



Research



Optimization of extraction conditions for phytochemicals from *Senna fistula* using cheminformatics

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Abstract

Senna fistula (commonly called golden shower tree) is a tropical plant renowned for its established medicinal properties. Additionally, it serves as a popular ornamental tree for homesteads and roadside plantings. But previous phytochemical studies on the plant adopted the conventional methods of extraction without optimal yield of the plant extract. This study investigated on the phytochemistry of *S. fistula* leaf, stem bark and flower using ethanol, aqueous and palm wine extracts. Also, optimization of extraction with the aid of design expert was carried out to determine the extraction condition that would give the optimum yield using different solvent. Result of qualitative analysis on phytochemicals showed presence of anthraquinones, flavonoids, saponins, terpenoids, phenols, cardiac glycosides and tannins. An extraction of 10 g of *S. fistula* for 24 h at 300 rpm, with an orbital shaker time of 30 min, gave an optimal yield of 3.6 g. The bio-active compounds found in *S. fistula* are subjects of more exploratory research with usable products that can benefit mankind as the focus.

Highlights

1. Phytochemical analysis of *S. fistula* flower revealed the present of bioactive phytochemicals which can be further harnessed for pharmaceutical purposes.
2. Orbital shaking time of 29 min at 300 rpm gave an optimum yield of 3.4 g in *S. fistula*.
3. Dichloromethane and ethyl acetate solvents produced the best retention factor for *S. fistula*.

Keywords Cheminformatics · *Senna fistula* · Legume · Golden-shower tree · Phytochemicals · Medicinal plant

1 Introduction

The right extraction technique is crucial for qualitative and quantitative research of bioactive chemicals from plant materials [1, 2]. The initial step in every study of a medicinal plant is extraction, which has a substantial impact on the outcome. "Sample preparation techniques" is another name for extraction methods [3]. This area of research

has received limited attention and is often conducted by researchers with limited experience [4].

The success of the analysis of any bioactive component depends largely on the extraction procedures, input parameters, and precise nature of plant parts [5]. It is true that development of contemporary chromatographic and spectrometric techniques has made the analysis of bioactive compounds easier. The matrix

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qualities of the plant portion, the solvent, the temperature, the pressure, and the duration are the most frequent variables influencing the extraction processes [6].

All plants contain phytopharmaceuticals, that have discovered widespread use in human, agriculture and veterinary medicine, and other fields. *Senna fistula* (syn. *Cassia fistula*) a legume commonly called the golden shower tree is a plant with reported medicinal efficacy [7, 8]. *Senna fistula* (Fabaceae, Caesalpinioideae) is a semi-wild leguminous plant that is highly renowned for its established medicinal effects. It is locally called "Aidan tooro" in Yoruba language, south western Nigeria [9]. It can be found in Asia, Africa, the West Indies, and Brazil, among other places [6, 10]. It is a popular ornamental tree for homesteads and roadside plantings. Many physiologically active chemicals (i.e. phytochemicals) can be extracted and identified from various sections of the plant. The plant parts have been reported to possess analgesic, antifungal, antibacterial, antipyretic, anti-inflammatory, larvicidal, anti-tumor, antioxidant, hepatoprotective, hypoglycemic activities, and laxative properties [11, 12].

But there are limitations in previous extraction techniques, such as choice of solvents, alteration in temperature, pH of plant extract, etc. [13, 14]. Therefore, the need for cheminformatic guided way of extracting bioactive compounds from plants.

The word "cheminformatics" has only been in usage since the beginning of the 1990s, regardless of the fact that computers have been used in chemistry since their inception, when the field sprung out of previous work in the domains of computation, chemistry, information science, and drug discovery, to mention a few. Chemoinformatics (most common in Europe), chemical informatics, and sometimes even chemi-informatics are all terms that have been used to describe the field as a result of these various influences. The use of this techniques guides extraction which maximizes resources, yield, and reduces environmental losses [15–18].

