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RESEARCH ARTICLE

In vitro biological activity of seed oil of *Withania coagulans* and synthesis of catalyst from residual biomass

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Abstract

Withania coagulans is a well-known medicinal plant reported to possess numerous biological activities. However, the plant remains underutilized compared to other medicinal plants due to lack of clinical and analytical studies. In the present study, *in vitro* biological activities of oil extracted from seeds of *W. coagulans* were studied along with synthesis and characterization of catalyst from residue obtained post oil extraction. The extracted oil exhibited DPPH-radical scavenging activity, protein denaturation activity and *in vitro* -α amylase inhibitory activity. These activities were comparable to the corresponding activities of standard compounds indicating diverse medicinal potential of the oil. The residue obtained post oil extraction was utilized for synthesis of catalyst. The synthesized catalyst was characterized to identify the nature of the catalyst. Catalyst synthesized from residue was found to be silica-based catalyst, crystalline in nature and EDX mapping revealed catalyst to comprise of C, O, Na, Si, P and Ca. Interpretation of results obtained depict potential utilization of *W. coagulans* for diversified traditional as well as industrial application.

Keywords

Withania coagulans ; seeds; biocatalyst; XRD; SEM; EDX

Introduction

Withania coagulans Dunal popularly known as Indian rennet or Doda paneer is a well-known medicinal plant with widespread pharmaceutical as well as medical properties including anti-inflammatory, antioxidant, antimicrobial, antitumor and anti-hyperglycaemic activities (1-3). *W. coagulans* is utilized in treatment of diabetes, cardiovascular, asthma, nervous exhaustion and constipation. This endangered plant species remains underutilized due to extremely slow propagation rate in nature and lack of commercial cultivation; however, the plant has been recognized as Indian rennet due to its cheese making property which can be explored effectively in dairy industry. Most of the studies reported so far pertaining to medicinal and pharmacological properties of *W. coagulans* have been confined to the extract of the plant. However, presently there exists a research gap pertaining to exploring medicinal potential of oil of the plant. In recent past, a large number of scientific approaches have also focused on utilizing agro waste for synthesis of different value-added products. One such approach is synthesis of cata-

lyst from residue recovered as by product post processing of plant raw material for different purposes. Numerous plant species are utilized for extraction of oil which results in production of substantial volume of residual waste. Management and disposal of such waste is a challenge as well as environmental concern. Utilization of such residue for synthesizing catalyst is considered to be a promising alternative. A recent study reported the synthesis of a heterogeneous catalyst from waste obtained from *sesamum indicum* and the utilization of this catalyst for the production of biodiesel from sunflower oil (4). Such practices not only prevent dumping of agro waste into the environment (after oil extraction from respective plant species) but simultaneously increase the commercial viability of the plant through synthesis of catalyst with specific applications. Another similar study described the synthesis of catalyst from waste of *Brassica nigra* plant which was also reported to play catalytic role in biodiesel synthesis (5). A review on different aspects of utilizing waste materials as source of catalyst implicated that a prime reason why scientific interest has gained momentum in utilizing agro waste types as feedstock for catalyst synthesis is due to the issues associated with management of agro waste (6). Catalyst possesses potential advantages like high precision, high activity under mild environment conditions and high yield number. Bio-catalysis has proved to be a convenient platform for many challenges in to synthetic organic chemistry (7). Silica based catalyst are among the common type of catalyst synthesized from plant feedstock. Sodium silicate is a silica precursor with the features of easy availability and low cost, which contributes to its economic viability in industrial purposes (8). Silica is very stable, chemically versatile, biocompatible, and can be extracted from diverse sources (9). In the present study, *in vitro* antioxidant, anti-inflammatory and anti-diabetic activity of oil of seeds of *W. coagulans* were studied and the residue recovered as by product after oil extraction was utilized for synthesis of silica-based catalyst along with its analytical characterization.

Materials and Methods

Material

Oil extraction

In the present study, the protocol reported in a previous article was utilized for extraction of oil from seeds of *W. coagulans* (10). After oil extraction the solid residue recovered was further utilized for synthesis of catalyst. Solid residue was calcinated at 7000 °C for 5 h and then refluxed for 3 h at 500 °C in (4 M) NaOH solution (200 mL) and pH was adjusted to 7. The solution was filtered, liquid portion was discarded and precipitates were calcinated for 1 h at 900 °C following which synthesized Catalyst was recovered.

