Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
https://www.phytojournal.com
JPP 2023; 12(6): 159-169
Received: 10-09-2023
Accepted: 13-11-2023

Prachi Pandey
Department of Pharmaceutics, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

Rahul Pal
Department of Pharmaceutics, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

KS Bharath
Assistant Professor, Department of Pharmacology, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of research and education, Kelambakkam, Tamil Nadu, India

Alagan Akilandeshwari
Associate Professor, Department of Pharmaceutics, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of research and education, Kelambakkam, Tamil Nadu, India

Manju Koli
Assistant Professor, Invertis University, Bareilly, Uttar Pradesh, India

Neelu
Research Scholar, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

Mohammad Rizwan
Assistant Professor, Department of Pharmaceutical Science, Sir J.C. Bose Technical Campus, Bhimtal, Kumaun University, Nainital, Uttarakhand, India

Abrarul Haque
Assistant Professor, Department of Pharmacognosy, School of Pharmacy, Sai Nath University (SNU) Ranchi, Jharkhand, India

Himmat Singh Chawra
Professor, Department of Pharmaceutics, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

Corresponding Author:
Prachi Pandey
Department of Pharmaceutics, NIMS Institute of Pharmacy, NIMS University, Jaipur, Rajasthan, India

Design of Experiments (DoE) manipulation in the formulation and optimization of a traditional Ayurvedic medicine derived from dried extract of *Senegalia catechu* enhanced through statistical analysis

Prachi Pandey, Rahul Pal, KS Bharath, Alagan Akilandeshwari, Manju Koli, Neelu, Mohammad Rizwan, Abrarul Haque and Himmat Singh Chawra

DOI: https://doi.org/10.22271/phyto.2023.v12.i6b.14779

Abstract

Introduction: In India, the regulatory body for catechu is the Food Safety and Standards Authority of India (FSSAI). The FSSAI is responsible for regulating the manufacture, sale, and distribution of food in India, including catechu. The FSSAI has set standards for the purity and quality of catechu, and it also monitors the market for adulterated catechu. The FDA (The Food and Drug Administration) is responsible for regulating the safety and efficacy of drugs and dietary supplements in the United States (US). The FDA has not approved catechu as a drug or dietary supplement, but it does regulate catechu as a food additive. The FDA has set limits on the amount of catechu that can be added to food

Objective: The primary objective of this research was to involvement of design of experiments (DoE) manipulation in the formulation and optimization of a traditional Ayurvedic medicine derived from dried extract of *Senegalia catechu* enhanced through statistical analysis.

Methodology: The dried extract of *Senegalia catechu* was collected and identified at the botanical herbarium garden. Subsequently, it underwent a drying process and was ground into a powder.

This powder was then subjected to extraction using the digestion method, utilizing water as the solvent at a temperature of 70 °C. The resulting extract underwent phytochemical screening. Following this, the extract was utilized in the pharmaceutical development process.

The optimization of the formulation for the dried extract of *Senegalia catechu* began with the establishment of the Quality Target Product Profile (QTPPs) for the final product. The desired outcome was an oral dispersible tablet (ODTs) that would enhance patient compliance and ensure rapid disintegration. These QTPPs served as the foundation for identifying the Critical Quality Attributes (CQAs), which included hardness, disintegration time, and mass uniformity. These attributes were utilized in all subsequent experiments. The experimental phase was divided into two main manufacturing processes is first one is direct compression and another one is wet granulation techniques. Each process was thoroughly investigated to optimize the drug product.

To assess the potential risks and their impact on product quality, a comprehensive risk assessment was conducted. For the direct compression technique, a 3^2 full factorial Design (FFD) of DoE was employed to analyze the influence of the super disintegrant (26%) and lubricant range (ranging from 0.32% to 6%) on the characteristics of powder flow. On the other hand, the wet granulation technique utilized a 3^2 FFD, DoE to investigate the effects of the superdisintegrant (Ranging from 1.5% to 4.8%) and binder (ranging from 4% to 9%) on both flow properties and tablet properties.

Results: The successful optimization of an Enhanced Traditional Medicine (ETM) was achieved through the design and evaluation of formulations in this study. The utilization of DoE proved to be an exceptional approach in optimizing ETM formulations, offering a range of tools that enhance comprehension of the formulation and manufacturing process. Additional research on this DoE methodology is necessary to assess the impact of additional process variables, including compression force and speed, as well as formulation variables such as palatability.

Conclusion: The influence of formulation variables on disintegration time, wetting time, and hardness was demonstrated through the development of optimization models. Consequently, employing DoE for the formulation optimization of a category for ETM containing dried extract of *Senegalia catechu* is a viable strategy to enhance drug product understanding while saving both time and money.

Keywords: Dried extract, ayurveda formulation, catechu, design of experiment, pain, dried extract, response surface methodology, Pharmacognosy, phytoconstituents

Introduction

In the last decade, there has been a growing interest in the use of *Senegalia catechu* as a source of natural antioxidants. *Senegalia catechu* contains polyphenolic compounds that have been demonstrated to safeguard cells against harm caused by free radicals.

These molecules can harm DNA and other cellular components, increasing the likelihood of chronic illnesses such as cancer, heart disease, and Alzheimer's disease [1].

Senegalia catechu, also known as *Acacia catechu*, has been used for its medicinal and dyeing properties for centuries. It is a plant native to India and Sri Lanka and is used in traditional Indian and Sri Lankan medicine. One of the most well-known traditional uses of *Senegalia catechu* is as an astringent, a substance that can dry and contract tissues. In traditional medicine, it is used to treat diarrhea, mouth ulcers, and sore throat. It is also used as a gargle to treat sore throat and as a mouthwash to freshen breathe [2]. The traditional observational visualization of *Senegalia catechu* shown as per the Fig. 01 as below followings:

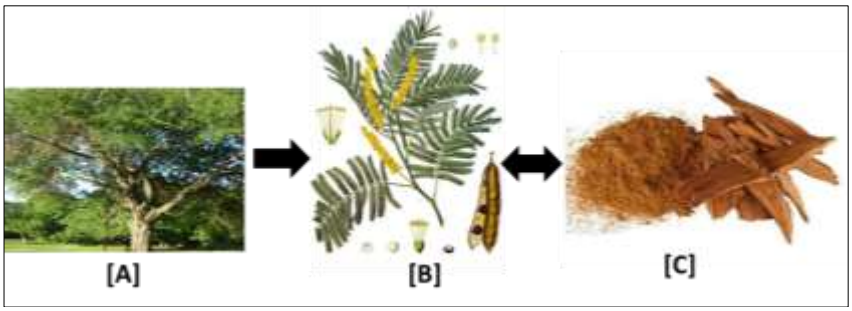

Fig 1: The origin of *Senegalia catechu* from the plant origin with bard dried extract; [A]. The original catechu plant, [B]. The lead origin and [C]. The dried extract powder

A *Senegalia catechu* is a plant with a long history of traditional use. It has been used as an astringent, a dye, and a source of natural antioxidants. More research is needed to confirm its effectiveness for treating various conditions, but it is generally considered safe when used in the recommended amounts [3].

