“Comparative analysis by total yield, antimicrobial and phytochemical evaluation of curcuminoid of district Kasur: With its potential use and characterization in electrospinning nanofibers”

Tanzeel Rehman Charan¹, Muhammad Aqeel Bhutto¹, Mihr Ali Bhutto¹, Azhar Ali Tunio¹, Ghulam Murtaza¹, Umair Aftab², Farhartullah Kandhro³ and Sheeraz Ahmed Khaskheli¹

Abstract

It has been reported through various studies that turmeric of different geographical regions has variabilities regarding the number of phytochemicals, some have a better curcuminoid yield, some have better antimicrobial and antioxidant activities, and even turmeric of some regions does not show compatibility with modern applications due to having unfavorable quality. So, here in this study, we have tried to demonstrate the potential and efficacy of locally produced turmeric powder from district Kasur, Pakistan,

¹Institute of Biotechnology & Genetic Engineering, University of Sindh, Jamshoro, Pakistan
²Department of Metallurgy & Materials Engineering, Mehran University of Engineering and Technology, Jamshoro, Pakistan
³College of Technology, Liaquat University of Medical and Health Sciences, Jamshoro

Corresponding author:
Tanzeel Rehman Charan, Institute of Biotechnology & Genetic Engineering, University of Sindh, Jamshoro 71100, Pakistan.
Email: trcharan@scholars.usindh.edu.pk

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through a comparative study with turmeric powder of international grade, analyzing their activity against pathogenic micro-organisms, presence of the number of phytochemicals, and presence of curcuminoid compounds like curcumin, extracted in four different solvents (ethanol, methanol, acetone and chloroform), and were verified by TLC and FTIR spectroscopy. This study justifies that the locally produced turmeric has better quality and potential as it gives a maximum curcuminoid yield of about 25% in Ethanol solvent and showed maximum zone of inhibition against E.coli about 14mm and 16mm against Staphylococcus aureus. While Antifungal activity was also observed high; as compared to the same amount of does of international grade turmeric extract. Further, electrospun nanofibers successfully generated from curcuminoid of Kasur Pakistan with PVA, present uniform, smooth nanofibers with an average diameter of 227.49 nm. Finally, this study suggests that turmeric of Kasur has similar or more potential as compared to turmeric of international grade, and can be used/replaced in nanotechnology labs for various applications.

**Keywords**

Medical textiles, polymer formation, electrospinning, fabrications, nano fibers

**Introduction**

Curcumin is a well-known traditional medicine, and has a long history of use in Asian countries such as India, Pakistan, Nepal, Bangladesh, China, and Indonesia, throughout the centuries; against various diseases and disorders like lung diseases, chronic kidney diseases, metabolic diseases, inflammatory diseases, cardiovascular diseases, liver problems, neurological disorders, digestive disorders and diabetes. Recently, it is also being successfully tested against AIDS, Alzheimer’s, cancer, COVID-19, etc. Curcumin is a yellow-colored polyphenol curcuminoid compound of the turmeric plant (*Curcuma longa* L) that can be extracted from its rhizomes along with two other curcumin derivatives demethoxycurcumin and bis-demethoxycurncumin, both are antioxidant agents; have the cytotoxic activity and showed the strong inhibitory effects on tumor cells growth. Curcuminoid extraction methods may be different depending on their applications and uses. Commonly nonpolar solvents like acetone, ethanol, methanol and chloroform have been used for extraction as curcuminoids are poorly soluble in hydrocarbon solvents.

The quality and quantity of curcuminoids and other compounds of turmeric plants depend upon the agro-climate zones of their harvest. The quality and potential of medicinal plants like turmeric longa of different regions are mostly assessed by their potential in antimicrobial, antioxidant activities and the presence of suitable medicinal phytochemicals, such as saponin, tannin, flavonoids, alkaloids, etc.

Electrospinning is the most advanced and popular technique to fabricate various nanofibers from natural and synthetic polymers. Electrospun nanofibers are widely used in various applications of biomaterials like wound dressings, antimicrobial membranes,
drug delivery systems and in edible food packing materials. Recently, electrospinning technology used successfully for encapsulating medicinal oils, antibiotics, hydrophobic chemicals and drugs on various nanofibrous polymers for different biological and medicinal purposes.