DPPH-radical scavenging assay

The antioxidant activity of essential oil of *W. coagulans* was performed by DPPH scavenging assay according to the method reported in a previous study (11). BHT, Rutin and Ascorbic acid were utilized as standards. UV absorbance was recorded at 517 nm and DPPH scavenging activity was determined by:

$$\text{Scavenging activity (\%)} = [(\text{Abs c} - \text{Abs s}) \div \text{Abs c}] \times 100$$

Where, Abs c= Absorption of control , Abs s= Absorption of sample

Protein denaturation assay

Anti-inflammatory potential was assessed as per an earlier reported methodology (12). To different concentrations of oil; 25, 50 and 75 µg/mL, 1 % aqueous solution of bovine albumin fraction was added followed by adjusting the pH to 7.0 and the samples were incubated for 20 min at 37 °C. After incubation, denaturations of the samples were done by heating at 57 °C for 20 min. All samples were cooled at RT. Diclofenac Sodium was utilized as standard and UV absorbance was recorded at 660 nm. Inhibition of protein denaturation was determined by:

$$\text{\% inhibition} = [(\text{Abs c} - \text{Abs s})/\text{Abs c}] \times 100$$

Where, Abs c= Absorption of control , Abs s= Absorption of sample

In vitro -α amylase inhibitory assay

Anti-diabetic potential of oil was studied using *in vitro* -α amylase inhibitory assay reported in a previous study (13). Acarbose was utilized as standard. Fresh starch solution (0.5 % w/v), was prepared by mixing 0.125 g of potato starch into 25 mL 20 mM sodium phosphate buffer, pH was adjusted to 6.9. α-amylase solution was prepared, with 2 units/mL, in ice-cold distilled water. Acarbose and prepared extracts were dissolved in buffer to attain desired concentrations. For the colour reagent, a solution was prepared by mixing sodium potassium tartrate (12.0 g tetrahydrate) with aqueous NaOH (8.0 mL of 2 M). Additionally, a solution of 96 mM 3,5-dinitrosalicylic acid was also prepared. All samples were mixed with starch solution allowed to react with the α-amylase solution for 3 min. Resulting mixture was then combined with the dinitro salicylic acid solution and placed in a water bath at 85 °C for 15 min. Reduction level of dinitro salicylic acid was measured at 540 nm.

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

Synthesis of catalyst

The residue recovered post oil extraction was thoroughly washed with tap water. Solid residue was treated with 20 % HCl for 5 h and again washed with tap water, filtered and left overnight to dry at room temperature. Residue was taken in crucible and calcinated at 700 °C for 5 h in muffle furnace. Calcinated residue was refluxed for 3 h at 50 °C in (4 M) NaOH solution (200 mL). After refluxing, complete precipitation by concentrated HCl till pH 7 was attained. The solution was filtered and washed with distilled water and left overnight for drying. The sample was calcinated again for 4 h at 900 °C. The prepared catalyst was collected.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

FTIR analysis of synthesized catalyst was conducted to identify different functional groups / classes of organic compounds present in catalyst. FTIR analysis was performed with model "Nicolet Summit LITE" (Instrument Serial: BFJ2010008). The catalyst was properly blended with potassium bromide (KBr) and gently pressed to obtain the pellet. The spectrum of catalyst was recorded at 400-4000 cm^{-1} scanning resolution.

X-ray Diffraction (XRD) analysis

The crystallinity and phase composition of the catalyst were determined using the XRD technique. The detector used in XRD was D/teX Ultra 250. The scan axis of XRD was Bragg's angle (2θ) in the range of 5^0 - 80^0 with a scan speed of 2 °/min.

Scanning Electron Microscopy (SEM)/Energy Dispersive X-Ray Spectroscopy (EDX)

Morphological study and elemental mapping of sample were studied by scanning electron microscope (model Carl Zeiss EVO 18).

Results and Discussion

Powdered seeds of *W. coagulans* were utilized for extraction of oil. Residue recovered after oil extraction was utilized to synthesize catalyst, approximately 1.2 g of catalyst was synthesized from 10 g of residue.

DPPH-radical scavenging assay

The results of the antioxidant assay showed that the oil obtained from *W. coagulans* exhibited potential antioxidant activity. Percentage inhibition of DPPH radical was found to be 81.4 % at 75 µg/mL concentration of oil utilized as test sample (Fig. 1A). However, radical scavenging was reportedly low when compared to standard antioxidants BHT, rutin and ascorbic acid. Irrespective of that, the observed antioxidant potential of oil of *W. coagulans* promises probable application of the plant as an antioxidative source. Several studies conducted have utilized DPPH assay to evaluate antioxidant potential of plant products including extracts, oil, resins, etc. In a recent study, antioxidant activity of leaves of *G. alyphum* was studied through DPPH, ABTS and FRAP assay and the ethyl acetate extract was reported to exhibit significant antioxidant activity (14). Another study analyzed the antioxidant potential of 6 different plant species and reported that *P. grantum* exhibited the highest antioxidant activity (15).

Protein denaturation assay

W. coagulans seed oil was also found to exhibit considerable anti-inflammatory activity compared to (standard) diclofenac sodium, aspirin and acetylsalicylic acid. Highest *in-vitro* anti-inflammatory activity of *W. coagulans* seed oil was found to be 88.09 % which was comparable to the maximum activity shown by standard (92.77 %) (Fig. 1B). A group of researchers studied anti-inflammatory potential of oil extracted from leaf of *Curcuma caesia* (12). The study suggested that the plant oil exhibited ant-inflammatory property with IC_{50} value of 182.5 µg/mL against sodium diclofenac Similarly, a recent study analysed anti-inflammatory potential of the aqueous extract of *B. graveolens* in male mice (strain NMRI Albinos) and reported that the extract exhibited improved anti-inflammatory activity when compared to Diclofenac sodium (16). Similar clinical evaluation of biological activities of *W. coagulans* is required so as to fully explore the medicinal potential of the plant.