In 2002, the FDA and the International Conference on Harmonization (ICH) developed the DoE methodology in accordance with QbD principles. This systematic approach enables the analysis of formulation and process variables and their impact. The main objective of this investigation was to enhance an ETM that integrates *Senegalia catechu* dried extract, utilizing the DoE involving methodology with conjunction with QbD.

Botanical Characteristics and Traditionally therapeutics uses: *Senegalia catechu*, also known as *Acacia catechu*, is a deciduous tree that can grow up to 15 meters tall. It is native to India, Sri Lanka, Myanmar, and Nepal. The bark is dark grayish-brown and exfoliating in long strips. The leaves are bipinnate, and the flowers are small and yellow. The fruit is a flat pod that is pointed at both ends [3, 4]. The botanical characteristics and other therapeutic uses discussed in the given Table. 01 as below followings:

Table 1: The botanical characterization and therapeutics uses of *Senegalia catechu* [2, 4].

Featuring Subsection	Plant description characteristics
Botanical Name	*Senegalia catechu* (L.f.) Willd.
Common Names	Catechu, khair, cutch, black cutch, cachou
Family	Fabaceae (Pea family)
Genus	Senegalia
Species	catechu
Plant Type	Deciduous tree
Height	Up to 15 meters (50 feet)
Bark	Dark grayish-brown and exfoliating in long strips
Native Range	India, Sri Lanka, Myanmar, and Nepal
Traditional Therapeutics Uses	Diarrhea, mouth ulcers, sore throat, wounds, skin ulcers, hemorrhoids, vaginal discharge, leucorrhea, menorrhagia
Chemical Constituents	Catechin, epicatechin, quercetin, kaempferol, gallic acid, ellagic acid

Senegalia catechu has been used in traditional medicine for centuries. It is known for its astringent, antiseptic, and antidiarrheal properties. It has been used to treat a variety of conditions including; Diarrhea, Mouth ulcers, Sore throat, Wounds, pyretic, Skin ulcers Hemorrhoids, Vaginal discharge, Leucorrhea and Menorrhagia [3, 5]. It has also been used as a mouthwash to freshen breath and as a gargle to treat sore throat.

Chemical Compositional Constituents: The heartwood of the tree is used to produce catechu, a brown or black extract that has been used in traditional medicine for centuries. Catechu is a rich source of polyphenolic compounds, which are antioxidants that have a variety of health benefits. The chemical composition of catechu varies depending on the source of the extract and the method of preparation [6]. However, the main chemical constituents of catechu include:

1. **Catechin:** Catechin, a powerful antioxidant, effectively shields cells from harm induced by free radicals. These molecules have the potential to harm DNA and other vital cellular elements, thereby elevating the susceptibility to chronic ailments like cancer, heart disease, and Alzheimer's disease.
2. **Epicatechin:** Epicatechin is another flavanol that is similar to catechin. It also has antioxidant properties and has been shown to have anti-inflammatory effects.
3. **Quercetin:** Quercetin is a flavonoid that is found in many fruits and vegetables. It is a potent antioxidant and has been shown to have anti-inflammatory and anti-cancer properties.
4. **Kaempferol:** Kaempferol is another flavonoid that is similar to quercetin.
5. **Gallic acid:** Gallic acid is a phenolic acid that is found in many plants. It is a potent antioxidant and has been

shown to have anti-inflammatory and anti-microbial properties.

6. **Ellagic acid:** Ellagic acid is a polyphenolic compound that is found in many fruits and berries. It is a potent antioxidant and has been shown to have anti-cancer and anti-viral properties [4, 6].

In addition to these main chemical constituents, catechu also contains other polyphenolic compounds, as well as tannins, alkaloids, and saponins. The various chemical constituents of catechu with its IUPAC name mentioned in the given Table. 02 as below followings:

Table 2: The list of chemical constituents of catechu with their chemical and IUPAC [5, 7]

Compound	IUPAC Name	Chemical Formula
Catechin	(2R, 3S)-3,7-Dihydroxy-2-(3, 4-dihydroxyphenyl)-5-chromanone	$C_{15}H_{14}O_6$
Epicatechin	(2R, 3R)-3, 5, 7-Trihydroxy-2-(3, 4-dihydroxyphenyl)-5-chromanone	$C_{15}H_{14}O_6$
Quercetin	2-(3, 4-Dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one	$C_{15}H_{10}O_7$
Kaempferol	3, 5, 7-Trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one	$C_{15}H_{10}O_6$
Gallic acid	3, 4, 5-Trihydroxybenzoic acid	$C_7H_6O_5$
Ellagic acid	2, 3, 7, 8-Tetrahydroxychromeno [5, 6b] chromene-5, 10-dione	$C_{14}H_6O_8$

These compounds also contribute to the antioxidant, anti-inflammatory, and antimicrobial properties of catechu. The chemical structure of all chemical constituents of *Senegalia catechu* shown as per the Fig. 02 as below followings:

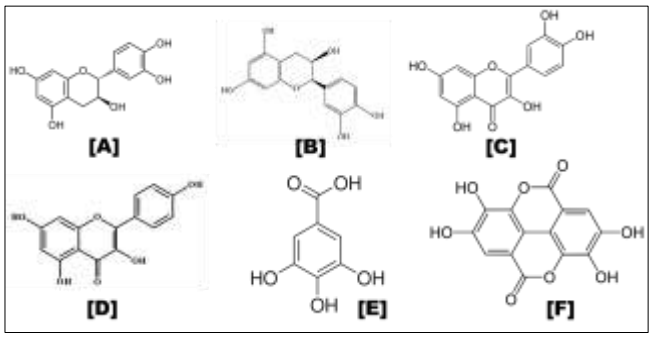

Fig 2: The all chemical constituents of *Senegalia catechu*; [A]. Catechin, [B]. Epicatechin, [C]. Quercetin, [D]. Kaempferol, [E]. Gallic acid and [F]. Ellagic acid

Pharmacological Applications: *Senegalia catechu*, also known as *Acacia catechu*, has been used in traditional medicine for centuries. It is known for its astringent, antiseptic, anti-inflammatory, and anti-diarrheal properties. The plant contains a variety of chemical constituents, including polyphenolic compounds, tannins, alkaloids, and saponins. These compounds are responsible for the plant's medicinal properties.