In the electrospun nanotechnology research lab, mostly turmeric of international grade purchased from the international market is used for the extraction of curcumin, curcuminoids, and turmeric oils for various biological and medicinal studies. However, Pakistan also produces very fine quality turmeric, from district Kasur, which is easily available in local markets. In the present study, we have comparatively assessed the quality and potential of turmeric harvest in district Kasur and compared its phytochemicals, antimicrobial characteristics with turmeric of international grade, extracted in most commonly applied four different solvents separately. Further, its extracted Curcuminoid was successfully applied to fabricated electrospun nanofibers with synthetic polymer and morphologically analyzed by SEM and FTIR spectroscopy for its potential use in future studies.

**Materials and methods**

**Chemicals and reagents**

Polyvinyl alcohol (PVA), sodium chloride (NaCl), potassium chloride (KCl) and potassium iodide (KI), dextrose, peptone, agar, acetone, methanol, ethanol, H₂SO₄, glucose, 80% phenol were of analytical grade and purchased from Sigma Aldrich. Ferric chloride, sodium hydroxide, acetic acid, potassium sodium tartrate, alkaline sodium carbonate, copper sulphate-potassium sodium tartrate, alkaline solution, folin-Ciocalteu, 10% aluminum chloride, 5% sodium nitrate, 0.1% ferric chloride (FeCl₃), 0.2 M phosphate buffer (Ph 6.6), acetic anhydride, all obtained from Merck, Germany. All the reagents and chemicals used were of analytical grade.

**Samples collection and Extraction process**

Turmeric rhizomes samples of district Kasur (KP) and international grade (IG) authenticated and recommended, verified by the Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. Washed and dried samples were cut into small pieces and crushed into an electrical grinder separately in order to make fine powder and passed through the sieve of 0.4 mm to obtain a uniform sample of both varieties, separately. The Soxhlet extraction method is the well-known, most applied method for the extraction of curcuminoids. Briefly, 60 g of turmeric powder were subjected to extract curcuminoids from both varieties of turmeric separately in four different solvents (ethanol, methanol, acetone, chloroform).
Determination of the total yield

The product yield in % and quantity of distillate solvents were calculated properly at the end of each process. The % of yield was calculated by using the following equation.

\[
\text{\% yield} = \frac{\text{total amount of extract}}{\text{total amount of turmeric powder}} \times 100
\]  

(1)

Evaluation by the presence of phytochemicals

Both samples of IG and PK were analyzed for the comparative analysis of the presence of phytochemicals such as Alkaloids, Saponin, Tannin, Flavonoids, Phytosterol, Phenol, Anthraquinone, Cardiac glycosides, Carbohydrates, Terpenoids and Steroids, through precipitation and coloration reactions methods, as reported in our earlier work.\(^{17}\)

Determination of Alkaloid (Wagner’s test)

To determine the presence of Alkaloids in samples, Wagner’s test was applied. Briefly, 50 mg of dried extract were mixed and stirred with 3 mL of HCl in the test tube and filtered thoroughly into another test tube. The presence of alkaloids was determined by the appearance of reddish-brown precipitates appear in the samples.\(^{18}\)

Determination of Saponin

Saponin was determined by the earlier reported method of Permatananda et al., briefly, 5 mL of distilled water was mixed with 2 mL of extract and shaken vigorously. The positive results were confirmed through the formation of foam in test samples for a long time.\(^{19}\)

Determination of Tannins

The presence of tannins was determined as earlier reported by Chanda et al., briefly, mixing 1 mL of distilled water with 1 mL of extract in a test tube, then few drops of ferric chloride solution were poured into the test tube. The appearance of blue color confirms the presence of gallic tannins and green black color confirms catecholic tannins.\(^ {20}\)

Determination of flavonoids

NaOH and HCl test: In 2 mL of turmeric extract, 2 mL of 10% NaOH were added and mixed gradually then treated with 2 mL of HCl. The presence of flavonoids was confirmed through the appearance of the yellow-orange color in the samples.
Zn test: In a test tube with 4 mL of extract few amounts of zinc dust were placed in the bottom, then a few drops of concentrated HCl were added to agitate the solution. The appearance of red color in samples confirms the presence of flavonoids.  

**Determination of sterol**

The presence of sterol was determined by the earlier reported method of Ikpeama et al., briefly, 2 mL of plant extract was added with 1 mL alcohol and 1 mL of H<sub>2</sub>SO<sub>4</sub>. The presence of sterols was confirmed through the formation of violet or green color in the samples.