In vitro α-amylase inhibitory assay

Oil of *W. coagulans* was found to exhibit *in vitro* -α amylase inhibitory activity which was comparable to standard Acarbose (Fig. 2). *In vitro* α-amylase inhibitory activity increased with increase in concentration of sample from 25 µg/mL – 75 µg/mL. At 75 µg/mL seed oil and acarbose exhibited *in vitro* α-amylase inhibitory activity of 86.14 and 93.33 % respectively. It is hereby interpreted that *W. coagulans* oil can be further explored as a potential source of natural antidiabetic compounds.

Results obtained from *in vitro* biological activities conducted indicate potential medicinal value of oil ex-

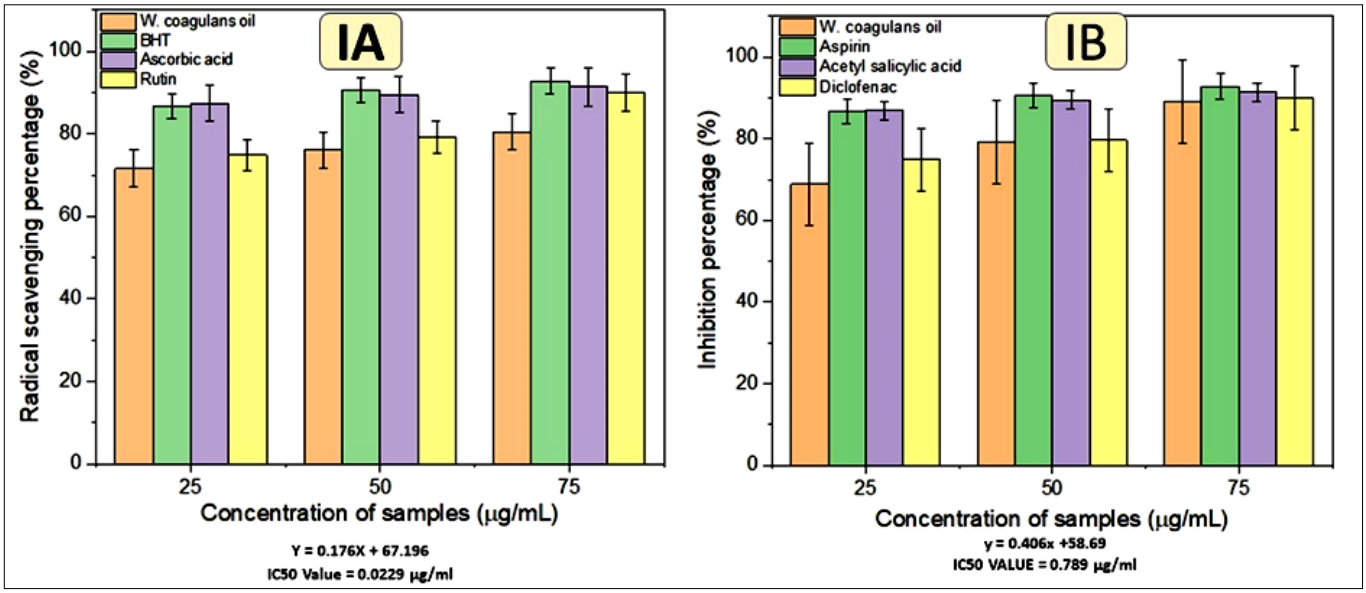


Fig. 1. (**A**) shows the DPPH-radical scavenging assay of *W. coagulans* seed oil and 1 (**B**) shows the Protein denaturation activity of *Withania coagulans* seed oil.

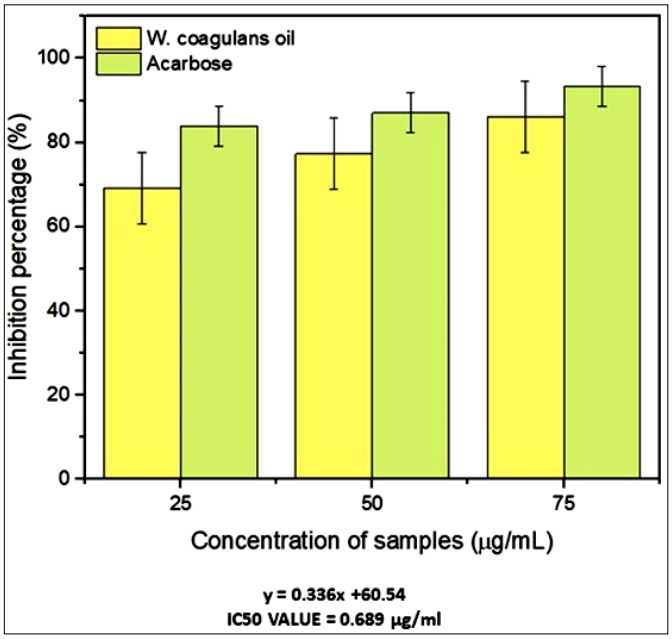


Fig. 2. *In vitro* -α amylase inhibitory activity of oil extracted from *W. coagulans* seeds.

