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Research Article

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Detoxification of Gunja Seeds with Ex Vivo Study

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ABSTRACT

Seeds of Abrus precatorius linn. (fabaceae) are used in different Ayurvedic therapeutics. Since they are poisonous, they are processed with various media like Godugdha (cow's milk), Kanji (sour gruel), Nimbu Swarasa (Lemon Juice), etc. before being incorporated into the Ayurvedic formulations. This study investigates the use of Gomutra (Cow Urine), normal saline (NaCl), and water as detoxification media. A stainless-steel vessel with a dolayantra was used to immerse the seeds for 3 hours of boiling during the detoxification process. Detoxification using Gomutra gave a reddish tinge to the seeds compared to the light brown color seen with NaCl medium. The physicochemical changes in detoxification that occurred in the gomutra treated gunja seeds were validated by TLC and Ex Vivo investigations (agglutination assay). To characterize the extract using TLC, the mobile phase was Toulene: ethyl acetate: glacial acetic acid (6.5: 3.3: 0.2). The removal of distinctive bands in the TLC profile of processed gunja seeds suggested that the gunja seed extract's composition had changed.

Key words: *Abrus Precatorius, Gunja seeds, Abrin, Gomutra, Dolayantra*

INTRODUCTION

Gunja (*Abrus precatorius Linn*.), known as Indian liquorice, is reputed as one of the world's most deadly but most beautiful seeds belonging to the family Fabaceae, classified as Upavisha (semi-poisonous medications) and widely employed in various Ayurvedic formulations with significant therapeutic value [1]. Due to the poisonous content present in gunja, it comes under the term "poisonous drug." In Ayurveda, it's stated that gunja can be used for therapeutic effect after proper detoxification [2]. Cow's milk, sour gruel, lemon juice, water, and other substances have previously been used to detoxify gunja [3]. In this study, we used *gomutra* and normal saline water for detoxification of gunja, and the confirmatory test known as the agglutination assay was carried out to confirm the agglutination factor present in gunja, which is Abrin, removed after the detoxification process using the new detoxification media, which is Cow's Urine [4]. This review paper shows the detoxification of gunja seed by using the TLC method and agglutination assay [5].

MATERIALS AND METHODS

Drug procurement

HEILEN BIOPHARM PVT. LTD. 1201, Matrix, Near Divya Bhaskar Press, SG Highway, Prahladnagar, Gujarat (India)

Detoxification of gunja seed powder

Instruments used: dolayantra

Two steel vessels are used to form the dolayantra with the help of an iron rod in such a way that the drug tied in cloth should not touch the bottom. The drug was tied into the muslin cloth by making the pottali, and it was deepened into the media in such a way that the pottali should not touch the bottom [6] **(Figure 1)**.

a)

b)

Figure 1. Dolayantra Instrument

Selection of media

Abrin is highly polar and this is connected with the drug by a disulphide bond which has to be broken to separate abrin from the drug. To break the disulphide bond, we need a high ionic-containing media, so cow urine and normal saline water were selected as the detoxification media [7]. To break this bond, we need to follow the shodhana procedure, which is termed swedana (boiling) in media [8]. From ancient times, cow's milk and kanji were used for the detoxification of gunja seeds [9,10]. Because milk and kanji contain several ions such as calcium, magnesium, sodium, potassium, inorganic phosphates, citrate, chloride, water, lipids, proteins, lactose, etc., The cow's urine contains water, sodium, nitrogen sulphur, vitamins (A, B, C, D, E), minerals, iron, magnesium, citric, calcium salts, phosphate, lactose, enzymes, and hormones, which is a high concentration of ions that should be used to break the disulfide bond between the drug components and abrin. So, we decided to use cow urine and normal saline water as a medium [11, 12].