Several literature surveys revealed that *S. fistula* is an important medicinal plant [9]. This plant is used by traditional medical practitioners for the treatment of various diseases [19, 20]. Therefore, optimizing the extraction of the phytochemicals from the plant parts will provide more valuable information which will assist scientists in obtaining maximum resources from plant species for the improvement of human and animal health and wellbeing.

The main purpose of this study was to use chemistry informatics (cheminformatics) to improve the extraction

of phytochemicals from *S. fistula* plant parts. To know what extraction condition will give the optimum yield using different solvent.

2 Materials and methods

2.1 Sample collection

The *S. fistula* samples were collected from Bowen University, Iwo, Nigeria (Latitude 7° 38' N, 4° 11' E) during its flowering period. The plant was authenticated, and assigned herbarium number BUH032. The collected leaves, stem and flowers were dried at room temperature, ground to smaller particulates and stored in airtight containers.

2.2 Maceration

Senna fistula powder weighting 100 g (leaves, stem bark, flower respectively) was put in 1000 ml glass jars and then soaked with three different solvents (distilled water, palm wine and ethanol respectively) of about 800 ml volume. The glass jars were placed on an orbital shaker at about 253–300 rpm for about 10–20 min. The extracts were filtered out and concentrated on a rotary evaporator under the reduced pressure conditions at 60 to 85 °C.

2.3 Thin layer chromatography

A capillary tube was used to take sample extract and create a spot on the chromatography plate. The chromatography plate was labeled at the very top with pencil for ease of noting the point of dropping. The developing chromatography plate was then dipped in a 250 ml beaker containing solvents (e.g., *n*-hexane, ethyl acetate, dichloromethane, ethanol) ratios. After this process, the plates were checked under an ultraviolet (UV) light and the various retention factor (rf) taken.

2.4 Phytochemical tests

Tests were carried out to discover the presence or absence of the following phytochemicals: flavonoids, saponins, phenols, alkaloids, tannins, cardiac glycosides, proteins, steroids, terpenoids, anthraquinones and carbohydrates. Method as described by Banu and Cathrine [21] and Harborne [22] were adopted for the phytochemical analysis.

Table 1 Phytochemical analysis of *S. fistula*

Phytochemicals	Sample	Ethanol	Water	Palm wine
Alkaloids	<i>S. fistula</i> leaves	–	–	–
	<i>S. fistula</i> flower	–	–	–
	<i>S. fistula</i> stem bark	–	–	–
Anthraquinones	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> flower	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
Flavonoids	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
	<i>S. fistula</i> flower	+	+	+
Saponin	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
	<i>S. fistula</i> flower	+	+	+
Tannins	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
	<i>S. fistula</i> flower	+	+	+
Cardiac glycosides	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
	<i>S. fistula</i> flower	+	+	+
Phenols	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
	<i>S. fistula</i> flower	+	+	+
Proteins	<i>S. fistula</i> leaves	–	–	–
	<i>S. fistula</i> stem bark	–	–	–
	<i>S. fistula</i> flower	–	–	–
Carbohydrates	<i>S. fistula</i> leaves	–	–	–
	<i>S. fistula</i> stem bark	–	–	–
	<i>S. fistula</i> flower	–	–	–
Steroids	<i>S. fistula</i> leaves	–	–	–
	<i>S. fistula</i> stem bark	–	–	–
	<i>S. fistula</i> flower	–	–	–
Terpenoids	<i>S. fistula</i> leaves	+	+	+
	<i>S. fistula</i> stem bark	+	+	+
	<i>S. fistula</i> flower	+	+	+

+ Present, – absent

Table 2 Variations in extraction time, shaking time and speed of shaking for *S. fistula* stem bark

Run	Extraction time (h)	Orbital shaking time (min)	Shaking (rpm)	Yield (g)
1	6	30	300	2.9
2	6	30	257	2.7
3	24	10	229	3.2
4	24	10	257	3.5
5	6	30	229	3.2
6	6	10	300	2.7
7	24	30	300	3.6
8	24	10	300	3.2