20), the symmetric stretching vibrations of Si–O–Si peaks at 1031.507 cm^{-1} correspond to the Si–O–Si peaks (21). In addition, the absorption peaks at 707.335 cm^{-1}, 582.884 cm^{-1} and 527.889 cm^{-1} represent Si-O bending, Si-O-Si bending and Si=O stretching vibrations respectively (20, 22). The sharp band observed at 442.872 cm^{-1} correspond to Si–O bending vibration (21, 23).

X-ray diffraction (XRD)

XRD analysis spectra of catalyst confirm the crystalline nature of catalyst, depicted in Fig. 5. According to the JCPDS-ICDD: 01-072-0079 (24) database, the intense peaks of the Na_2SiO_3 at 2θ degree reflections were centered at 16.9°, 25.1°, 29.5°, 34.9, 37.3°, 49.1°, 64.2° and 66.9° with reflections planes Na_2SiO_3 (200, 111, 310, 400, 311, 002, 131), Quartz (113) and Cristobalite (201, 204) respectively. A similar study synthesized catalyst (BIO-MCM-41) from residue recovered post oil extraction from rice straw (25). The study reported the synthesized catalyst to enhance the yield of extracted oil when compared to non-catalytic

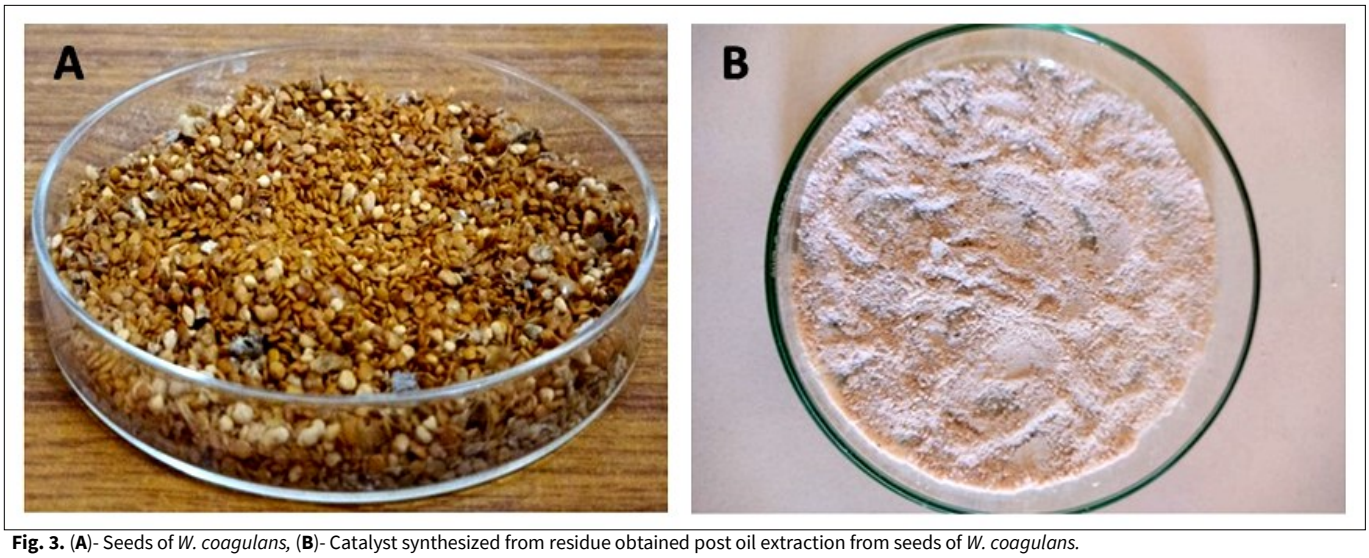


Fig. 3. (**A**)- Seeds of *W. coagulans,* (**B**)- Catalyst synthesized from residue obtained post oil extraction from seeds of *W. coagulans.*

tracted from seeds of *W. coagulans*. Earlier studies conducted have primarily focussed on exploring biological properties of extracts of seed / fruit of *W. coagulans* (17, 18). Due to this, there exists a profound research gap pertaining to studies conducted on *W. coagulans* oil. A major obstacle w.r.t *W. coagulans* is critically endangered status of plant along with extremely low germination rate. In another study, an effective way of enhancing germination rate of *W. coagulans* was reported (3). This, as well as other micropropagation methods reported can be utilized for mass propagation of plant to yield sufficient fruit / seed to be further utilized as feedstock for oil extraction. The extracted oil can be subsequently utilized for medicinal and industrial purposes.