Anti-Inflammatory Activities: *Senegalia catechu* has been shown to have anti-inflammatory activities in both *in vitro* and *in vivo* studies. The plant's polyphenolic compounds, such as catechin and quercetin, are thought to be responsible for these activities. These compounds have been shown to inhibit the production of inflammatory mediators, such as prostaglandins and leukotrienes.

Antiulcer: *Senegalia catechu* has been shown to have antiulcer activity in animal studies. The plant's tannins are thought to be responsible for this activity. Tannins have been shown to protect the stomach lining from damage caused by gastric acid.

Wound healing: *Senegalia catechu* has been shown to promote wound healing in animal studies. The plant's polyphenolic compounds are thought to be responsible for this activity. These compounds have been shown to stimulate the production of collagen, a protein that is essential for wound healing.

Antipyretic: *Senegalia catechu* has been shown to have antipyretic activity in animal studies. The plant's tannins are thought to be responsible for this activity. Tannins have been shown to reduce fever by inhibiting the production of prostaglandins [6, 9].

Senegalia catechu exhibits a diverse array of pharmacological activities, making it a highly promising plant. Additionally, the compounds found in catechu play a significant role in its antioxidant, anti-inflammatory, and antimicrobial properties.

Methodology

Experimental Study Design: The experimental FFD of the study was conducted in accordance with the QbD approach. The raw material used for the study, which was the catechu plant. The identification of the raw material was carried out at the botanical Herbarium. The research was conducted at the phytochemistry laboratory [8-10]. All the involvement of the DoE latest software for their optimization purpose and statistical analysis respectively.

Additional excipients: The orodispersible tablet was formulated using Talc, Magnesium stearate, gelatine, lactose, corn starch, methyl parahydroxybenzoate, sucrose, crospovidone, and povidone. IMPM magazine generously provided these ingredients as gift samples. Furthermore, commercially sourced strawberry powder was obtained for the formulation.

Herbal drug processing: The *Senegalia catechu* bark was obtained from a botanical herbarium garden. After being carefully dried in a well-ventilated room for a few weeks, it was finely ground using an electrical mill and subsequently stored in a plastic bag.

Extraction method: The process of extracting ground bark using water as a solvent through digestion method at 70 °C. 3kg of dried powder was mixed with 50ml of water and the process was repeated to increase the yield. The resulting extract was combined with starch and dried in plates placed in an oven at 70 °C to obtain a powder. The dried crude extract and starch were then ground and stored in air tight plastic bags until further use. The percentage of extract in the starch extract mix after drying was calculated using equation 1 and the percentage yield of extraction was determined using equation 2 [9, 11].

$$\% \text{ Extract in Strach extract mixure} = \frac{\text{Mass of extract in gm}}{\text{starch extract mix}} \times 100 \quad [01]$$

The percentage of extract in starch-extract mix as per formula:

$$\% \text{ Yield Extract} = \frac{\text{Mass of dry extract in gm}}{\text{Initial mass of dry unprocessed powder}} \times 100 \quad [2]$$

Phytochemical analysis: The phytochemical analysis of *Senegalia catechu* has shown that the plant contains a wide variety of compounds (Table. 03), including:

- **Polyphenolic compounds:** Catechin, epicatechin, quercetin, kaempferol, gallic acid, and ellagic acid.
- **Tannins:** Catechin tannins, gallic acid tannins
- **Alkaloids:** Berberine, palmatine
- **Saponins:** Triterpenoid saponins
- **Other compounds*:*** Carbohydrates, proteins, lipids

Table 3: The list of phytochemical analysis of *Senegalia catechu* for the formulation

Phytochemical	Name of detailed Test	Obtained resultants
Alkaloids	Mayer's test, Dragendorff's test	Negative (-ve)
Tannins	Ferric chloride test	Positive (+ve)
Saponins	Frothing test	Positive (+ve)
Flavonoids	Shinoda test, Alkaline reagent test	Positive (+ve)
Steroids	Liebermann-Burchard test	Negative (-ve)
Terpenes	Liebermann-Burchard test	Positive (+ve)
Phenolic compounds	Ferric chloride test	Positive (+ve)

Polyphenolic compounds are the most abundant type of compound in *Senegalia catechu*. They are responsible for the plant's antioxidant, anti-inflammatory, and anti-microbial properties [12].

Test of polyphenolic compound: There are several tests that can be used to identify and quantify polyphenolic compounds. These tests can be divided into two main categories:

Qualitative tests

- **Colorimetric assays:** These assays are based on the ability of polyphenolic compounds to react with certain reagents to produce a color change. For example, the Folin-Ciocalteu reagent is a commonly used reagent for detecting polyphenolic compounds. This reagent reacts with polyphenolic compounds to produce a blue color.
- **Precipitation assays:** These assays are based on the ability of polyphenolic compounds to precipitate with certain reagents. For example, lead acetate is a commonly used reagent for precipitating polyphenolic compounds. This reagent reacts with polyphenolic compounds to form a white precipitate.

Quantitative tests

- **Spectrophotometric assays:** These assays are based on the ability of polyphenolic compounds to absorb light at certain wavelengths. For example, the absorbance of polyphenolic compounds at 280 nm is commonly used to quantify the concentration of these compounds in a sample.
- **Chromatographic assays:** These assays are based on the ability of polyphenolic compounds to separate based on their different affinities for different stationary phases. For example, high-performance liquid chromatography (HPLC) is a commonly used technique for separating and quantifying polyphenolic compounds.

In addition to these general tests, there are also a number of specific tests that can be used to identify and quantify individual polyphenolic compounds [12, 15]. These tests are typically based on the unique chemical properties of each compound.