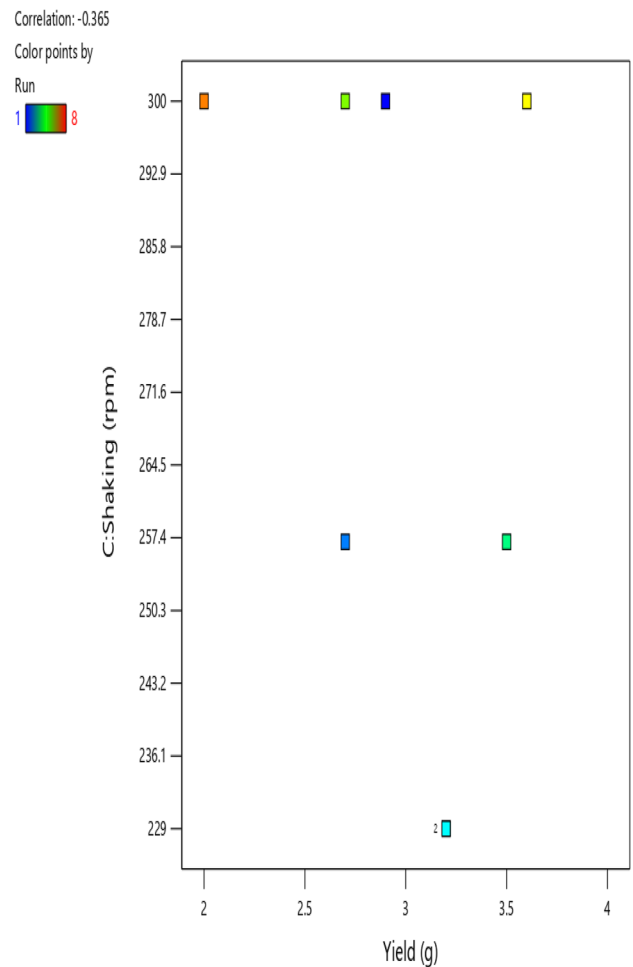


Fig. 1 Graph of shaking speed (rpm) against yield (g) of *Senna fistula*

2.5 Experimental design

A 2³ factorial table was chosen in the Design Expert Software v12.0 (Stat-Ease Free Download for Windows), and the relevant factors were inputted into the table given. The

factors were extraction time (h), orbital shaking time (min) and shaking (rpm). These factors would then give result as yield of extract (g).