Fourier Transform Infrared Spectroscopy (FTIR) analysis of catalyst

FTIR analysis (Fig. 4) of catalyst revealed the presence of organic compounds belonging to different classes. Appearance of broad peaks at 3269.401 cm^{-1} were attributed to Si-O-H stretching vibrations caused by the absorbed water molecules on the surface. The sharp band observed at 1449.408 cm^{-1} is assigned to Si=O stretching vibrations (19,

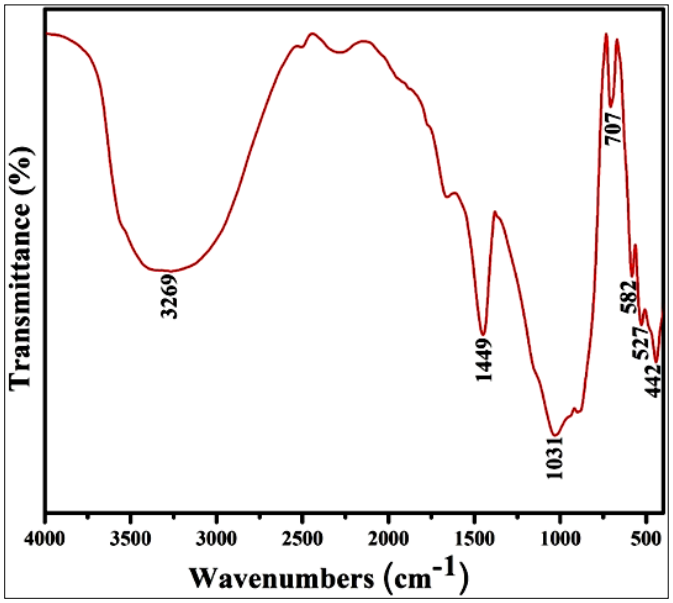


Fig. 4. FTIR graph of the catalyst (*W. coagulans*) depict major peaks corresponding to characteristic chemical groups.

process. Moreover, the oil obtained from plant-based biomass (as in the present study) are suitable alternative to be utilized in different industries for diverse applications.

