optimization of concentration of *Giloy* stem extract

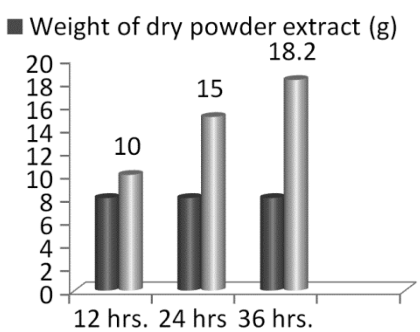

Fig. 2 — Optimization of extraction time of *Giloy* stem extract

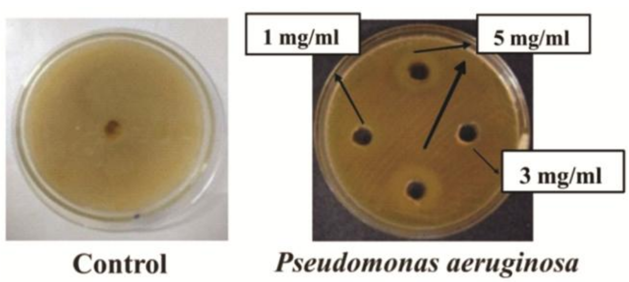

Plate 1 — Zone of inhibition produced at different concentrations of *Giloy* extract.

acidic solution, Wagner's reagent was added. The formation of brown precipitate indicates the presence of alkaloids.

ii. ***Detection of tannins:*** Ferric chloride test was carried out for the detection of tannins. To the filtrate of the extract, a few drops of ferric chloride solution were added. The formation of a blackish precipitate confirms the occurrence of tannins.

iii. ***Detection of flavonoids:*** Lead acetate test was performed for the detection of flavonoids. A few drops of lead acetate solution were added to the extract. The formation of yellow colour precipitate indicates the presence of flavonoids.

iv. ***Detection of saponin:*** Foam test was employed for the detection of saponin. 0.5 g of the extract was vigorously shaken with 2 mL of water. If the foam produced persisted for about 10 min, it indicates the presence of saponin.

v. ***Detection of phenols:*** For the detection of phenols, Ferric chloride test was performed. The extract was treated with 3-4 drops of ferric chloride solution. The formation of bluish black color confirmed the occurrence of phenols.

Table 2 — Standardization of pH and temperature for application of *Giloy* stem extract on cotton fabric

Variable	Bacterial Count (CFU/mL)
pH	
4.5	4.3×10^{8}
5.5	3.8×10^{8}
6.0	4.0×10^{8}
Temperature	
30°C	5.6×10^{8}
45°C	4.9×10^{8}
60°C	4.1×10^{8}

It is clear from Figure 1 that the optimum concentration for maximum yield of *Giloy* stem extract on the basis of maximum yield percentage was obtained with 1.5 g/L and therefore, selected as optimum concentration for the extraction of *Giloy* stem extract and further taken.

For optimizing extraction time for *Giloy* extract, maximum yield of *Giloy* stem was observed. It is evident from Figure 2 that the optimum time for maximum yield of *Giloy* stem extract was 36 h and therefore, selected as optimum extraction time for the *Giloy* stem extraction and continued for research.

Antibacterial activity of *Giloy* stem extract against *Pseudomonas aeruginosa*

As represented in Table 1, sterilized distilled water kept as control had no inhibition whereas the concentrations of 1 mg/mL and 3 mg/mL exhibited moderate zones of inhibition i.e., 8.2 mm and 11.9 mm, respectively while 5 mg/mL concentration had a strong zone of inhibition i.e., 19.00 mm against *Pseudomonas aeruginosa* (Plate 1). It was found that antibacterial activity increased with increase in concentration of the extract. A study conducted by Deorankar *et al.*[18] reported similar instances of antimicrobial activity of plant extract (*Clitoria ternatea*). The ethanol and aqueous extracts of the root of *C. ternatea* showed good efficacy against *S. aureus*, *E. coli* and *P. aeruginosa.* The results are well supported by the findings of Nageswari *et al.*[19] who reported an increase in zone of inhibition for increased concentrations (10, 20, 30, 40 and 50 mg/mL) of aqueous extracts of different plants. It is evident that with the increase in the concentration of the extract the efficacy increases which may be due to higher concentration of phytochemicals that dissolve in the solution.

