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Research Article

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Extraction and isolation of gallic acid from self-generated fermentation system of *Terminalia chebula*

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ABSTRACT

Myrobalan (Terminalia chebula) is a potential raw material for the production of gallic acid. It is a rich source of tannins and contains up to 40% of total tannins as gallotannins. The present study involves the fermentation of Myrobalan used as a substrate for fermentation using arishta containing tannase producing microorganism resulting in the biotransformation of Myrobalan tannins to gallic acid which is extracted, isolated and characterized. The self-generated fermentation system, arishta was prepared by forming a decoction of Myrobalan which was allowed to ferment for one month. Then after the fermentation was complete the gallic acid was extracted and isolated by partitioning it with ethyl acetate. The gallic acid crystals obtained upon recrystallization was characterized by phytochemical screening, TLC, IR, NMR and XRD. The completion of fermentation process was confirmed when the pH of the formulation was found to be 4.28. The extract was screened for the presence of tannins, which was confirmed by the appearance of bluish black precipitate upon reaction with Ferric chloride solution. Further the structural characterization of gallic acid was confirmed by TLC, FTIR, NMR and XRD.

Keywords: Myrobalan, Gallic acid, Arishta, Bioconversion, Characterization

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INTRODUCTION

3,4,5- Trihydroxy benzoic acid commonly known as gallic acid finds important use as the basic intermediate for an anti-bacterial drug, 'Trimethoprim'. It is also used in pharmaceutical industry as astringent and styptic agent. Gallic acid esters have been in use various industries as antioxidant, photographic developer, tanning industries, etc[1]. The main hindrance in the development of a successful bioconversion process is the sensitivity of the microorganisms to tannic acid and the oxidation of the unused tannic acid[2]. At present gallic acid is produced industrially by acid hydrolysis of naturally occurring gallotannins. The present methods for extraction are expensive, produce low yield of desired product and also produce toxic effluents by acid hydrolysis hence an enzyme based eco-friendly technology for gallic acid production is urgently required [3].

Arishtas are self-generated herbal fermentations of traditional system and are considered as unique and valuable therapeutics in Ayurveda. They are prepared by allowing the herbal juices and their decoctions to undergo fermentation [4]. In the present study fermentation is brought about by the addition of a source of sugar and *dhataki* (*Woodfordia fruiticosa* Kurz) flowers to act as a natural source *Aspergillus niger* which is responsible for fermentation and release of tannase enzyme [5]. Tannase (Tannin acyl hydrolyses-EC 3.1.1.20) is the enzyme responsible for the bioconversion of hydrolysable tannins (gallotannins) to gallic acid. It is an extracellular, inducible enzyme that hydrolyzes ester bond in hydrolysable tannins thus releasing gallic acid and glucose [6]. Keeping in view the importance of gallic acid and development of an ecofriendly process, the present study was undertaken. Myrobalan is a potential raw material for the production of gallic acid, thus for the present study Myrobalan substrate - Chebulic Myrobalan) was taken for the microbial bio-conversion of gallotannins from Chebulic [3].

EXPERIMENTAL SECTION

Plant Material

Dried Myrobalan fruits (100g) were ground to a coarse powder and stored. Other ingredients of the Arishta like Grape fruit and Dhataki flowers were obtained from local vendors.

Preparation of Arishta [7][8]

For the preparation of the Arishta, the powdered Myrobalan fruits (*Terminalia chebula*), Grape fruit (*Vitis vinifera*), Dhataki flowers (*Woodfordia fruticosa*) were taken in quantities according to the formula shown in Table 1.

Table 1: It shows Formulation composition of Arishta

Ingredients	Quantity Taken	Role
Terminalia chebula	100g	Source of Gallic acid.
Vitis vinifera	50g	Induces fermentation, added to support and enhance the process of fermentation.
Woodfordia fruticosa	20g	Host for Fungi-*Aspergillus species.* Helps to produce tannase enzyme. Acts as inductor.
Jaggery	100g	Acts as complex nutrient for bacteria.
Water	1000ml	Act as a solvent.