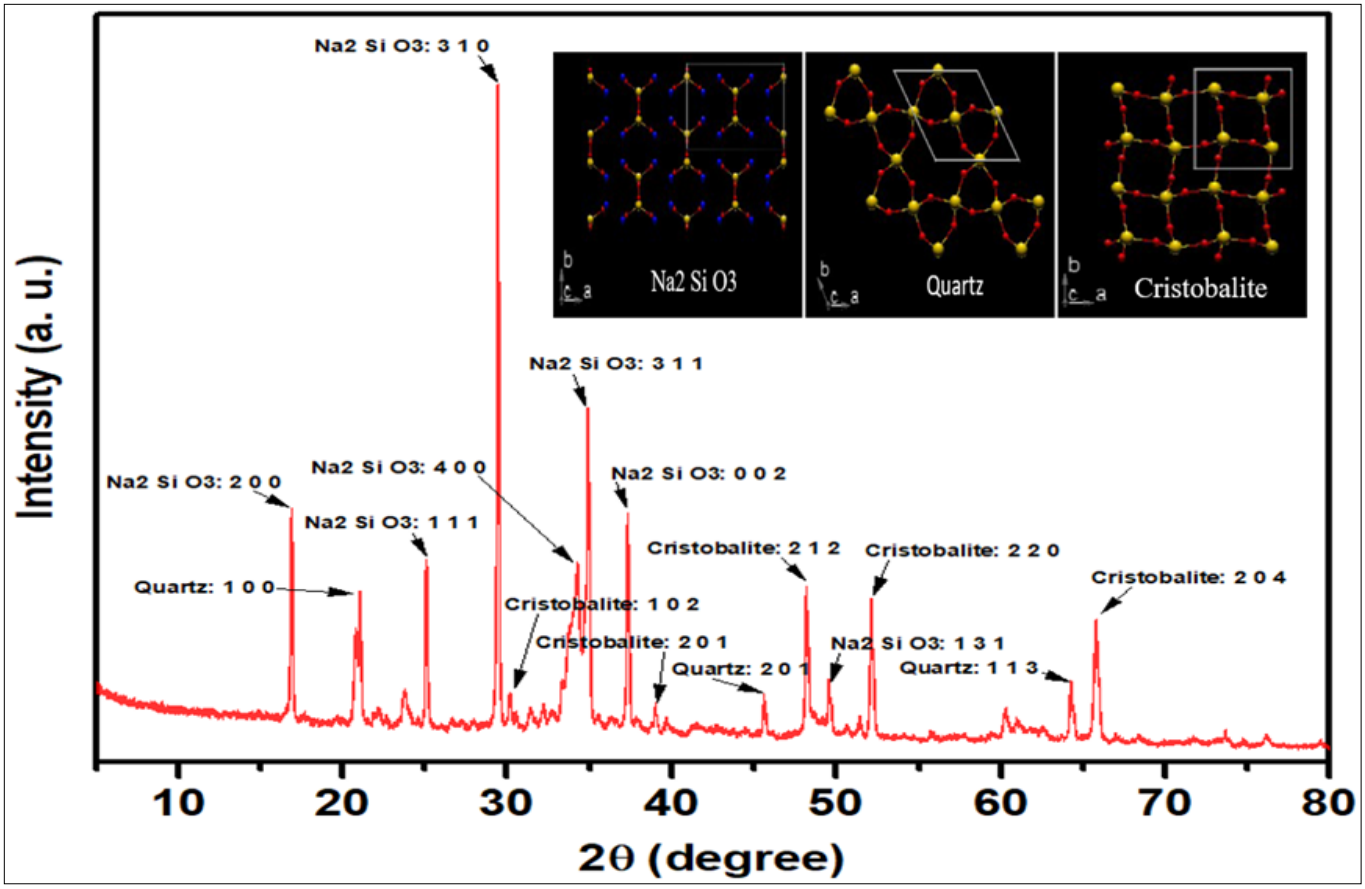


Fig. 5. XRD diffraction pattern of the catalyst synthesized from residue obtained post oil extraction from *W. coagulans* seeds.

Scanning Electron Microscope (SEM) analysis

SEM analysis was utilized for surface morphology study of the catalyst. The Fig. 6 illustrates distribution of catalyst particles on different magnification. At 500x magnification catalyst particles were visible in crystal particle with uniform particle distribution. When the magnification was enhanced to 1000x uniform distribution of particles was observed along with agglutinated ones. In further observations at 5000x and 15000x, agglutinated and clumped distribution of particles were recorded. The catalyst was ob-

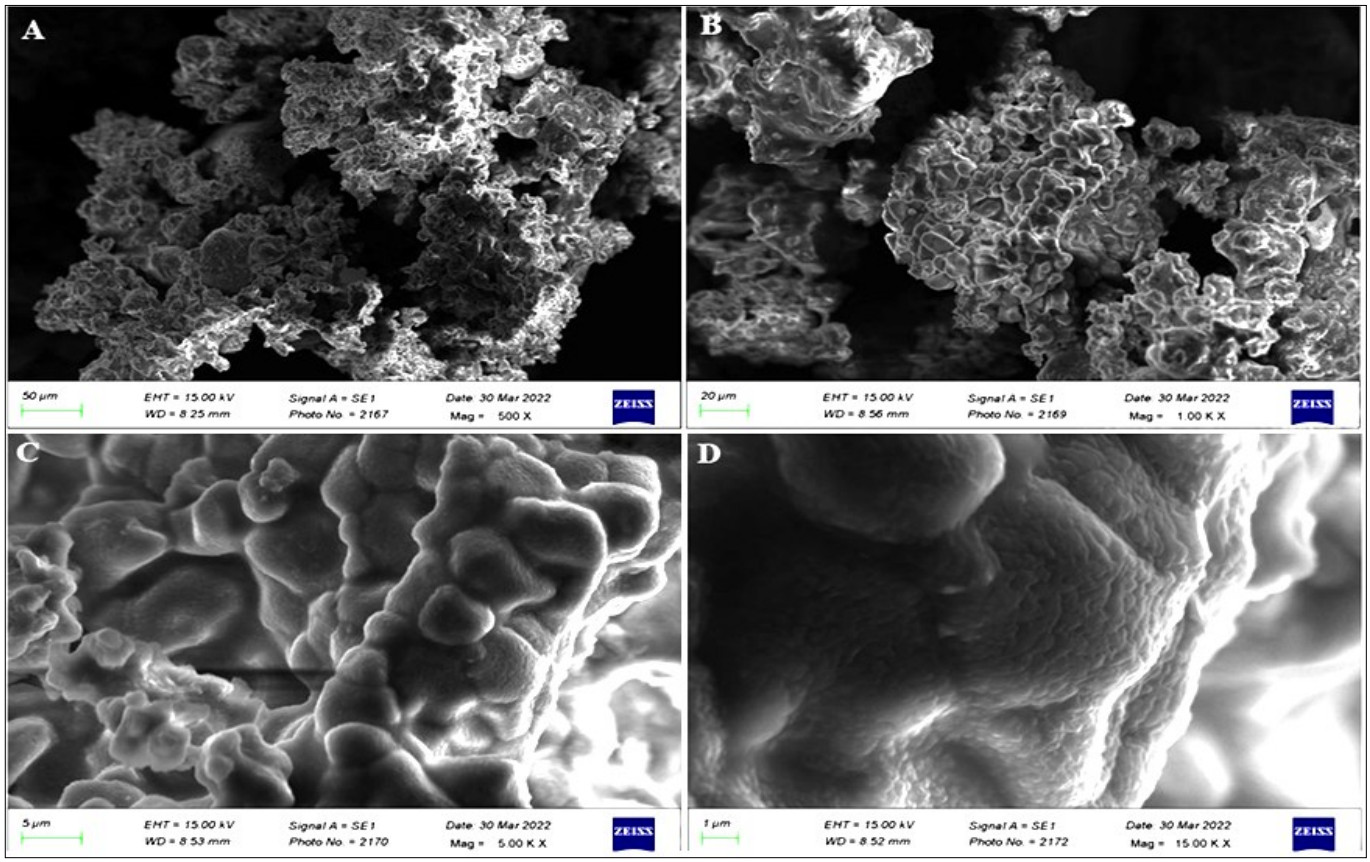


Fig. 6. SEM analysis of the catalyst at different resolutions like (A) 500X, (B) 1000X, (C) 5000, (D) 15000X.