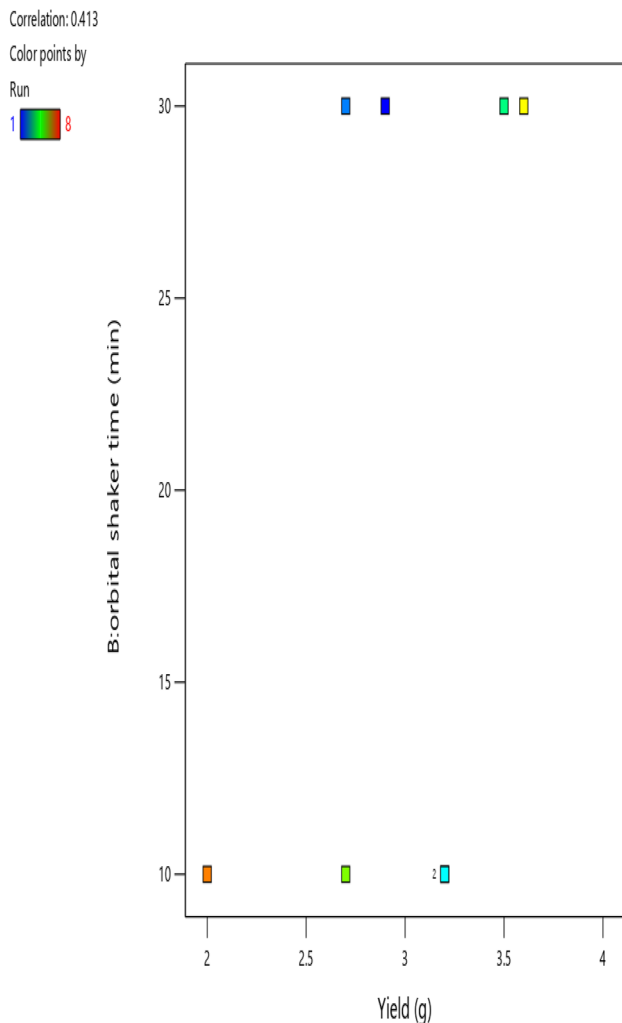


Fig. 2 Graph of orbital shaking time (min) against yield (g) of *Senna fistula*

Variations in the extraction time, shaking time and speed of shaking for *S. fistula* stem bark was made and also 10 g of the mass of dry sample of *S. fistula* stem bark and a constant volume of 100 ml distilled water, while extraction time, shaking time and speed of shaking was varied. The results that gave the best yields were selected out of the pool of results given.

3 Results and discussion

Qualitative analysis done on the distilled water, ethanol and palm wine extracts of *S. fistula* leaves, stem bark and flowers revealed the presence of anthraquinones, flavonoids, saponins, terpenoids, phenols, cardiac glycosides and tannin. While alkaloids, proteins, carbohydrates and

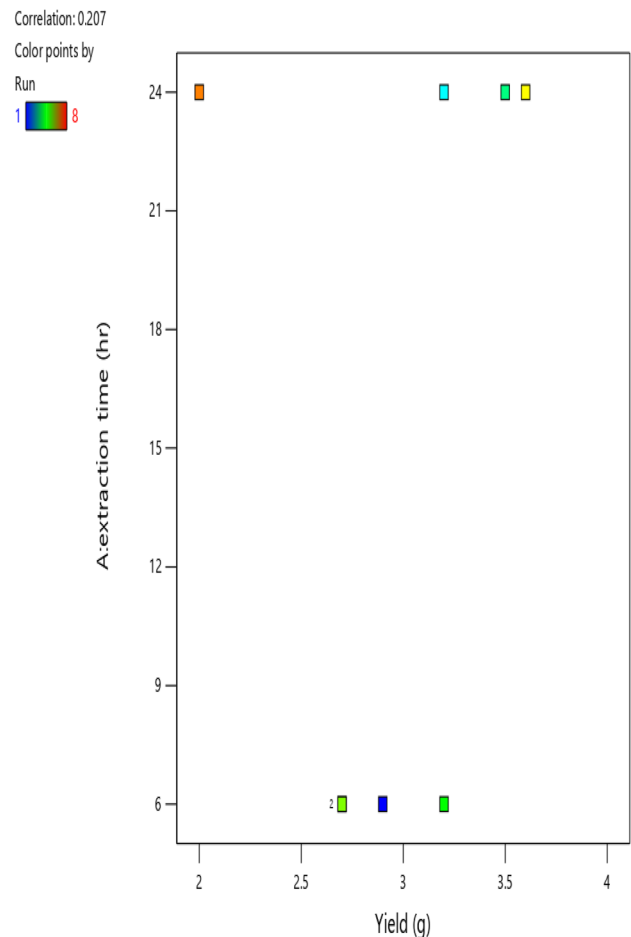


Fig. 3 Graph of extraction time (min) against yield (g)

steroids were absent in all extracts (Table 1). Previous reports on phytochemical constituents of *S. fistula* have been extracts from the leaf [23, 24], stem bark [25] and seed pulp [26, 27]. Bioactive compounds from *S. fistula* were reported to be more in polar solvents than in non-polar solvents [27]. This research further highlighted the bioactive phytochemicals present in *S. fistula* flower, such as anthraquinones, flavonoids, saponins, tannins, cardiac glycosides, phenols, and terpenoids. The benefit of which can be further harnessed for pharmaceutical purposes.

An increase in extraction time (24 h), orbital shaking time (30 min) and speed of shaking (300 rpm) resulted in the highest yield of 3.6 g (Table 2). Time of orbital shaking was reported to have positive influence on the yield plant extract [28, 29]. In this study increase in time of orbital shaking at room temperature of increased yield of extracts.

