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A framework for harmla alkaloid extraction process development using fuzzy-rough sets feature selection and J48 classification

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Abstract
Medicinal plants as the pivotal source of alternative and complementary medicine have recently supported some hopes in alleviating of symptomatology associated with many diseases. The optimization and development of an efficient method for extracting effective medical substances from wild plants have great importance from both medical and economic prospectives. Therefore, the growing significance of using machine learning algorithms has become an influential positive factor in pushing exploration the pharmacological activities from medicinal plants. Peganum harmala is a widespread species growing as a wild plant in Egypt. It is proved to be useful as an anti-hemorrhoid, anthelmintic, and central nervous system (CNS) stimulating agent in folk medicine. Alkaloids, mainly harmine, harmaline, harmol, and harmalol, represent the major active constituent of the seeds of Peganum harmala. In this paper, a real-world case study of Peganum harmala involving extraction of alkaloids from its seeds using machine learning algorithms is presented. Therefore, dried powdered seeds of Peganum harmala were extracted using 70% methanol by the conventional maceration method. The extraction process was carried out 80 times for three runs using 11 variables, including the volume and concentration of organic solvent, HCl, temperature, and PH. This study proposes a fuzzy rough technique with J48 classification model to find the best extraction procedure for the Peganum harmala. The accuracy is evaluated using 10-fold cross-validation. The experimental results of this proposed intelligent model showed a better understanding tool to present the scientific rule for increasing harmala alkaloid yield range to be around 5%.

Keywords
Peganum harmala, Extraction process, Fuzzy-rough sets, Feature selection, J48.

1.Introduction
Peganum harmala (*Zygophyllaceae*) is an herb native to the dry area of the Mediterranean east to northern India. The plant originated in Central Asia, but now grows wild in Africa, Middle East, India, South America, Mexico, and Southern USA [1]. Seeds of *Peganum harmala* are growing widespread in Egypt. They are used as an anti-hemorrhoid and central nervous system (CNS) stimulating agent in folk medicine. Seeds stimulate the CNS, cause paralysis and are poisonous in strong doses [2]. Harmine and the resembled alkaloids are hallucinogens, serotonin antagonists, short-term monoamine oxidase (MAO) inhibitors and CNS stimulants. Little doses (25-50 mg) have a cerebral stimulant effect. Sometimes, it may cause drowsy state for half hour. Bigger dosages up to 750 mg may cause hallucination. Its intensity changes depending on persons using it [3].

These alkaloids have a broad spectrum of pharmacological actions, including binding to benzodiazepine receptors, convulsive or anticonvulsive actions, tremor genesis, anxiolytic and behavioral effects, anti-oxidative action, and immunomodulatory effects.

Peganum harmala is one of the plants, which purifies the atmosphere, because of its antiseptic property. It is usually used when the epidemic appears by burning the seeds and use its smoke. The burning process is done by placing it on burning coal. In an epidemic, the air becomes septic and pathogens spread out. In old age, it was presumed that the bad smell of the smoke could get rid of the ghosts.

Peganum harmala seeds also produce a red dye. So, it was a source of the richly colored textile dye. Various fractions of *Peganum harmala* and its alkaloids (Harmine, Harmaline, Harmol, and Harmalol) has been reported to have Antimicrobial activity. Harmaline, which is the major alkaloid

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present in Harmala seeds, has been noted for its psychotropic effects [4]. Harmine ($C_{13}H_{12}N_2O$) induces a fall in blood pressure, chiefly due to the weakening of the cardiac muscle is also more toxic for protozoa than quinine. Harmaline ($C_{13}H_{14}N_2O$) is reported to be used as an abortifacient, narcotics, aphrodisiac, stimulant and sedative, emmenagogue, emetic, vermifuge, and soporific [5]. There were also some reports concerning the cardiovascular actions of harmala alkaloids [6].

Alkaloids in the seeds of *Peganum harmala* are derived from β-carboline (βCs) and tetrahydoβ-carboline (THβCs). These alkaloids are occurring naturally in foods as a chemical condensation between indoleamines and aldehydes or α-keto acids. This reaction may occur during food production, processing, and storage. The occurrence of harman and norharman are known in well-cooked meat and fish at ng/g level. Minor amounts of them were reported in some alcoholic beverages and foodstuffs [7].

The field that deals with the subjective processing of the information, communication tasks of medical usage, education, and research is called "Medical Informatics." It is an unnatural interdisciplinary field, with a high interest and applied focus, but it also handles various essential research problems in addition to planning and policy issues. It uses the devices, resources, and methods need optimizing the acquisition, retrieval, storage and using the information on health and biomedical fields [8-12].