The QTPPs: The inception of dispersible tablet in the pharmaceutical sector initiated with the recognition of the preferred characteristics and dosage form based on the target product profile. Ongoing clinical trials are being conducted for *Senegalia catechu* ODTs. The pharmaceutical objective is to create an orodispersible tablet that improves patient adherence and guarantees a swift onset of action [16]. The tablet's manufacturing process necessitates a sturdy and consistent approach, ensuring the production of a pharmaceutical product that satisfies the essential quality characteristics. The expected quality specifications for the drug product are outlined in Table. 04.

Table 4: The list of QTPPs *Senegalia catechu* orodispersible tablet [14, 17]

QTPPs elements		Target	Justification
Dosage form		Tablet	Ease of production
Dosage design		Orodispersible tablets lacking any scoring or coating.	Increased patient compliance
Route of administration		Oral	Ease of administration
Dosage strength		240 mg	Suitable for clinical trial for antianaemia properties
Drug product quality attributes	Physical attributes	The patients find the color and shape to be satisfactory, with no presence of unpleasant odor or visible imperfections.	Patient compliance
	Content uniformity	USP standards	USP standards
	Disintegration	Less than 3 minutes	European pharmacopoeia standards
Hardness		100 N - 150 N Robust tablet able to transport and handling.	USP standards

The Quality Attributes (QAs): The QTPP serves as the foundation for establishing the CQAs, Critical Process Parameters (CPPs), and Control Strategy. By referring to the desired characteristics of the product, the initial CQAs that define the acceptable quality were determined. These CQAs were defined based on empirical evidence obtained from prior experiments and the knowledge gained from similar products. Table. 05 provides a classification of the quality attributes that have been identified as CQAs.

Table 5: The list of involving CQAs for *Senegalia catechu* orodispersible tablets [15, 18]

QAs	Target	CQA	Justification
Appearance	Uniform, light brown, round, biconvex tablets	Visual inspection	To ensure that the tablets are of consistent quality and free from defects.
Dimension	Diameter: 8.0 mm ± 5%; Thickness: 3.0 mm ± 5%	Calipers	To ensure that the tablets are of the correct size and shape.
Hardness	100 N - 150 N	Hardness tester	To ensure that the tablets are sufficiently hard to withstand handling and shipping.
Friability	Not more than 1.0%	Friability tester	To ensure that the tablets are not too fragile and will not break or chip during handling and shipping.
Weight variation	Not more than 5%	Weighing balance	To ensure that the tablets are of uniform weight and that each tablet contains the correct amount of active ingredient.
Dissolution	Not less than 85% of the active ingredient dissolved within 30 minutes in water at 37 °C	Dissolution tester	To ensure that the active ingredient is released from the tablets in a timely manner so that it can be absorbed into the bloodstream.
Potency	95% - 105% of the labeled amount of active ingredient	HPLC	To ensure that the tablets contain the correct amount of active ingredient.

Design of Experiments (DoE): A 3^2 FFD with two factors and three variables was employed for direct compression in DoE, necessitating nine experiments. The two factors, P1 (disintegrant level) and P2 (lubricant level), were denoted by -1, 0, and +1, signifying low, middle, and high values, respectively. Table. 06 displays these values as below followings:

Table 6: The Coded Design of Experiments for Direct compression [16, 19]

Factor	Level		
	-1 (Low)	0 (Medium)	1 (High)
P1 = Disintegrant (Lactose) %	2	3.5	5
P2 = Lubricant (Magnesium Stearate) %	0.25	2.5	5

The Table. 07 provides a comprehensive breakdown of the design, outlining the 9 different formulations. These formulations are categorized under the superdisintegrant sodium croscarmellose and the lubricant magnesium stearate, with their respective weight percentages.

Table 7: The 3^2 FFD used for optimization of orodispersible tablets by direct compression [20]

Formulation code	Super disintegration Sodium croscarmellose	Lubricant (Magnesium stearate) [% w/w]
R1	-1	0
R2	0	1
R3	-1	1
R4	1	-1
R5	0	-1
R6	-1	0
R7	1	0
R8	1	1
R9	0	-1

Table 8: The list of basic composition of *Senegalia catechu* by direct compression [21]

Ingredients	Function	Percentage (%)	Quantity (mg)
Senegalia catechu	Main Ingredients	61	250
Sodium croscarmellose	Super disintegration	22	82
Colloidal silicon dioxide	Glidant	5.5	4.8
Gelatine	Binder	8.3	6.9
Magnesium stearate	Lubricant	3.26-4.5	1.5-23
Lactose	Filler	--	39.49-73.93
Microcrystalline cellulose	Bulking agents	--	43.99-49.45
Total	--	100	428.68

Table 9: The list of details for the formulations for direct compression of *Senegalia catechu* [22]

Raw materials (%)	R1	R2	R3	R4	R5	R6	R7	R8	R9
Senegalia catechu	60	60	60	60	60	60	60	60	60
Microcrystalline cellulose	20	20	20	20	20	20	20	20	20
Lactose	2	3.5	2	5	3.5	2	5	5	3.5
Sodium croscarmellose	4.8	2.6	2.6	2.6	4.8	0.26	4.8	0.26	2.6
Colloidal silicon dioxide	1	1	1	1	1	1	1	1	1
Magnesium Stearate	0	0	0	1.9	1.9	1.9	1.9	1.9	1.9
Gelatine	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
Total	100	100	100	100	100	100	100	100	100

Moving on to Table. 08, it presents the overall composition of the nine formulations, listing the ingredients, their functions, percentages, and quantities in milligrams. The selection of excipient levels is in line with past knowledge and supported by relevant literature. The final mass of the formulation amounts to 440 mg. For a more detailed analysis of the formulations [18, 20].

Table. 09 offers a comprehensive overview of each of the nine formulations, providing specific details and information.

A 3^2 FFD was utilized to optimize the orodispersible tablet of *Senegalia catechu*. The dependent responses measured included disintegration time, hardness, friability, wetting time, and water absorption ratio. The levels of two independent factors, crospovidone concentration and gelatine concentration, were varied at two different levels. The high and low levels of each factor were denoted as +1 and -1, respectively. The optimization of orodispersible tablets was carried out using experimental design 3^2, which employed wet granulation. The coded design for the four formulations (R10-R13) of wet granulation is presented in Table. 10, while Table. 11 provides further details. The range of lactose, a superdisintegrant, was varied from -1 to +1 [21-24].