Figure 1 shows that the two best yields 3.6 g and 3.5 g occur at 300 and 257 rpm respectively. While Fig. 2 shows the best two yields 3.6 g and 3.5 g occur at 30 min of

Fig. 4 Graph of orbital shaking time (min) and extraction time (min)

Factor Coding: Actual

Yield (g)

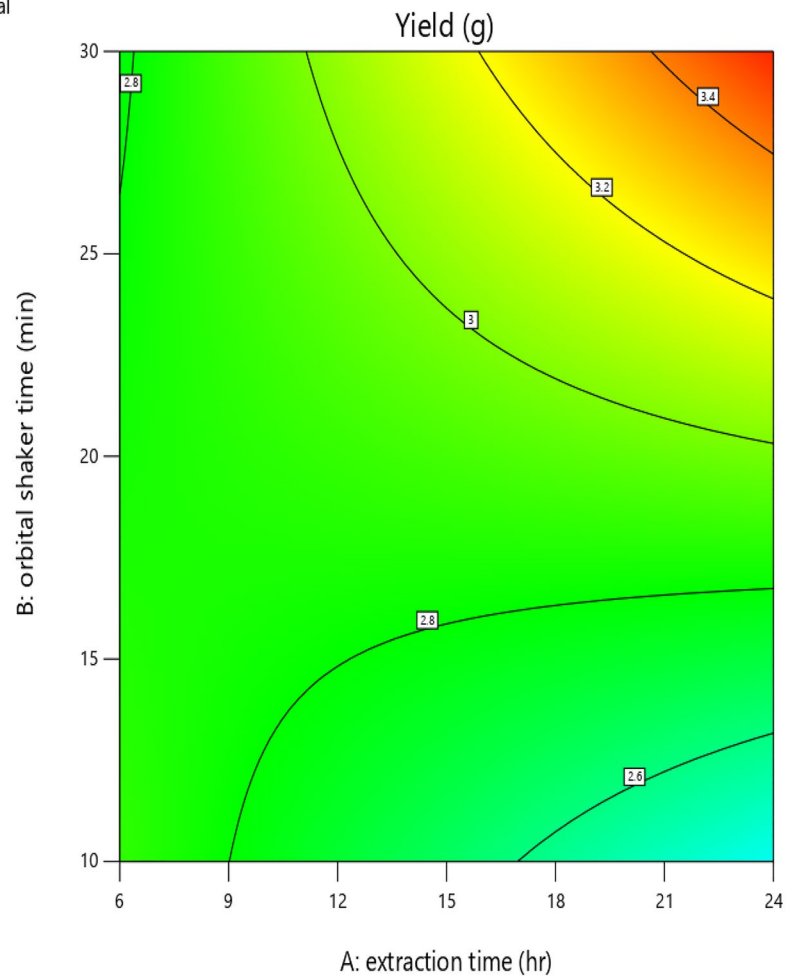
2  3.6

X1 = A

X2 = B

Actual Factor

C = 275



orbital shaker time also Fig. 3 shows the optimum yields 3.6 g and 3.5 g to occur at 24 h of extraction time. Therefore, we can conclude that carrying out an extraction of 10 g of *S. fistula* for 24 h at 300 rpm, with an orbital shaker time of 30 min, an optimal yield of 3.6 g would be obtained.

From the graphs in Figs. 1, 2, 3, 4, 5, and 6, we could predict and derive the values below by assigning and estimating using the graphs.

Table 3 is useful as a model for extraction process of phytochemicals from *S. fistula* stem bark and it can also be used to optimize an existing model's yield. From the run table, the highest yield 3.6 g occurred as a result of extraction time (24 h), orbital shaking time (30 min) and shaking (300 rpm) while the lowest yield 2 g occurred as a result of extraction time (24 h), orbital shaking time (10 min) and shaking (300 rpm). From the predictive table, the highest yield 3.4 g was gotten as a result of extraction time (22 h), orbital shaking time (29 min) and shaking (300 rpm) while the lowest yield 2.4 g occurred as a result of extraction

time (24 h), orbital shaking time (11 min) and shaking (298 rpm).