This study proposes an intelligent model involving a simple, rapid, and economical method for extraction of alkaloids from harmala seeds based on a fuzzy rough and J48 classification technique. It integrates fuzzy rough and J48 in three phases. The first phase is preparing the input data to construct the information table. The second phase is to minimize the features to get the minimum important ones and reject the excessive attributes for saving preparing time. The last phase is the classification stage, which is based on J48. The accuracy is evaluated using 10-fold cross-validation. The experimental results of this proposed intelligent model showed a better understanding tool to present the scientific rule for increasing harmala alkaloid yield range to be around (5%).

2.Materials and methods

We introduced a system for exploring the most important rules affecting extraction process. Therefore, this paper proposes a Fuzzy Rough with J48 classification model. It integrates fuzzy rough and J48 in three phases, as shown in *Figure 1*. The first phase is preparing the input data to construct the information table. The second phase is to reduce features to get the minimum important ones and exclude the redundant attribute for saving training time. The last phase is the classification stage, which is based on J48. In the following subsections, we will introduce basic stages of our proposed technique in more detail.

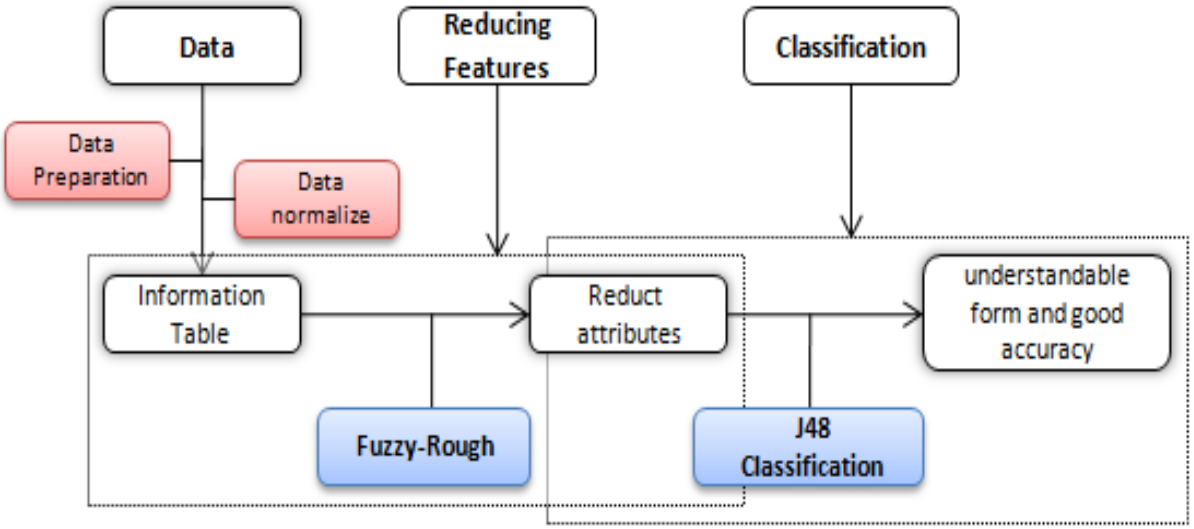


Figure 1 The block diagram of the proposed system

2.1Data preparation and information table construction

At this stage, we prepare the dataset to be presented in a suitable form for processing. The dataset should be prepared to remove the redundancy, represent the non-numerical attributes in an appropriate numerical form, check for missing values, and rescale the real values for decision attribute, as shown in *Figure 2*.

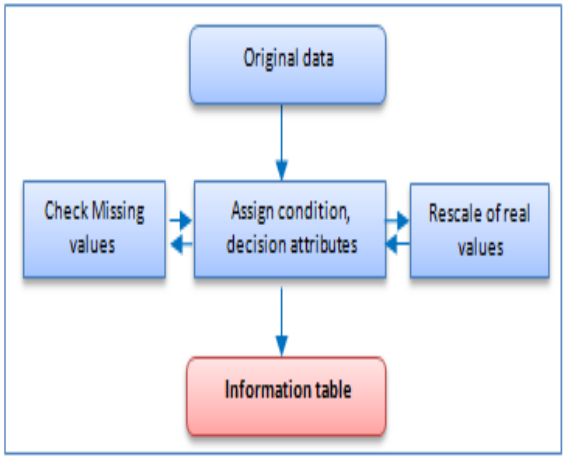


Figure 2 The architecture of data preparation and information table construction phase

This was done to construct the information table for extracting knowledge in the form of a two-dimensional table. For dealing with real data, we do not have to use discretization algorithms. To avoid loss of data, we use fuzzy rough feature selection, which can deal with real data effectively.

2.2Features reduction

Features reduction is a critical stage in building classification systems. Certainly, it has a good effect on the classification accuracy by limiting the number of input features to the classifier [13-18]. We eliminate the redundant attributes by using the fuzzy-rough quick reduct algorithm [19]. It is based on dependency function to choose, which important attributes to be added to the current reduct until the addition of any remaining attribute does not increase the dependency.