Air dried fruits of *Terminalia chebula* were coarsely ground. To this *Vitis vinifera,* Dhataki, Guda and water was added. The mixture was allowed to boil for 20 mins. The decoction was poured in a jar and it was allowed to ferment for 4 months. The pH of the formulation was monitored.

Isolation of Gallic acid [6]

Gallic acid was isolated using the organic solvent ethyl acetate in a separating funnel. The fermented material was removed and water was added to it. This was heated to about 60 – 70^{o}C. Ethyl acetate was added to the extract in a separating funnel. Gallic acid being soluble in organic solvent was extracted into the organic phase. Gallic acid was separated from ethyl acetate by rotary vacuum evaporation. The pressure and temperature at which this separation was done was 200mbar and 70^{o}C. Diethyl ether was added to it and was evaporated in a rotary evaporator to obtain pure Gallic acid.

Characterization of Gallic acid

Preliminary Phytochemical Screening [9]

Preliminary phytochemical screening was done by testing the extract with Ferric chloride solution. To 10 ml of the extract a small quantity of Ferric chloride solution was added.

Thin Layer Chromatography (TLC)

For TLC the solvent system was selected as ethyl acetate and formic acid in the ratio 9:1. The R_f value of standard gallic acid and the isolated gallic acid was calculated and compared.

Fourier Transform Infra Red (FTIR) Spectroscopy

FTIR study was carried out to analyze the functional groups present in the compound. Sample was fixed in Potassium bromide disc and was scanned between 400-3500cm^{-1}.

Nuclear Magnetic Resonance (NMR) Spectroscopy

Dimethy Sulfoxide (DMSO) was preferred to be the choice solvent for H^1 - NMR analysis for the given sample. The Chemical shifts values of the isolated compound were observed.

X-ray Diffraction (XRD)

The isolated Gallic acid was also analyzed by XRD using X-ray Diffractometer (Bruker). XRD of the sample was recorded at room temperature

RESULTS AND DISCUSSION

Myrobalan is a rich source of gallotannins [2]. Arishtas are fermentation products [4]. The tannase enzyme released during fermentation process causes the breakdown of gallotannins into gallic acid and other tannins [5] [6].The completion of fermentation process was confirmed when the pH of the formulation was found to be 4.28. This gallic acid was extracted, isolated and characterized from the arishta preparation.

Characterization of Gallic acid:

Preliminary Phytochemical Screening:

A bluish black precipitation was observed thus confirming the presence of tannins.

TLC

The R_f value of isolated Gallic acid was found to be 0.78 which matches with the R_f value of standard Gallic acid. It was thus confirmed that the product obtained from *Terminalia chebula* was Gallic acid. The results are shown in Fig.1.