In this study, the crude extract of *S. fistula* resulted in good separation from the TLC analysis. The mobilities of all compounds were calculated and recorded after separation from the various *S. fistula* extracts in an appropriate composition solvent system, as shown in Tables 4 and 5. Using their *r_f* values as a guide, Fig. 7 illustrates the separation of the mixture compounds. A sample's chemical components can be separated using the TLC technique to establish each component's chemical identity [30]. Using visible and ultra violet light, five distinct photographs of the mobile phases on TLC plates revealed colors with the calculated *r_f* value as tabulated. The UV 254 nm light showed colours ranging from black to red and some kind of fluorescence; evidence that *S. fistula* is rich in phytochemicals.

Out of all the mean *r_f* values calculated, it was found that dichloromethane:ethyl acetate at ratio 8:2 produced the best separation of retention mean at 0.0677. When comparing two different compounds under identical

Fig. 5 Shaking (rpm) against orbital shaker time (min)

Factor Coding: Actual

Yield (g)

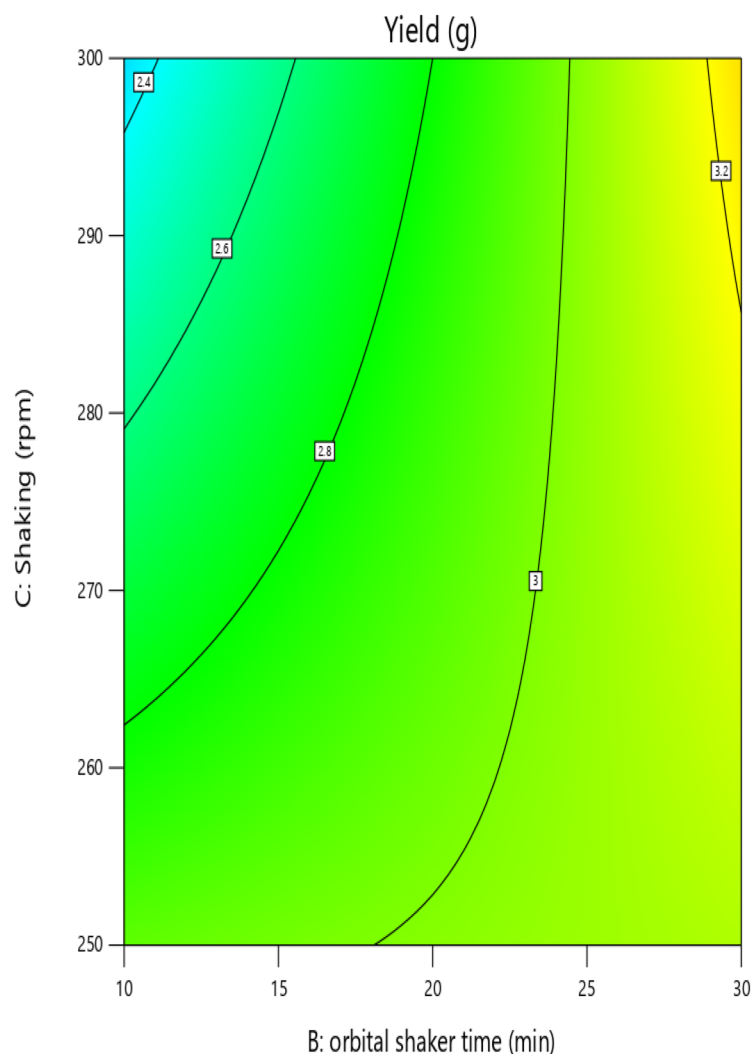
2  3.6

X1 = B

X2 = C

Actual Factor

A = 15



conditions, the compound with the large r_f value is less polar because it does not adhere to the stationary phase as much as the polar compound does. R_f can be used to identify compounds based on their characteristics compared to other compounds which has a lower r_f value.

Visible light, UV light at 245 nm and UV light at 365 nm identified various color spots on the TLC plates. The UV light identified the fluorescent spots. Evidence for identifying the features of the samples included r_f values, TLC plate color, size, and shape of detection zones under visible, 245 nm, and 365 nm UV light [31, 32].