Standardization of variables for application of *Giloy* stem extract

As presented in Table 2, temperature and pH was standardized for best results on the basis of minimum bacterial count. Different temperature ranges (30°C 45°C and 60°C) and pH ranges (4.5, 5.5 and 6.0) were analyzed and it was found that highest antibacterial activity was observed at 60°C with a bacterial count of 4.1 x10^8 and highest antibacterial activity was observed at pH 5.5 with a bacterial count of 3.8±10^8. It may be because certain phytochemicals are pH and temperature dependent which get activated for specific temperature and pH conditions.

Bacterial resistance of *Giloy* stem extract finished fabric against growth of *Pseudomonas aeruginosa*

The antibacterial activity of *Giloy* stem extract finish against growth of *Pseudomonas aeruginosa* on finished samples was counted quantitatively by AATCC-100 test method. The antibacterial activity of finished grey cotton samples finished by exhaust and pad-dry cure methods and inoculated with *Pseudomonas aeruginosa* was compared to its controlled samples. The bacterial resistance was determined by comparing the bacterial colony counts and calculating the percentage reduction of controlled and finished samples after different time periods (24 h, 48 h, 72 h and 96 h). It is discernible from the results in Table 3 and plate 2 and 3 that after 24 h the per cent reduction in bacterial growth for treated samples by exhaust and pad dry cure method was 95.22 and 96.00% respectively. The reduction in bacterial count of finished fabric may have been a result of the presence of certain anti-oxidants and bioactive compounds of *Giloy* stem. The antibacterial activity of some secondary metabolites has been reported earlier and revealed that these secondary metabolites inhibit the growth of micro-organisms in many ways such as by inhibiting protein synthesis, breaking the peptide bonds, inferring with nucleic acid synthesis, functioning as chelating agents, interfering with cell wall construction, blocking metabolic pathways, or preventing microorganisms from utilizing available nutrients[20]. It was also observed that the antibacterial activity decreased with increase in time.

Efficacy of *Giloy* stem extract finished fabric with respect to washing

The efficacy of *Giloy* stem extract finish was analyzed against antibacterial activity of pathogenic

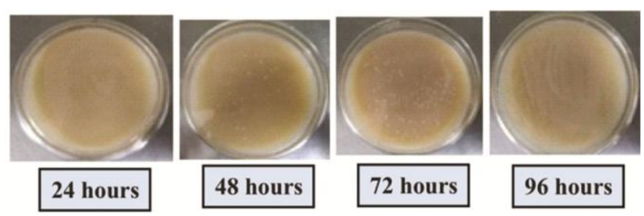

Plate 2 — Bacterial resistance of *Giloy* stem extract on finished cotton fabric against growth of *Pseudomonas aeruginosa* (Exhaust method)

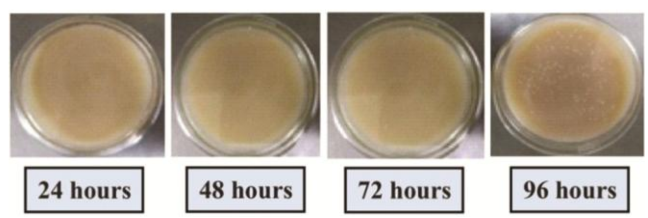

Plate 3 — Bacterial resistance of *Giloy* stem extract on finished cotton fabric against growth of *Pseudomonas aeruginosa* (Pad dry cure method)

Table 3 — Antibacterial activity of *Giloy* stem extract finished fabric against growth of *Pseudomonas aeruginosa*

Pathogenic Bacteria / Incubation Period / Application methods**	*Pseudomonas aeruginosa* * Bacterial count (CFU/mL) (% Reduction)			
	24 h.	48 h.	72 h.	96 h.
Exhaust method	4.3x10^8 (95.22 %)	7x10^8 (92.22%)	8.2x10^8 (90.8%)	10.3x10^8 (88.55%)
Pad dry cure method	3.6 x10^8 (96.0 %)	6x10^8 (93.33%)	7.6x10^8 (91.55%)	9.6x10^8 (89.33%)
Controlled fabric	Confluent growth			