Table 10: The experimental design 3^2 employed for the optimization of orodispersible tablets through wet granulation [23]

Factor	Levels	
	-1 (Low)	1 (High)
Disintegration (Lactose) %	2	5
Binder (Gelatine) %	5	10

Table 11: The Coded Design of Experiments for wet granulation [24]

Formulation code	Super disintegration Sodium croscarmellose	Binder: Gelatine
R10	-1	-1
R11	1	1
R12	1	-1
R13	-1	1

Table. 12 presents the comprehensive formulation of the four batches. *Senegalia catechu* exhibited the highest concentration (53.59%) as active ingredient, whereas corn starch served as the super disintegrant at a proportion of 18.18%.

Table 12: The General formulation for wet granulation for *Senegalia catechu* [25]

Raw Material	Role	Percentage (%)
Senegalium catechu	Active ingredient	95-105
Microcrystalline cellulose	Bulking agent	50-60
Lactose	Disintegrant	10-20
Sodium croscarmellose	Superdisintegrant	1-3
Colloidal silicon dioxide	Glidant	0.5-1.5
Magnesium stearate	Lubricant	0.5-1.5
Gelatine	Binder	2.5-3.0

Formulation for wet granulation: The wet granulation formulation for the 4 batches can be found in Table. 13 below, which outlines the raw material percentages for R10-R13. These batches consist of two phases, namely the internal/intragranular phase and the external/extra granular phase. It is important to highlight that there were no notable distinctions observed between the *Senegalia catechu* and corn starch used in R10-R13 respectively.

Table 13: The list of specific recipe for wet granulation of *Senegalia catechu* [22, 24]

Raw material %	R10	R11	R12	R13
Internal /Intragranular Phase	--	--	--	--
Senegalia catechu	53.52	53.53	54.54	54.54
Microcrystalline cellulose	19.19	19.19	18.18	18.18
Lactose	4.5	9.8	4.6	9.5
External/Extra Granular Phase	--	--	--	--
Magnesium stearate	0.4	0.4	0.4	0.4
Sodium croscarmellose	1.6	1.6	1.6	1.6
Colloidal silicon dioxide	2.5	2.5	2.5	2.5
Gelatine	1.89	1.89	1.89	1.89
Microcrystalline cellulose	12.17	4.11	9.18	5.17

Statistical Analysis

The measured variables were collected and inputted into Microsoft Excel 365 to obtain raw data. To compare the groups, the Graph Pad Instat version 5.1 software was utilized, employing one-way analysis of variance (ANOVA) followed by DoE experimental design software [13]. The results were presented as mean ± standard deviation, with P-values ≤0.05 indicating statistical significance. The means were employed for analysis of compounds [25].

Results and Discussion

The process of water extraction by digestion resulted in a percentage yield of 43.95%, which can be considered satisfactory. However, it was observed that increasing the ratio of water to drug extract from 12.5:1 (Batch 1) to 16.7:1 (Batches 2-7) led to a higher percentage yield. Table. 14 provides detailed information on the extraction of *Senegalia catechu* through digestion.

Table 14: The extraction of catechu by digestion with their amount and yield [22, 24]]

Formulation numbers	Initial Weight (kg)	Amount of extract (gm.)	Yields (%)
1	4	1456.66	39.5
2	3	1201.09	42.8
3	3	1190.91	42.9
4	3	1394.21	51.6
5	3	1284.5	43.7
6	3	1080.0	39.9
7	3	1209.5	42.75
Total	22	9308.8	43.95

Phytochemical screening: The plants were endowed with colour, taste, and inherent defense against pests through phytochemical screening. The examination was performed on the aqueous solution derived from the stem bark of *Senegalia catechu*, and the results are displayed in Table 15. The evaluation demonstrated the absence of alkaloids and triterpenes, while confirming the presence of potent medicinal components such as tannins, saponins, phenols, flavonoids, and coumarins [25].

Table 15: The list of phytochemical screening of the aqueous stem bark extract of catechu was conducted

Phytochemicals	*Senegalia catechu*
Alkaloids	---
Saponins	++
Phytosterols and triterpenes	---
Phenols	+++
Tannins	++++
Flavonoids	+++
Reducing sugars	+++
Coumarins	++

+= Detectable presence ++= slightly abundant +++= abundant ++++ = very abundant-no variation occurs

Pre-compression studies: An analysis was performed to assess the flow properties, bulk and tapped densities, Carr's index (CI), Hausner's ratio (HR), and the angle of repose [26] before compressing the powder mixture into tablets. The pre-compression parameters of the powder mixture can be found in Table 16 as below mentioned.

Table 16: The involved list of pre-compression parameters of powder blends

Method	Formulation Batch code	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of repose	Carr's index (CI)	Hausner's ratio (HR)
Direct compression	R1	0.66	0.99	32.33	22.0	1.21
	R2	0.68	0.89	35.41	26.7	1.36
	R3	0.69	0.92	36.21	29.6	1.39
	R4	0.67	0.92	29.12	25.9	1.39
	R5	0.63	0.84	31.81	26.8	1.34
	R6	0.63	0.87	32.79	27.5	1.37
	R7	0.64	0.81	35.33	24.0	1.29
	R8	0.64	0.88	43.99	22.9	1.32
	R9	0.67	0.80	29.12	27.8	1.21
Wet granulation	R10	0.88	0.75	37.44	9.8	1.08
	R11	0.58	0.74	39.78	20.5	1.24
	R12	0.58	0.72	29.82	19.21	1.26
	R13	0.67	0.70	21.09	15.1	1.12

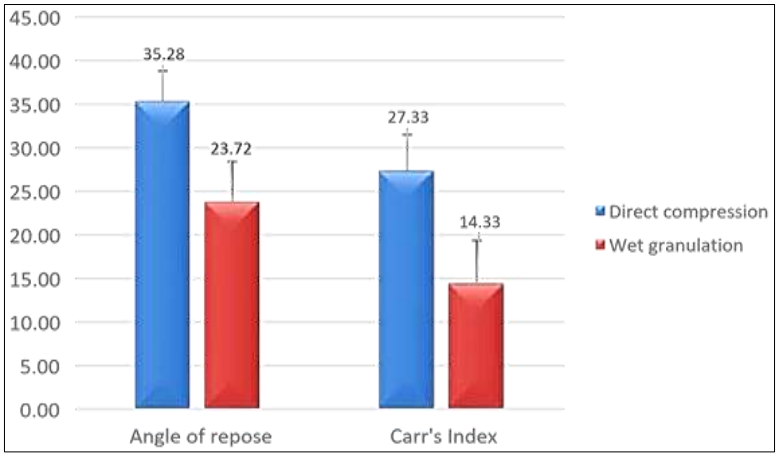

Fig 3: The comparison of Angle of repose and CI for direct compression and wet granulation

The pre-compression parameter for wet granulation of *Senegalia catechu* involves the angle of repose and CI. The comparison of these parameters is illustrated in Fig. 03 as depicted below.