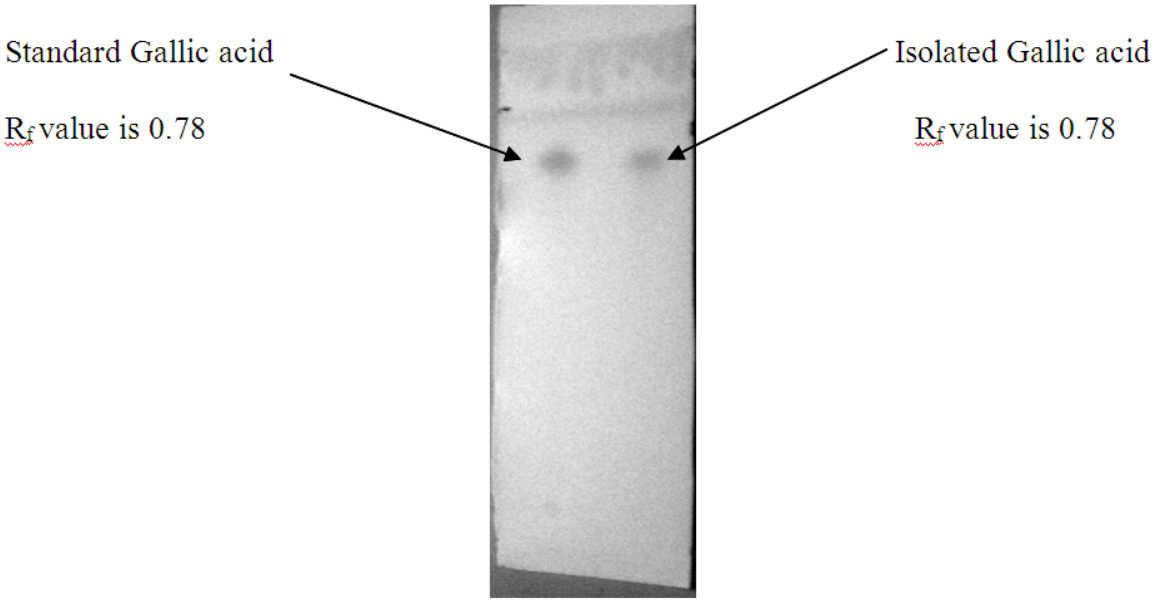

Fig. 1: It shows TLC of Standard and Isolated Gallic acid

Fourier Transform Infra Red (FTIR) Spectroscopy [10]

The functional groups were recorded using Fourier transformation-infra-red (FT-IR) spectroscopy. The FTIR spectrum results of the isolated gallic acid are shown in Fig. The IR showed 3478 – 3385cm^{-1} (H bonded hydroxyl group), 1681 cm^{-1} (C=O, oxidic carbonyl), 1450 cm^{-1}, 1523 cm^{-1} (aromatic ring) regions. The results are shown in Fig.2.

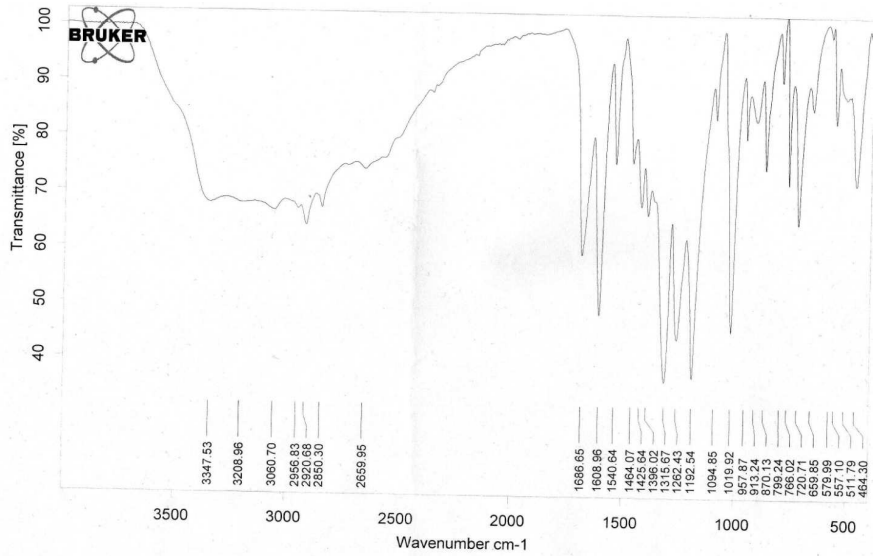

Fig.2: This shoes FTIR of Isolated Gallic acid

Nuclear Magnetic Resonance (NMR) Spectroscopy

The chemical shift values obtained from proton NMR were δ12.253ppm, δ 9.208ppm, δ 6.914ppm, δ 3.374ppm, δ 2.506ppm, and δ 1.234ppm thus confirming the presence of gallic acid. The results are shown in Fig.3.