Thin-layer chromatography

Procedure

Three samples are used for spot, such as,

1. Sample with the raw drug.
2. A cow's urine detoxified sample
3. Normal saline detoxified sample.

Preparation of samples: 2 gm of each sample was macerated in 20 ml of methanol for 24 hours. After that, samples are filtered and concentrated to 5 ml each **(Table 1)**.
Preparation of the mobile phase:
The mobile phase was made by
Toluene: Ethyl acetate, glacial acetic acid (6.5:3.5:0.2)

Table 1. Calculations of Rf Values

Rf Value of Raw Drug	Rf Value of Gomutra Treated Drug	Rf Value of Normal Saline Treated drug
0.7	0.64	0.63
0.9	0.89	0.89
-	0.93	0.95

Thin-layer chromatography
where,
a = Raw Drug Sample **(Figure 2)**
b = Cow's Urine Treated Sample **(Figure 3)**
c = Normal Saline Treated Sample **(Figure 4)**

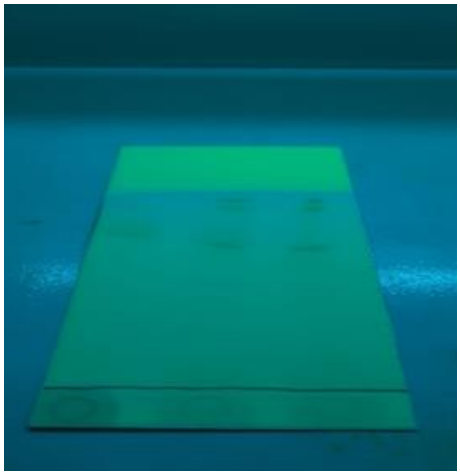


Figure 2. TLC of Raw Drug Sample

Figure 3. Cow's Urine Treated Sample

Figure 4. Normal Saline Treated Sample

Ex-Vivo study [7] *(agglutination assay)*
A candidate having o positive blood group was selected.

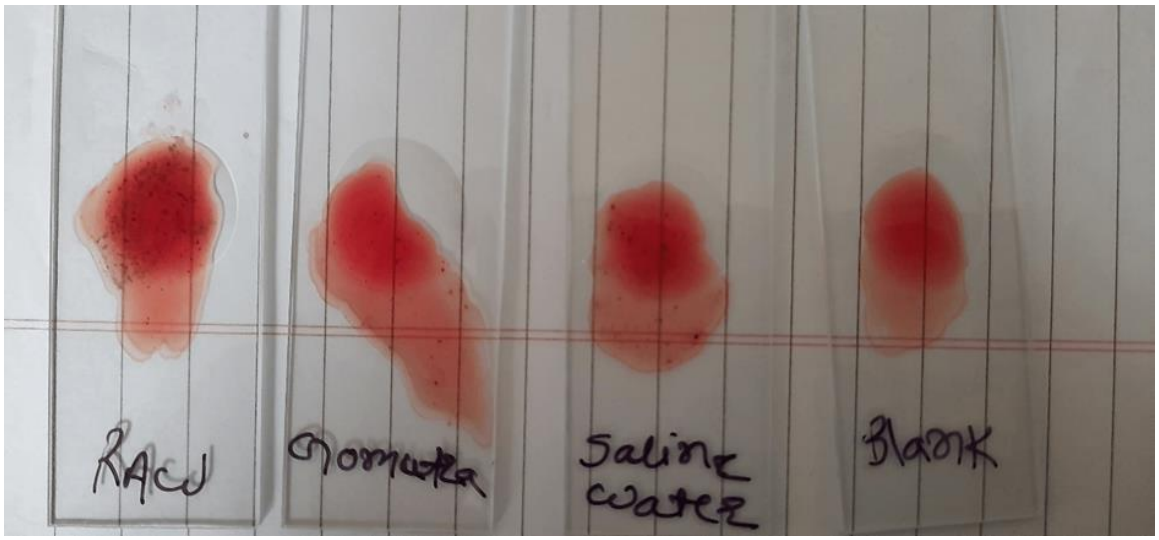
Procedure

- 500 mg of each sample was taken and triturated with 3 mL of distilled water.
- All three samples were centrifuged for 20 minutes at 1000 rpm.
- One drop of O-positive blood was taken on the glass slide.
- One drop of distilled water was added to the blood.
- Then, the supernate liquid was found after centrifugation was taken and one drop of each sample was added into the blood sample and mixed with the blunt end of the capillary tube.