**Determination of phenol**

The presence of phenol was determined by the reported method of Chanda and Ramachandra. Briefly, 3 mL of extract were treated with four drops of FeCl<sub>3</sub> solution. The appearance of the bluish-black color of solutions indicates the presence of phenol.

**Determination of Anthraquinone**

The presence of anthraquinone was determined as earlier reported by Sawant and Godghate. Briefly, in a test tube, 1 mL of H<sub>2</sub>SO<sub>4</sub> was used to hydrolyze 4 mL of extract then 1 mL of benzene with 1 mL of NH<sub>3</sub> added to samples. The test was confirmed positive when rose-pink color appears in the samples.

**Determination of glycosides**

The presence of glycosides was determined as described in keller-killani test. In brief, plant extract and glacial acetic acid at fraction of 3:2 were mixed in a test tube, then one drop of FeCl<sub>3</sub> was added to the solution. The test was considered positive after indicating a brown color ring in the test samples.

**Determination of carbohydrates**

For carbohydrates, 3 mL of extract were treated with approximately 4–5 drops of Iodine solution and the appearance of blue color in the solutions indicates the presence of Carbohydrates.

**Determination of Triterpenoids and Steroids**

For the determination of the presence of triterpenoids and steroids, 4 mL of extract were treated with 2 mL of concentrated H<sub>2</sub>SO<sub>4</sub> and results were determined by observing red color at the lower layer as steroids while yellow color at the lower layer indicates the presence of triterpenoids.
**Determination of curcuminoids by thin layer chromatography (TCL)**

The extracts of acetone were tested through Thin Layer Chromatography (TCL) for the justification of curcuminoids compounds: curcumin, bisdemethoxycurcumin and demethoxycurcumin. Briefly, pre-coated silica gel plates were used for the TLC test, and the Camag twin-trough glass tank with saturate mobile phase (benzene: ethyl acetate 18:2) and 10 cm of height was developed for each plate. The spotted results on plates were visualized under U.V light and noted for determination of curcumin demethoxycurcumin and bis-demethoxycurcumin.25

**FTIR spectroscopy of curcuminoid**

The Thermo-Scientific FTIR Spectrometer (Nicolet iS10. In the region of 3500-750 cm−1 at room temperature) was used to determine the molecular structure of extracted curcuminoid. All the spectra were processed by using OMNIC FTIR software, and further plotted using Origin pro 2019b.

**Antimicrobial activities**

Pre-cultured, the Gram-negative *E. coli* and Gram-positive *Staphylococcus aureus* bacteria in Mueller Hinton agar were obtained from IBGE (Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan).26 A small portion of pre-cultured microorganisms through the inoculation loop was transferred into prepared 10 mL Luria Bertani (LB) media and stored in an incubator at 37°C for 24 h as previously described in our work.17,27 The turbidity of LB media was adjusted using 0.5 McFarland standard at a concentration of 10⁸ Cells/mL. The fungal specimens of pre-cultured *Aspergillus niger* and *Penicillium crysogenum* in Petri-dishes of potatoes dextrose agar were also obtained from IBGE.26 In vitro, antibacterial and antifungal activities were analyzed as reported in our previous work.17,27 However, the minimum dose 50,000 µg/mL of curcuminoids in each sample was used to analyze the antimicrobial activities.

**Electrospinning parameters**

Initially, 10% PVA solution was prepared in de-ionized water and developed into a complete homogenous mixed solution at a magnetic stirrer for 2 h at 80°C. For the preparation of PVA-Curcuminoid nanofiber material, 10% of curcuminoid with respect to the PVA content was mixed homogenously in the prepared PVA solution again at a magnetic stirrer for further 4 h. The prepared solution of PVA-Curcuminoid was electrospun using an in-house electrospinning setup. Electrospinning parameters were optimized to obtain the better synthesis of nanofibers at,15 KV, DC power supply, the distance between collector and tip of the needle was fixed at 10 cm and the fellow rate was fixed at 1 mL/h. While 2 mL of solution was electrospun every time. All the samples of nanofibers were collected on aluminum foil. The procedure was followed under room temperature and in controlled humidity.28
Morphological characteristics of electrospun nanofibers

Morphological analysis for size, diameter and structure of nanofibers were observed through scanning electron microscopy at an accelerating voltage of 20 kv. The images were captured through the scanning electron microscope (SEM) JSM-6380L JEOL. While ImageJ software was used to examine and measure the average diameter of the nanofibers by analyzing at least 50 nanofibers randomly in SEM images and data were recorded in ± standard deviation. The molecular structure of nanofibers was determined through FTIR spectroscopy.