Summary of pre-compression parameters: The Table. 01 presents a summary that compares the pre-compression parameters of powder blends using direct compression and wet granulation techniques. The findings from the pre-compression investigations conducted on the powder blends for tablet production methods, namely direct compression and wet granulation, are summarized in Table 17. It is evident from the results that the powder blend utilized in wet granulation demonstrates enhanced flow properties in comparison to direct compression [25, 27].

Table 17: The list of findings derived formulation the flow of powder in various formulations

Formulation Methods	Batch Observations	Results
Direct compression	R1	Passable and good
	R2	Very poor and poor
	R3	Good and very poor
	R4	Passable and poor
	R5	Very poor and poor
	R6	Very poor and poor
	R7	Very poor and good
	R8	Very poor and poor
	R9	Very poor and very poor
Wet granulation	R10	Good and good
	R11	Excellent and excellent
	R12	Good and good
	R13	Good and excellent

Advancement of modelling of Disintegration time (DT): The ANOVA table efficiently partitions the variability in DT into separate components for each effect (Table. 18). Afterwards, it evaluates the statistical significance of each effect by comparing the mean square with an estimate of the experiment errors. Including the specific situation, three effects demonstrate p-values less than 0.05, indicating their significant deviation from zero with a 95.0% confidence level [28].

Table 18: The Analysis of variance (ANOVA) for disintegration time

Evaluating terms	Sum R^2	Df	Mean of squares	F-Values	p-value
Super Disintegrant	4669.21	1	4068.41	4228.45	----
Binder level	55138.9	1	43259.9	51127.88	----
Super disintegrant level x Binder level	4385.21	1	13365.21	2318.72	----
Total error	7.78	84.09			
Total	62020.8	11	--	--	--

When fitted, the model showcases an impressive R-Squared statistic, indicating its ability to effectively explain 98.95% of the variability in disintegration time. For a more appropriate comparison between models with varying numbers of independent variables, the adjusted R-squared statistic also stands at an impressive 99.98%.

Wetting Time: The wetting time of the Table. 13 batches exhibited the longest duration for the R13 formulation, as depicted in Fig. 3. R13, characterized by a low sodium croscarmellose content and a high binder (Gelatine) content, demonstrated the highest wetting time of 66.0 seconds, while R12, with a high crospovidone content and a low binder content, exhibited the shortest wetting time of 19.43 seconds. The wetting time of all tablet formulations ranged from 19.5 to 73.0 seconds [29]. The wetting time values for the wet granulation formulation of *Senegalia catechu* are illustrated in Fig. 04 below.

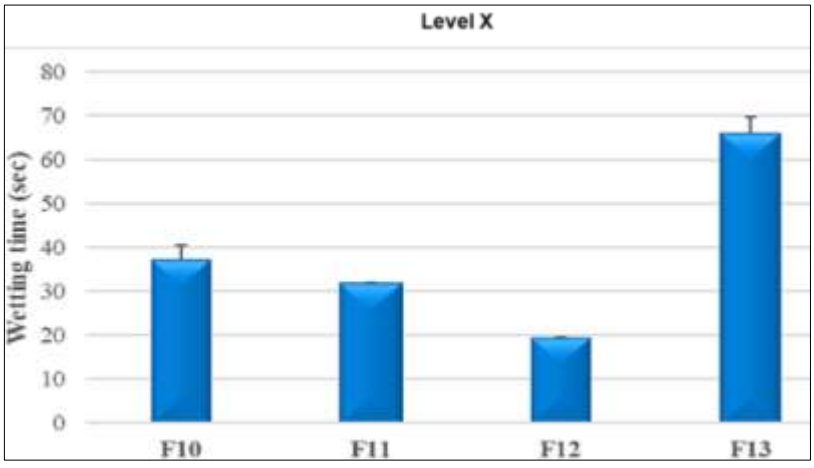

Fig 4: The representation of wetting time values for wet granulation formulations

Optimization of Wetting Time: The R13 formulation, as shown in Fig. 05, displayed the longest wetting time among the four tablet batches. This can be attributed to its low crospovidone content and high binder content. In fact, R13 demonstrated an impressive wetting time of 66.0 seconds. On the other hand, R12, which had a high crospovidone content and low binder content, exhibited the shortest wetting time of 19.43 seconds. The wetting time of all tablet formulations ranged from 19.5 to 73.0 seconds. The response surface plot in Fig. 06 illustrates the water absorption of *Senegalia catechu* in relation to wetting time.

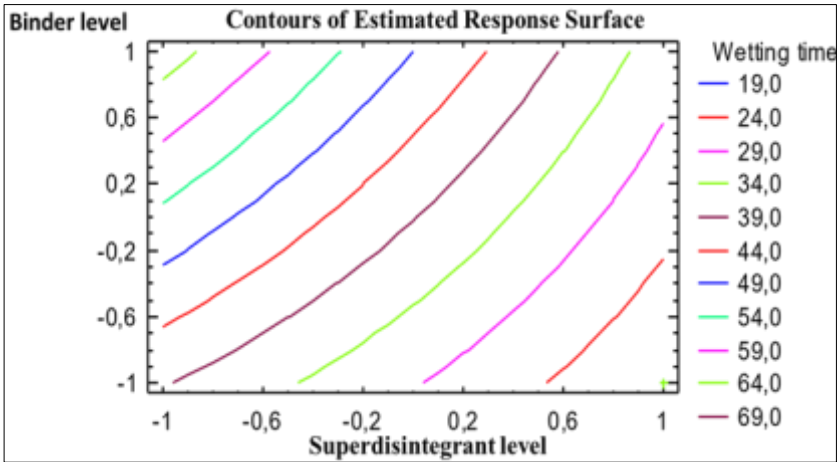

Fig 5: The contour plot for wetting time of *Senegalia catechu* vs. super disintegration and binder/wetting levels