The use of solid phase extraction technique such as TLC plate helps in the isolation of bioactive compounds by their retention properties on solid phase. This method is effective for sampling, extracts preservation and as an indicator of bioactive compounds present in plant extract

[33]. This method is inexpensive, non-laborious and an improved precision and accuracy methods of solvent extraction [34]. The TLC plates obtained for *S. fistula* in this study can be stored and later used as a reference for extraction of bioactive compounds based on their retention properties.

Previous publications documented the phytochemical constituents of *S. fistula* using polar solvents such as methanol [24], ethanol [28], and water [28] for the extraction process and its absorption properties [35]. There are no reports on the use of palm wine (indigenous solvent) as an extraction solvent for the phytochemical analysis of *S. fistula*. Although in this study, the palm wine extract showed the same phytochemical constituents as with aqueous extract. The use of *S. fistula* flowers in this study is also an additional raw material for medicinal use of this plant.

Fig. 6 Shaking (rpm) and extraction time (min)

Factor Coding: Actual

Yield (g)
2  3.6

X1 = A

X2 = C

Actual Factor

B = 20

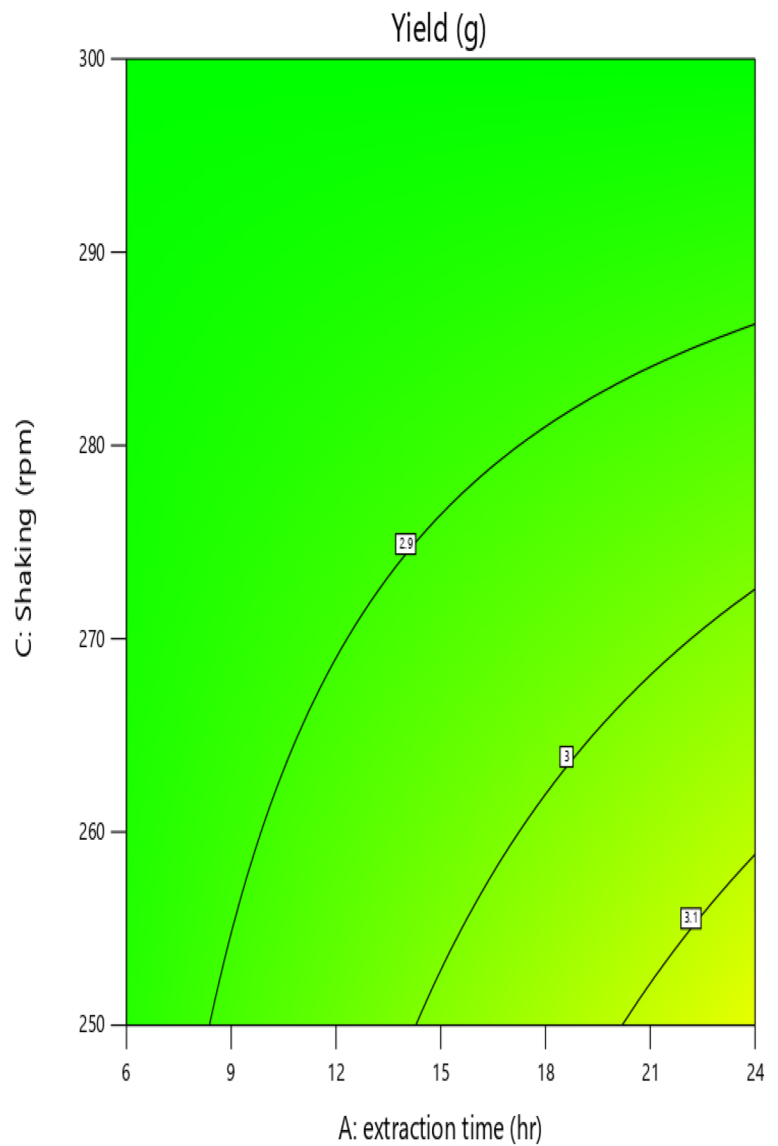


Table 3 Table of yield and extraction time derived from the graph of shaking time versus shaking speed