Results and discussion

Determination of the total yield

The total curcuminoid yield of both varieties of turmeric in four different solvents was separately calculated after each Soxhlet extraction and their total amount of yields and respective percentages are presented in Table 1 and compared in the comparative graph in Figure 1.

The obtained results indicate that the turmeric of KP has a little bit more content of curcuminoid, extracted in various solvents as compared to the counterpart turmeric of IG. The ethanolic extracts give the maximum amount of curcuminoid as we obtained 20.55% and 25% from turmeric of IG and KP respectively. In the previous work of Sawant and Godghate, obtained 25% curcuminoid yield in ethanol. However, yields % from other solvents were remarkably small as compared to the turmeric of KP. It may be due to any one of our applied variations in the Soxhlet method, like change in temperature, time, quality of solvents, or quality of turmeric. While in all the extraction processes we applied the same amount of 200 mL of solvent and at the end of the extraction processes re-obtained 160–190 mL through the rotary distillation process.

| Solvents  | Turmeric powder | Extract (g) | Percentage (%) | Extract (g) | Percentage (%) |
|-----------|-----------------|-------------|----------------|-------------|----------------|
| Ethanol   | IG 60           | 12.33 ± 1.24| 20.55          | 15 ± 0.8    | 25             |
| Acetone   | IG 60           | 10.33 ± 0.94| 17.2           | 11.6 ± 0.47 | 19.3           |
| Methanol  | IG 60           | 8.4 ± 0.45  | 14             | 11.3 ± 0.94 | 18.83          |
| Chloroform| IG 60           | 8 ± 0.4     | 13.33          | 10.66 ± 0.47| 17.76          |

Given values are means of triplicated determination (n = 3) ± standard deviations.
A comparative evaluation of the presence of phytochemicals in all samples of turmeric extracts was screened out. The obtained results indicate that samples of turmeric KP and IG have approximately the same kinds of phytochemicals, these results are presented in Table 2 and the bar chart in Figure 2(b). However, we didn’t work on a detailed study on the quality and quantity of these phytochemicals obtained from all the samples.

While in previous various studies on phytochemicals of different regions showed variable contents of phytochemicals in turmeric extracts, therefore in this study we have determined phytochemicals present in both samples and evaluate both samples with each other. Phytochemicals are chemical substances that are naturally present in medicinal plants, possessing some important chemical and medicinal properties. Like saponin help in decreasing blood lipids and reducing the risk of cancer, tannin was found helpful for feed efficiency and protein digestibility in animals, flavonoids have anti-inflammatory and antioxidant effects, alkaloids are also well known as cardioprotective and anti-inflammatory agents.8–11

The obtained results of this study confirm the presence of 11 different phytochemicals, the majority of phytochemicals were present in acetone extracts of both turmeric samples.

**Determination of curcuminoinds by TLC**

The obtained TLC results showed no difference in both samples of turmeric for curcumin, demethoxycurcumin and bis-demethoxycurcumin. The observed Rf value of curcumin from both samples of acetone extract was 0.8, for demethoxycurcumin was 0.7 and for bis-demethoxycurcumin was 0.6. The Rf value was calculated through the following equation
Previously various researchers like Wahyuni et al., Gupta et al., Revathy et al., Janßen and Gole, mentioned different mobile phases at different frictions to assist curcuminoid compounds. However, in this study, we have applied benzene:ethylacetate (18:2 ratio) as reported by Gupta et al., and adopted $R_f$ of IG as standard to compare the same compound present in KP. The above-mentioned results show the presence of the same quantity and quality of curcuminoid compounds in both varieties of turmeric.

**FTIR spectroscopy of curcuminoid**

FTIR spectrum of isolated curcuminoid from local turmeric KP was compared with available literature. FTIR report in Figure 3, shows a characteristic stretching band of O-H at 3303 cm$^{-1}$. The peak at 2929 cm$^{-1}$ represents C-H Stretching and the 1622 cm$^{-1}$ peak was assigned to C = C symmetric aromatic ring stretching. The peak at 1510 cm$^{-1}$ represents C = O, while the enol C-O peak was obtained at 1270 cm$^{-1}$ and benzoate trans-C-H vibration was at 962 cm$^{-1}$. The TIR spectrum of the isolated curcuminoid showed similarity with the FTIR spectrum reported in the literature.