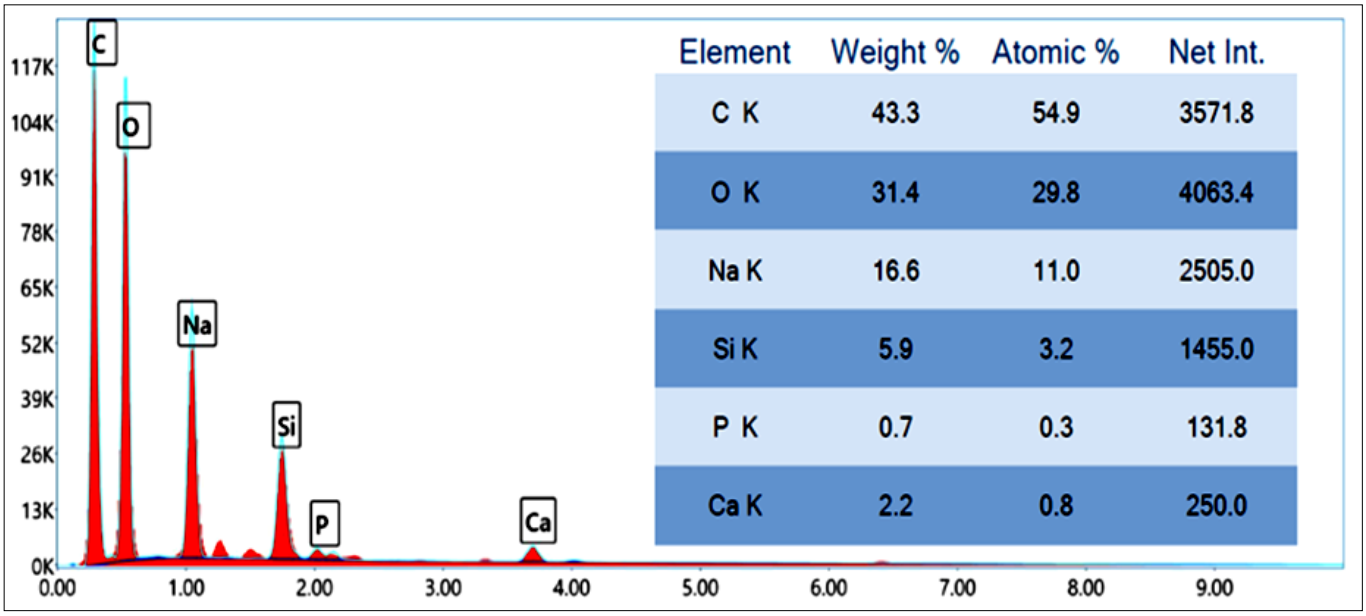
served to be microporous in nature.

Energy Dispersive X-Ray Spectroscopy (EDX) and EDX mapping

EDX spectroscopy analysis was utilized for identification of composition of catalyst. EDX analysis (Fig. 7) depicts well-dispersion of catalyst particles. Elemental characterization of catalyst revealed it to be composed of carbon, oxygen, sodium, silicon, phosphorus and calcium elements. EDX mapping of catalyst revealed the catalyst to comprise of mixture of carbon, oxygen, sodium, silicon, phosphorus and calcium (Fig. 8). Interpretation of Fig. 8, depicts overlapping of sections of different element identified during characterization of distribution of elements – carbon (Fig. 8.2), oxygen (Fig. 8.3), sodium (Fig. 8.4), silicon (Fig. 8.5), phosphorus (Fig. 8.6) and calcium (Fig. 8.7). The results of EDX mapping also indicate the formation of mixed oxides of sodium, silicon and carbon along with the formation of individual oxides. Utilization of residue obtained post processing of plant material is generally considered to be leftover waste which is simply discarded. Utilization of such residue for processes such as catalyst synthesis is considered to be a promising approach, since the synthesized catalyst can be utilized for diverse applications along with restricting the disposal of waste into environment. A previous review has also emphasized upon potential of biocatalysts to be explored as an alternative for developing new drugs and pharmaceuticals (26). Validation through clinical trials can act as a potential support for further commercial application of plant-based catalyst.

Conclusion

The present study reports an efficient approach to utilize



Element	Weight %	Atomic %	Net Int.
C K	43.3	54.9	3571.8
O K	31.4	29.8	4063.4
Na K	16.6	11.0	2505.0
Si K	5.9	3.2	1455.0
P K	0.7	0.3	131.8
Ca K	2.2	0.8	250.0

Fig. 7. Elemental analysis of the catalyst.