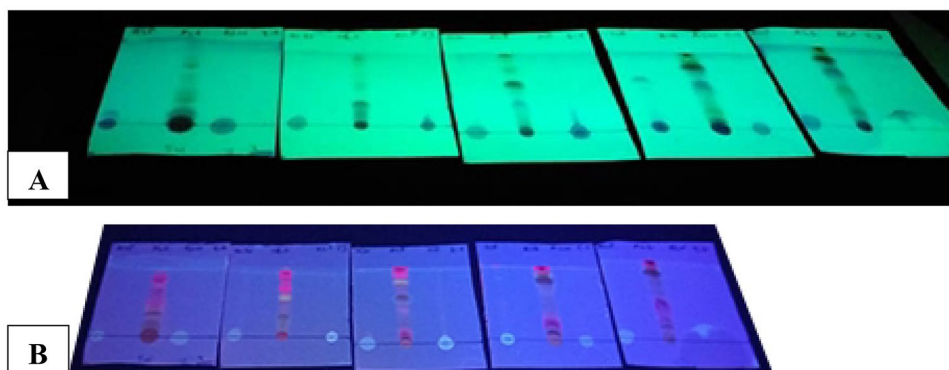
Yield (g)	Run	Extraction time (h)	Orbital shaking time (min)	Shaking (rpm)
2.4	1	24	11	298
2.6	2	20	13	289
2.8	3	15	17	277
2.9	4	14	20	274
3.0	5	16	23	270
3.0	6	16	19	263
3.1	7	22	30	255
3.2	8	22	29	293
3.4	9	22	29	300

Table 4 Retention factor of chromatography of selected ethanol extract samples of *S. fistula* for 100% ethyl acetate

Sample	Solute fronts (cm)	Solvent fronts (cm)	Retention factor
Leaves	0.5, 1.4, 2.8, 4.3, 4.9	5.1	0.098, 0.2725, 0.549, 0.8431, 0.9608
Stem bark	2.2	6.5	0.3385
Flower	0.6, 3.5, 5.2, 5.8	5.9	0.1017, 0.5932, 0.8814, 0.9831

Table 5 Retention factor of chromatography of mixed solvents extract samples of *S. fistula*

Sample	Solvent	Ratio	Solute front (cm)	Solvent front (cm)	Retention factor
Leaves	Dichloromethane/ethyl acetate	8:2	0.3, 0.5, 0.8, 1.2, 2.5, 4.3	4.6	0.0652, 0.1086, 0.1739, 0.2604, 0.5434, 0.9348
Leaves	Dichloromethane/ethyl acetate	8:2	2.2, 4.0	4.2	0.5238, 0.9524
Stem bark	Dichloromethane/ethyl acetate	8:2	0.4	6.4	0.0625
Flower	Dichloromethane/ethyl acetate	8:2	0.5, 0.7, 2.3	6	0.0833, 0.1167, 0.3833
Flower	Ethanol/ethyl acetate/hexane	3:4:3	4.6, 6	6.5	0.7076, 0.9231
Flower	Dichloromethane/ethanol/hexane	5:3:2	0.7, 4.5	5.7	0.1228, 0.7895

Fig. 7 Thin layer chromatography (TLC) of *Senna fistula* **A** TLC without UV light and **B** TLC under UV light

4 Conclusion

Senna fistula is a plant that is abundant in popular phytochemical components like flavonoids, saponins, tannins, phenols, cardiac glycosides, and terpenoids, according to the phytochemical analysis. This study also demonstrated the use of design expert in the extraction procedure, thus furthering into the area of computational chemistry. The bio-active substances found in *S. fistula* should be the subject of more exploratory research with usable products that can benefit mankind as the focus. Additionally, more research should be done on how to increase the use of cheminformatics in chemical extraction procedures and other fields so as to optimize the extraction procedures.

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Author contributions D.O., I.O. and E.F. conceptualized the research, D.O. and T.O. carried out the experiment. D.O. and I.O. drafted the manuscript and wrote the final manuscript. All authors reviewed the manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest with regards to the content of this article.

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