**Antimicrobial activities**

we have evaluated in vitro antimicrobial activities of all samples. The inhibitory effects of curcuminoids of all the turmeric extracts from ethanol, acetone, methanol and chloroform on selected species of bacteria and fungi were observed through agar well diffusion method. The results were recorded after measuring the area of the circular zone of

$$R_f = \frac{\text{Distance traveled by the spot of sample}}{\text{Distance traveled by the spot solvent}}$$

| Phytochemicals | Ethanol | Acetone | Methanol | Chloroform |
|---------------|---------|---------|----------|------------|
|               | IG      | KP      | IG       | KP         | IG        | KP         | IG        | KP         |
| Alkaloids     | +       | +       | +        | +          | +         | -          | +         | +          |
| Saponin       | -       | +       | +        | +          | -         | -          | +         | +          |
| Tannin        | -       | -       | +        | +          | -         | -          | -         | -          |
| Flavonoids    | +       | +       | +        | +          | +         | +          | +         | +          |
| Sterol        | -       | -       | +        | +          | +         | +          | -         | -          |
| Phenol        | -       | -       | +        | +          | +         | +          | -         | -          |
| Quinone       | +       | +       | +        | +          | +         | +          | +         | +          |
| Cardiac glycosides | -     | +       | +        | +          | -         | -          | -         | -          |
| Carbohydrates | +       | +       | +        | +          | +         | +          | +         | +          |
| Terpenoids    | +       | +       | +        | +          | +         | +          | +         | +          |
| Steroids      | -       | +       | -        | -          | +         | +          | -         | -          |
inhibitions around the drug-loaded wells. The results are presented by mean of triplicate determination ($n = 3$) ± standard deviation in Table 3.

Study on *E. Coli* the maximum zone of 15 mm with an average mean of 14±0.81 mm was observed from methanol extract of KP, which is also high as compared to other...

**Figure 2.** (a) Samples of testing, is Anthraquinone, ii Cardiac glycosides, iii Flavonoids Zn test, iv flavonoids HCl test, v phenol, vi Phytosterol, vii terpenoids, viii Saponin. (b) chart of total present photochemicals in various extracts.
extracts. Study on *Staphylococcus aureus*: showed maximum sensitivity to an acetone extract of KP, its maximum zone of inhibition was 17 mm with an average mean of 16 ± 0.81 mm.

The maximum zone of inhibition of 18 mm against *Aspergillus niger* with an average mean of 17 ± 0.81 mm was observed from chloroform extract of KP, followed by 15.33 ± 1.2 mm and 15.33 ± 1.6 mm of acetone extract of KP and chloroform extract of IG respectively. The maximum average mean of inhibitory zone against *Penicillium crysogenum* was observed from acetone extract of KP about 16 ± 0.8 mm as compared to its counterpart IG which showed maximum activity 15.3 ± 1.2 from acetone. The observed results confirm the potential antimicrobial activity of both KP and IG turmeric extracts, presented in Table 3, however KP show slightly better results as compared with IG. The comparative scatter chart in Figure 4(a) to (c) present the comparative analysis with error bars.

Therefore, the above presented all the results validate and prove that extract of KP from various solvents has similar and, in some respect, a little bit more antimicrobial potential even though it can produce more yield as compared with turmeric extract of IG.

**Electrospinning**

For the evaluation of potentiality, suitability and characteristics of extracted curcuminoid from turmeric of KP. The plant extract (acetone) without any purification and treatment was applied with PVA (Poly-vinyl alcohol) for the synthesis of electrospinning nanofibers. This study is only confined up to curcuminoid, the initial extract of turmeric, which also possesses volatile oils along with curcumin and curcumin derivatives.