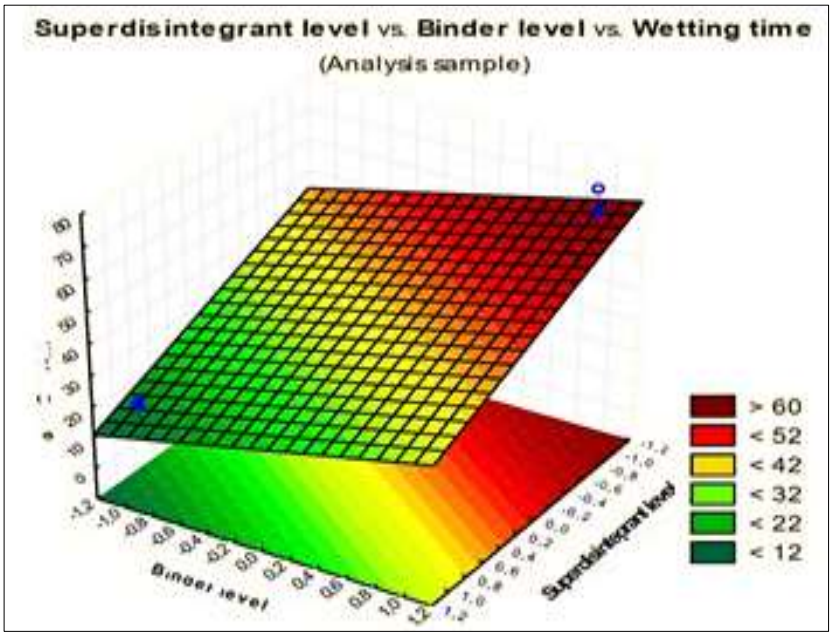

Fig 6: The response surface plot (RSP) depicting the wetting time water absorption ratio is illustrated in a schematic manner

The yield obtained from maceration by other researchers was significantly lower, with percentages of 12.3% [6, 24] and 27.3% [13, 19] when rotated to enhance the yield. In contrast, our study achieved a relatively higher percentage yield of 42.96%. This difference can be attributed to the grinding of the plant material, which increases the surface area for extraction and consequently enhances the extraction rate. The previous studies have recommended a solvent to sample ratio of 10:1 (v/w) as the ideal ratio [3, 15, 17, 29].

Water, a widely used universal solvent, was selected for our research to extract various plant products. The components that can be easily extracted with water include anthocyanins, starches, tannins, saponins, terpenoids, polypeptides, and lectins [28, 32], all of which were of particular interest in our study. However, it is worth noting that alternative solvents may uncover additional phytochemicals [33, 36].

Conclusion

The results of the study suggest that *Senegalia catechu* can be more effectively extracted through aqueous digestion compared to other methods. The extract was found to contain a variety of phytochemicals, including tannins, saponins, phenols, flavonoids, and coumarins, which have been shown to have pharmacological properties in previous research. The flow properties of the powder blend were significantly impacted by direct compression and wet granulation techniques, with wet granulation proving to be superior in producing free-flowing granules for *Senegalia catechu*. Furthermore, the amount of lubricant and diluent used in the formulation process was found to have a significant impact on powder flow, highlighting the importance of proper selection of these variables to optimize powder flow.

The tablet properties of *Senegalia catechu*, including disintegration time, hardness and wetting time were found to be significantly influenced by the levels of superdisintegrant

and binder. However, the water absorption ratio was not significantly affected. The study's statistical design enabled a limited number of experiments, yet the model derived from it can accurately forecast response values within the experimental space. The wet granulation formulation was effectively optimized through the implementation of this design. Within this obtaining resulting of research, *Senegalia catechu* stem bark extract ODTs were successfully prepared using a QbD as DoE approach and a wet granulation method. The formulation's design and evaluation ultimately led to the successful optimization of an ETM. The effectiveness of DoE in optimizing ETM formulations and offering valuable insights into the formulation and manufacturing process was demonstrated. Future studies regarding this formulation should utilize DoE to explore the impact of additional process variables, such as compression force and speed, as well as formulation variables like palatability.

Authors Contribution

This project was undertaken through a collaborative endeavor involving all four authors, encompassing the conceptualization and design of study, data collection, compilation, analysis and interpretations, as well as their composition and critical evaluation endorsement of the final manuscript.

Competing Interests: Nil.