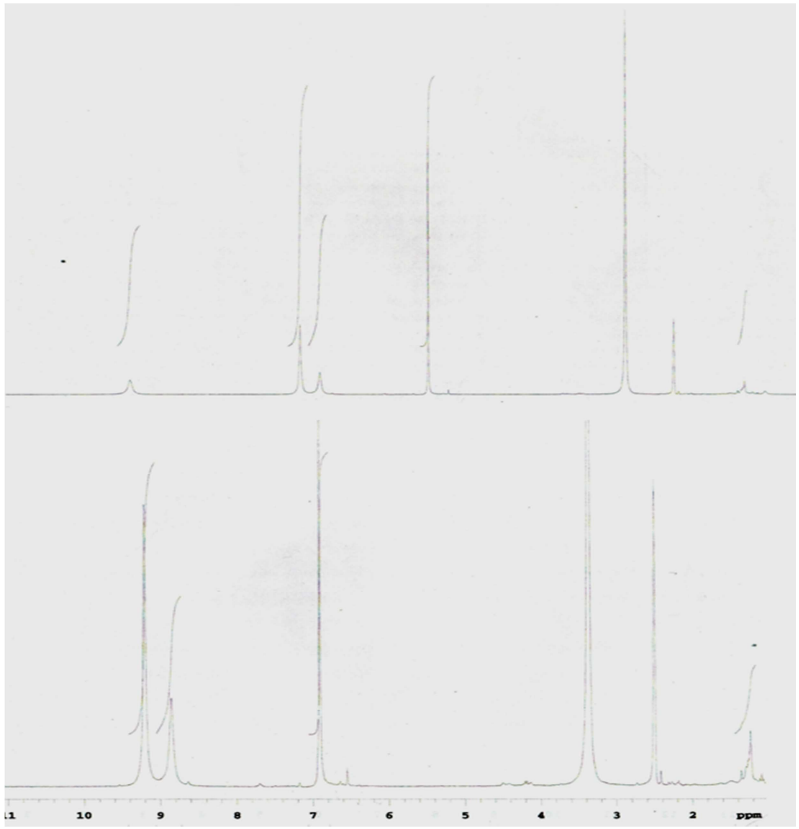

Fig.3: It shows H^1-NMR Spectra of standard and isolated gallic acid

X-ray Diffraction (XRD):

Powder X ray diffraction (PXRD) was performed in order to determine the crystallinity of the isolated sample of Gallic acid. The results are shown in Fig.4.

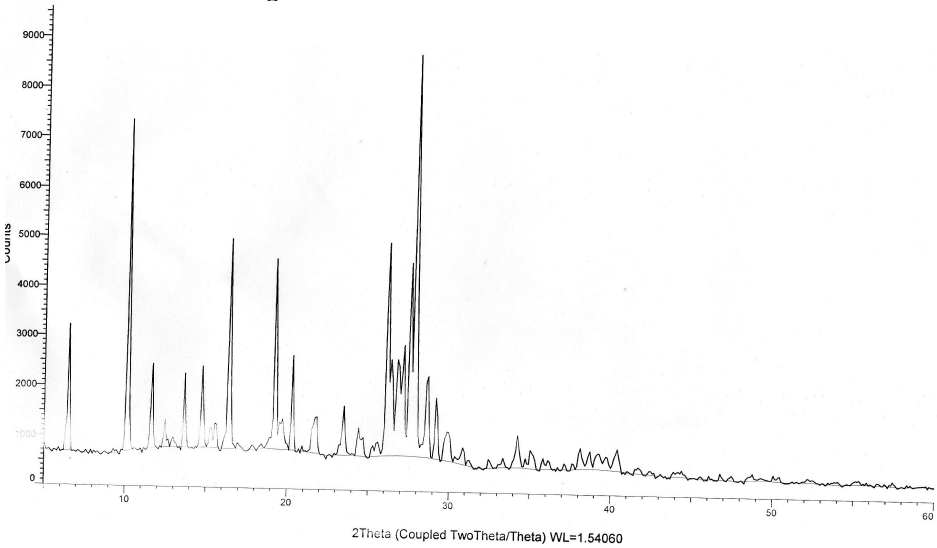

Fig.4: It shows XRD of gallic acid

CONCLUSION

Conventionally gallic acid is produced by acid hydrolysis of tannic acid but the procedure is expensive, product may contain impurities and the yield is low [1]. Alternatively, gallic acid can be produced by the microbial hydrolysis of tannic acid by tannase, an inducible enzyme, secreted by microorganisms overcoming the above problems.

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