After some initial optimization, the nanofibers of PVA with curcuminoid were successfully electrospun from an aqueous solution. The samples of nanofibers mates around
Table 3. Detailed Results of antibacterial and antifungal activity of curcuminoid of IG and KP of various solvents.

| Extract of turmeric in | Bacteria | Fungi |
|------------------------|----------|-------|
| IG                     | KP       | IG    | KP   |
| E. coli               | S. aureus| A. niger | P. cry |
| Ethanol               | 9.66 ± 0.4 | 11 ± 0.81 | 13 ± 0.81 | 13 ± 1.4 | 12.66 ± 0.9 | 14 ± 1.6 | 13.43 ± 1.2 | 15.33 ± 0.4 |
| Acetone               | 11.3 ± 0.4 | 13.33 ± 0.47 | 14 | 16 ± 0.8 | 13 ± 1.4 | 15.33 ± 1.2 | 15.3 ± 1.2 | 16 ± 0.8 |
| Methanol              | 13 ± 0.81 | 14 ± 0.81 | 10 ± 0.8 | 11 ± 1.6 | 10 ± 0.8 | 13 ± 0.81 | 9.6 ± 0.9 | 9.66 ± 0.94 |
| Chloroform            | 11 ± 0.8 | 11.66 ± 1.2 | 10.66 ± 1.4 | 12.33 ± 0.4 | 15.33 ± 1.69 | 17 ± 0.81 | 13 ± 0.9 | 12.33 ± 0.4 |

Given values are means of three replicates (n = 3) ± standard deviations.
the size of $113.1 \pm 20.4$ cm$^2$ were collected on vertical standing aluminum foil, depicted in Figure 5(a).

**Morphological characteristics of electrospun nanofibers**

The morphology of PVA and PVA-Curcuminoid nanofibers presented in Figure 5(b) and (c), was assessed through SEM and observed smooth nanofibers, without any crystal formation and beads. The average diameter of the nanofibers was analyzed by using ImageJ software from the surface and cross-sectional SEM images. The average diameter of nanofibers of PVA and PVA-Curcuminoid were $281 \pm 68$ nm and $227 \pm 49$ nm respectively.
FTIR spectroscopy of nanofibers

FTIR spectra of PVA and PVA-Curcumin nanofibers are presented in Figure 5. The spectrum of PVA has major peaks from O—H stretching at 3249 cm$^{-1}$, stretching and vibration of C-H from alkyl group at 2941, -CH stretching (CH$_3$) at 2916 cm$^{-1}$, CH$_2$ bending at 1417 cm$^{-1}$, and C-O stretching at 1083 cm$^{-1}$. The spectrum of fibers of PVA-Curcuminoid presents the similar peaks and features of PVA and curcuminoid that indicate minor interactions occurred in these two compounds Figure 6.

The size, diameter, and chemical structure of nanofibers are the important parameters that help to determine the potential values of synthetic nanofibers. Previous various studies have highlighted the importance and use of PVA-Curcumin nanofibers in various applications.
In another study, Dede et al., presented that electrospun biofibers loaded oils of medicinal plants have various use and importance in various biological fields and applications. Herein this study, the fabricated nanofiber contains three curcuminoid compounds and turmeric oils, which suggest future use of these fibers in various applications such as wound dressing, food packing and antimicrobial membranes.

Overall result reports present that PVA-Curcuminoid nanofibers were successfully fabricated with better properties that support the potential of locally produced curcuminoids for modern applications, particularly in nanofibers technologies.

**Conclusion**

It has been reported in various studies that turmeric powders of different regions have different quality and potential, which can be assessed by analyzing their activity against pathogenic micro-organisms, presence of the number of phytochemicals, and presence of curcuminoid compounds like curcumin. Herein this study, we have comparatively evaluated locally produced turmeric powder (KP) with turmeric powder purchased from the international market (IG). The observed results of this study provide the following conclusions that turmeric powder of KP generates maximum curcuminoid yield, showed maximum antimicrobial activities against selected pathogens and have the same number of phytochemicals as were compared with turmeric of IG. Further electrospun nanofibers generated from curcuminoid of KP with PVA justify the potential of curcuminoid in modern nanofibers applications. So, the overall results suggest that turmeric of KP has similar or more potential as compared to turmeric of IG, and can be use/replace in nanotechnology labs.

**Declaration of conflicting interests**

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**ORCID iDs**
Tanzeel Rehman Charan  
https://orcid.org/0000-0003-4332-5342
Azhar Ali Tunio  
https://orcid.org/0000-0002-0797-4436

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**Appendix**

**Abbreviations**

- KP: Kasur Pakistan (turmeric of District Kasur)
- FTIR: Fourier transform infrared spectroscopy
- IG: Turmeric of international grade
- LB: Luria Bertani media
- MHA: Mueller Hinton Agar
- PDA: Potatoes Dextrose Agar
- PVA: Polyvinyl alcohol
- SEM: scanning electron microscope