References

1. Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, *et al*. *Areca catechu* L. (Arecaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. Journal of ethnopharmacology. 2015;164:340-356.
2. Ansari A, Mahmood T, Bagga P, Ahsan F, Shamim A, Ahmad S, *et al*. *Areca catechu*: A phytopharmacological legwork. Food Frontiers. 2021;2(2):163-183.
3. Patil PR, Rakesh SU, Dhabale PN, Burade KB. Pharmacological activities of *Areca catechu* Linn: A review. Journal of Pharmacy Research. 2009;2(4):683-687.
4. Salehi B, Konovalov DA, Fru P, Kapewangolo P, Peron G, Ksenija MS, *et al*. *Areca catechu*-From farm to food and biomedical applications. Phytotherapy Research. 2020;34(9):2140-2158.
5. Stohs SJ, Bagchi D. Antioxidant, anti-inflammatory, and chemoprotective properties of *Acacia catechu* heartwood extracts. Phytotherapy research. 2015;29(6):818-824.
6. Kumari M, Radha, Kumar M, Zhang B, Amarowicz R, Puri S, *et al*. *Acacia catechu* (Lf) Willd: A review on bioactive compounds and their health promoting functionalities. Plants. 2022;11(22):3091.
7. Adhikari B, Aryal B, Bhattarai BR. A Comprehensive Review on the Chemical Composition and Pharmacological Activities of *Acacia catechu* (Lf) Willd. Journal of Chemistry; c2021. p. 1-11.
8. Osborne PG, Ko YC, Wu MT, Lee CH. Intoxication and substance use disorder to *Areca catechu* nut containing betel quid: A review of epidemiological evidence, pharmacological basis and social factors influencing quitting strategies. Drug and alcohol dependence. 2017;179:187-197.
9. Sharma P, Lingha R. A recent update on the pharmacognostical as well as pharmacological profiles of the *Acacia catechu* heartwood: A mini review. J Ayurveda Holist. Med. 2021;7:188-192.
10. Satpudke S, Pansare T, Khandekar S. Review on Khadira (*Acacia catechu* Willd.) with special reference to Prameha (Diabetes). Int. J Herb. Med. 2020;8:1-5.
11. Pal R, Pandey P, Rizwan M, Koli M, Thakur SK, Malakar RK, *et al*. The Utilization of Response Surface Methodology (RSM) In the Optimization of Diclofenac Sodium (DS) Liposomes Formulate through the Thin Film Hydration (TFH) Technique with Involving Computational Method. Journal of Advances in Medicine and Medical Research. 2023;35(22):287-300.
12. Hakim L, Kumar L, Gaikwad KK. Screen printing of catechu (*Senegalia catechu*)/guar gum based edible ink for food printing and packaging applications. Progress in Organic Coatings. 2023;182:107629.
13. Bhattarai R, Sharma P, Wagle B, Adhikari A, Acharya S. Revision and compilation of health management plan of Khair (*Acacia catechu*). Grassroots Journal of Natural Resources. 2020;3(1):15-28.
14. Duval A, Averous L. Characterization and physicochemical properties of condensed tannins from *Acacia catechu*. Journal of agricultural and food chemistry. 2016;64(8):1751-1760.
15. Peng W, Liu YJ, Wu N, Sun T, He XY, Gao YX, *et al*. *Areca catechu* L. (Arecaceae): A review of its traditional uses, botany, phytochemistry, pharmacology and toxicology. Journal of ethnopharmacology. 2015;164:340-356.
16. Ba'ai NM, Hashim HZ. Waste to wealth: the innovation of *Areca catechu* as a biomaterial in esthetics seed-based jewelry. In Proceedings of the International Symposium on Research of Arts, Design and Humanities (ISRADH 2014). Springer Singapore; c2015. p. 373-381.
17. Pal R, Pandey P, Nogai L, Anand A, Suthar P, Sahdev Keskar M, *et al*. The Future Perspectives and Novel Approach on Gastro Retentive Drug Delivery System (Grdds) With Currrent State. Journal of Population Therapeutics and Clinical Pharmacology. 2023;30(17):594-613.
18. Liu H, Li D, Huang Y, Lin Q, Huang L, Cheng S, *et al*. Addition of bacterial consortium produced high-quality sugarcane bagasse compost as an environmental-friendly fertilizer: Optimizing arecanut (*Areca catechu* L.) production, soil fertility and microbial community structure. Applied Soil Ecology. 2023;188:104920.
19. Sitorus A, Nugraha D, Rustamli D, Muslimin M, Hasan AR, Bulan R, *et al*. Design and Construction of a Cutting Machines for Fresh Betel Nut (*Areca catechu* Linn). In 2019 5th International Conference on Computing Engineering and Design (ICCED). IEEE; c2019. p. 1-4.
20. Al-Alwani MA, Hasan HA, Al-Shorgani NKN, Al-Mashaan ABS. Natural dye extracted from *Areca catechu* fruits as a new sensitiser for dye-sensitised solar cell fabrication: Optimisation using D-Optimal design. Materials Chemistry and Physics. 2020;240:122204.
21. Pal R, Pandey P, Waheed S, Thakur SK, Sharma V, Chanana A, *et al*. Transdermal drug delivery system (tdds) as a novel tool for drug delivery; c2022.
22. Lewis ED, Crowley DC, Guthrie N, Evans M. Role of *Acacia catechu* and *Scutellaria baicalensis* in enhancing immune function following influenza vaccination of healthy adults: a randomized, triple-blind, placebo-controlled clinical trial. Journal of the American Nutrition Association. 2023;42(7):678-690.

23. Pandey P, Pal R, Rizwan M, Saxena A, Koli M, Nogai L, *et al*. The recent approaches in nano-technology with applications of 3-d printing (3dp) in diverse advanced drug delivery system (dds).
24. Lakshmi T, Ramasamy R, Thirumalaikumaran R. Preliminary phytochemical analysis and *in vitro* antioxidant, FTIR spectroscopy, anti-diabetic activity of *Acacia catechu* ethanolic seed extract. Pharmacognosy Journal. 2015, 7(6).
25. Al-Mohizea AM, Raish M, Ahad A, Al-Jenoobi FI, Alam MA. Pharmacokinetic interaction of *Acacia catechu* with CYP1A substrate theophylline in rabbits. Journal of Traditional Chinese Medicine. 2015;35(5):588-593.
26. Singh N, Tailang M, Mehta SC. A review on herbal plants as immunomodulators. International Journal of Pharmaceutical Sciences and Research. 2016;7(9):3602.
27. Pal R, Pandey P, Rai B, Koli M, Chakrabarti M, Thakur P, *et al*. Chitosan: as highly potential biopolymer obtainable in several advance drug delivery systems including biomedical applications. Environmental science. 3, 4.
28. Karki R, Bajgai AK, Khadka N, Thapa O, Mukhiya T, Oli HB, *et al*. *Acacia catechu* Bark Alkaloids as Novel Green Inhibitors for Mild Steel Corrosion in a One Molar Sulphuric Acid Solution. Electrochem. 2022;3(4):668-687.
29. Trishala A, Lakshmi T, Rajeshkumar S, Gurunathan D, Roy A. Physicochemical Profile of *Acacia catechu* Bark Extract-An *In vitro* Study. Indian Journal of Public Health Research & Development. 2019, 10(11).
30. Aryal B, Adhikari B, Aryal N, Bhattarai BR, Khadayat K, Parajuli N, *et al*. LC-HRMS profiling and antidiabetic, antioxidant, and antibacterial activities of *Acacia catechu* (Lf) willd. BioMed research international; c2021.
31. Khurana EKTA, Singh JS. Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: a review. Environmental conservation. 2001;28(1):39-52.
32. Rahaman A, Debnath B, Chaudhury UG, Mahakal N. A comprehensive review and aoushadhi prayog of *acacia catechu* willd. With special reference to nighantus; c2022.
33. Lakshmi T, Rajeshkumar S, Roy A, Gurunadhan D, RV G. Antibacterial Activity of Taxifolin Isolated from *Acacia catechu* Leaf Extract-An *In vitro* Study. Indian Journal of Public Health Research & Development. 2019, 10(11).
34. Pal R, Pandey P, Maurya VK, Saxena A, Rizwan M, Koli M, *et al*. Optimization and formulation of doxorubicin (dox) loaded liposome well-used in chemotherapy involving quality by design (qbd): A transitory research.
35. Yaneva Z, Ivanova D, Popov N. Clinoptilolite Microparticles as Carriers of Catechin-Rich *Acacia catechu* Extracts: Microencapsulation and *In vitro* Release Study. Molecules. 2021;26(6):1655.
36. Beck SL, Dunlop RW. Micropropagation of the Acacia species-A review. *In vitro* Cellular & Developmental Biology-Plant. 2001;37:531-538.