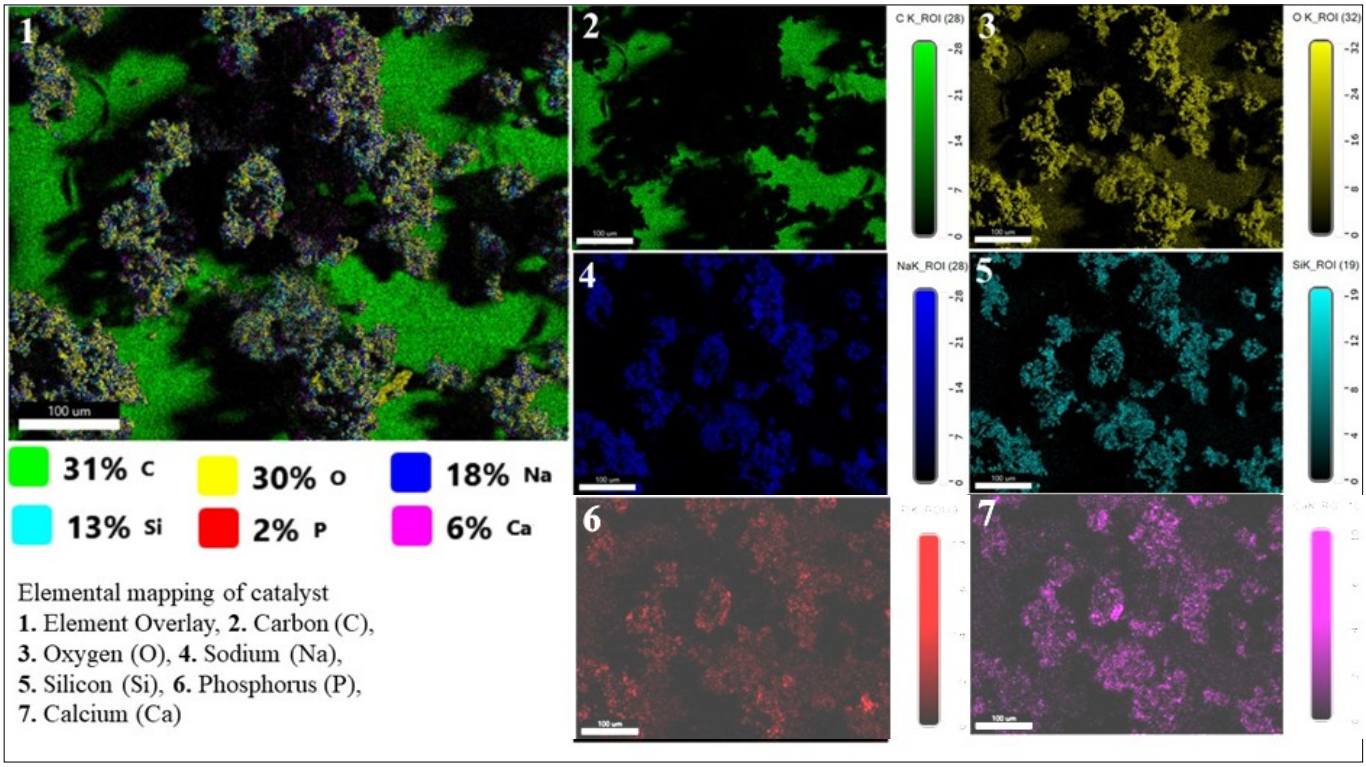


Fig. 8. Elemental mapping of the catalyst.

plant biomass as a feedstock for integrated and interdisciplinary applications. The study implies utilizing plant based raw material to procure herbal extract / oil and utilize for medicinal purposes and simultaneously subjecting the plant residue for synthesis of catalyst which can further be explored for diverse industrial applications. Findings of the present study depict medicinal potential of oil extracted from seeds of *W. coagulans* in terms of anti-inflammatory, antidiabetic and anti-oxidant activity. The oil can be utilized for respective applications after ascertained clinical studies. Synthesis of catalyst from diverse sources has gained momentum in the recent past owing to numerous potential applications of plant-based catalyst. From the results obtained in the present study it is hereby interpreted that the residue obtained after oil extraction from seeds of *W. coagulans* can be effectively utilized for synthesis of catalyst. Utilization of residual waste for catalyst synthesis will not only limit the release of such organic residue into the environment but also synthesized catalyst can be further utilized for industrial and medicinal application and waste water treatment.

Abbreviations

FTIR: - Fourier Transform Infrared Spectroscopy

SEM: - Scanning Electron Microscopy

EDX: - Energy Dispersive X-Ray Spectroscopy

XRD: - X-ray Diffraction

NaOH: - Sodium Hydroxide

Na_2SiO_3: - Sodium Silicon Oxide

DPPH: - 2,2-diphenylpicrylhydrazyl

BHT: - Butylated Hydroxytoluene

KBr: - Potassium Bromide

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Authors' contributions

NS and RS identified the research problem, conducted literature survey and designed the experimental study. PR, PC, HB conducted the experimental study. AC and NK carried out data analysis. Initial draft of manuscript was written by RS and NS. All authors approved the final draft of manuscript.

Compliance with ethical standards

Conflict of interest: Authors declare that there exists no conflict of interest.

Ethical issues: None

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