*Dilution mean of 10^5 and 10^8 CFU/mL
** Concentration 5 g/L

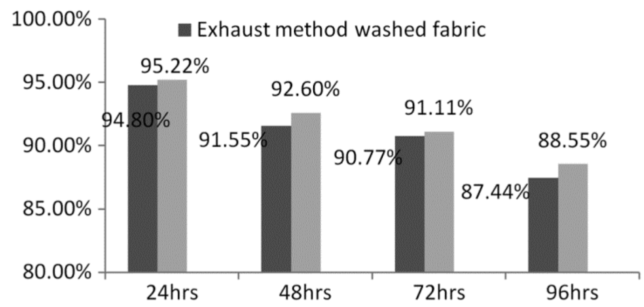

Fig. 3 — Efficacy of *Giloy* stem extract finish with respect to washing

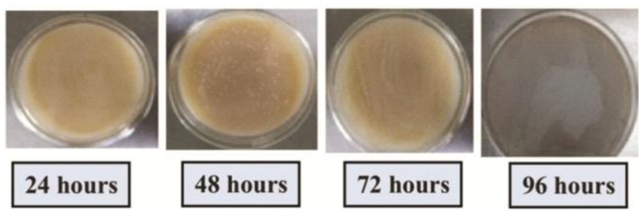

Plate 4 — Efficacy of *Giloy* stem extract on finished washed cotton fabric against growth of *Pseudomonas aeruginosa* (Exhaust method)

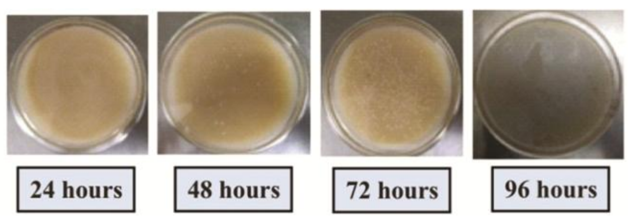

Plate 5 — Efficacy of *Giloy* stem extract on finished washed cotton fabric against growth of *Pseudomonas aeruginosa* (Pad dry cure method)

bacteria *Pseudomonas aeruginosa* after five wash cycles in Launder-o-meter using standard test method. Post washing, percent reduction was checked after 24 h, 48 h, 72 h and 96 h of inoculation of bacterium with dilution factors 10^5 and 10^8. It is clear from Figure 3 and plates 4 and 5 that the percentage reduction in bacterial growth (after 24 h of inoculation) on washed cotton sample finished by exhaust and pad dry cure methods with 5 g/L concentration of *Giloy* stem extract with dilution factors 10^5 and 10^8 was found to be 94.80% and 95.22% respectively. It was observed that the finish retained upto ten wash cycles and then needs to be finished again.

Conclusions

Antimicrobial finishing of textile material is gaining much interest for scientific researches. It has been observed that owing to the inert properties of natural fabrics, these are susceptible to microbial attack and deterioration. Plants are a great source of various bio active agents that are capable enough of providing resistance towards micro-organisms. Plants are renewable and can be grown easily. Also, the extraction of metabolites and active agents is quite economic and reliable. Herbal finishes are eco-friendly in nature and have beneficial effects on human health. For this purpose, various parts of the plants including roots, stems, leaves, flowers, fruits and seeds exhibiting antibacterial properties can be used to prepare extracts. Plants possess bioactive compounds such as tannins, phenols, flavonoids, essential oils, alkaloids etc. *Giloy* stem is a great source of various anti oxidants and bio active compounds which makes it suitable enough to be used as a finishing agent for bacterial resistance apart from its medical use only. It is clear from the study that *Giloy* stem extract applied with 5 g/L concentration exhibited excellent antibacterial activity on finished fabric against human pathogenic bacteria i.e., *Pseudomonas aeruginosa.*, therefore, can be effectively used as antibacterial finish on the textiles. The efficacy of the extract on cotton fabric was found better with pad dry cure method as compared to the exhaust method. Though, the finished fabric resulted in good efficacy even against washing hence it is recommended for medical purposes, infants garments and other uses where antibacterial property of the fabric is required.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Authors' Contributions

The concepts and research design of the article has been contributed by NA, written, assembled and edited by KM. All the technical assistance and authentication was provided by KM and SA whereas the technical editing and review of the manuscript was done by NC. All the authors have read and approved the final manuscript.

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