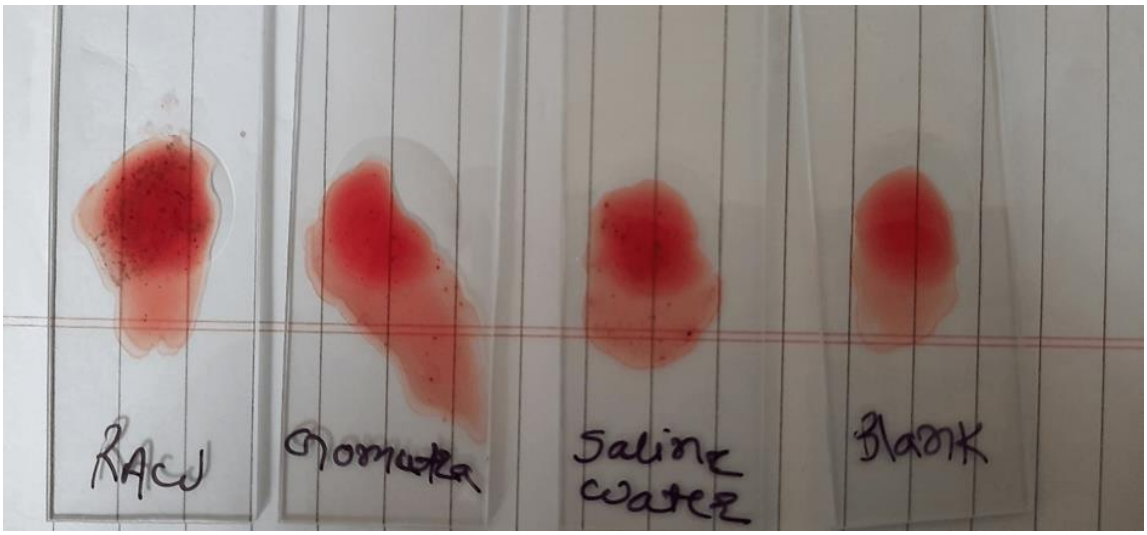
Observations and results
The blood sample spotted with raw drug supernatant liquid was agglutinated within 2 minutes. Other blood samples were similar to the blank, but slight changes were found. After 10 and 20 minutes, photographs were taken. The observable results showed that the agglutination factor was present in the raw sample and it was not present in other samples.

Agglutination assay

At the time of spotting



After 10 minutes



After 20 minutes

RESULTS AND DISCUSSION

In this study, a new detoxification medium was developed, which is cow's urine. After the selection of media, detoxification was carried out in Dolayantra, in which drugs are detoxified. After detoxification there, confirmatory tests were carried out in which observations and results were compared between the raw drug sample, normal saline water detoxified sample, and cow's urine detoxified drug sample. As per TLC interferences, it is observed that detoxified samples show one extra spot on the upper side, but raw samples have a high Rf value, which confirms that changes are occurring concerning the polarity of the drug. As per the EX VIVO study, it was observed that blood spotted with raw drug samples got agglutination, and detoxified drug samples did not show any agglutination.

CONCLUSION

As per the result found in the confirmatory test, it is concluded that we can use cow's urine as a detoxification medium instead of cow's milk, kanji, lemon juice, water, and normal saline water. According to Ayurveda Cow urine is also used for the treatment of various diseases, so this medium could be safe to use and more effective than other media. Cow's urine, kanji, lemon juice, purified water, normal saline water must be economically high in price, so we can get it free of cost and it will be available easily. So cow urine can be the better option as a detoxification medium for *Abrus precatorius*.

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CONFLICT OF INTEREST : None

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ETHICS STATEMENT : None

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