Fuzzy-rough sets have achieved more accurate results than Pawlak rough sets by translating a crisp, rough set to a fuzzy set and extended the indiscernibility relation to a fuzzy equivalence relation. So, we do not have to make any transformation for dealing with real data, such as discretization. We used fuzzy rough as a preprocessing stage to get minimum reduct. *Figure 3* shows the fuzzy rough reduct algorithm steps. Then, the result is supplied as input to the classifier to get a balance between training data and classification performance.

```
Input
C, the set of all conditional features
D, the set of decision features
Output
Red =a reduct of C

Begin
        Initialized Red=ϕ, R=C
        While γ_RED (D) ≠ γ_C(D), R≠0
                // the attribute with greatest increase in dependency degree chosen // and added to the
                potential reduct the process iterates and the two // dependency degrees calculated and
                go on

                Select an attribute a ∈ R with sig (a, Red, D) = max (sig (a, Red, D)) where a_i ∈ R
                R=R-{a}
                Red=Red ∪ {a}
        Output Red
End
```

Figure 3 The fuzzy rough quick reduct algorithm

2.3J48 Classification

Classification is an organization of data in the data collection according to given class labels. The classification algorithm builds a model based on training set where all data are associated with known class labels. Then, this model is used to classify new data. The classification task was performed using the J48 algorithm, which is a predictive machine-learning model based on the decision tree. It has many advantages, such as it can classify both categorical and numerical data, easy to understand, and easily translated into a set of rules. The general structure of decision tree contains internal nodes to denote the different data attributes, the branches between the nodes define the possible values that these attributes can have according to the data while the final value of the dependent variable belongs to the terminal nodes. *Figure 4* shows the learning algorithm steps.

A 10-fold cross-validation was used in the training dataset. The classification model is evaluated using confusion matrix, which contains statistical measures used to describe the ability of the classifier to discriminate between cases with positive and negative classes. Confusion matrix presents a visualization of the classification performance based on a table, which contains columns represent the instances in a predicted class and rows represent the instances in an actual class. The classification accuracy is the proportion of the total number of predictions that were correct. It is determined using the equation:

$$\text{Accuracy} = \frac{\text{TP} + \text{T N}}{\text{TP} + \text{T N} + \text{FP} + \text{F N}} \quad (1)$$

Where true positive (TP) refers to the number of cases, which are classified correctly in that class. The true negative (TN) is the number of cases, which are rejected correctly from that class. The false positive (FP) is the number of cases, which are rejected incorrectly from that class. Finally, the number of cases, which are classified incorrectly in that class belongs to the false negative (FN).

```
Input
TR: training set
Target: target set
Attr: Red set get by fuzzy Rough
Output Root
Begin
Create a Root node for the tree
If TR has the same target attributes value t_i
        Then Return the single node tree
If Attr= empty then Return the single node tree
Otherwise
{
        Select attribute A from Attr that best classify TR based on an entropy based measure set A the attribute
        for root
        For each legal value A, v_i. Do
        {
                Add a branch below, corresponding to A= v_i
                Let TRv_i Be the subset of TR that have A= v_i
                If TRv_i is empty
                Then add a leaf node below the branch with target value= most common value of Target in TR
                Else below the branch, add subtree learned by
                Tree-learning (TRv_i, target, Attr-{A})
        }
}
Return (Root)
End
```

Figure 4 The decision tree learning algorithm

- **Chemicals and plant materials:**

Peganum harmala seeds were purchased from local markets in Mansoura city, Egypt in April 2015, as shown in *Figure 5* [20]. The seeds of the plant were ground into fine powder using a coffee blender. All chemicals used were in analytical grade.

- **Extraction process:**

We used the method of kartal with some modifications [21]. Ten grams of dried and powdered seeds of *Peganum harmala* were macerated four times with 50ml 70% methanol at 50˚C in a water bath for 1h. Then, the extracts were combined and evaporated to dryness. The residue was dissolved in 100ml, 2% HCl, filtered throughout a "Fisher brand QL100, 90mm filter paper". The filtrate was extracted twice with 50ml petroleum ether. The aqueous acid layer was basified (pH 10) with 50ml dil. NH_4OH and then extracted 4 times with 50ml Methylene Chloride. The methylene chloride layers were combined and evaporated to dryness, and the total alkaloids were weighed out. The previous method was carried out 80 times with 11 variables for three Runs as follows:

V1: The amount of used methanol.
V2: The concentration of methanol.
V3: The temperature of the water bath.
V4: The time in the water bath.
V5: The amount of the used HCL.
V6: The percentage of the used HCL.
V7: The amount of used Pet. Ether.
V8: Number of times used by Pet. Ether (2, 3, 4 times (runs)).
V9: The PH of the used base (NH4OH).
V 10: The number of times used by methylene chloride.
V 11: The amount of the used methylene chloride.

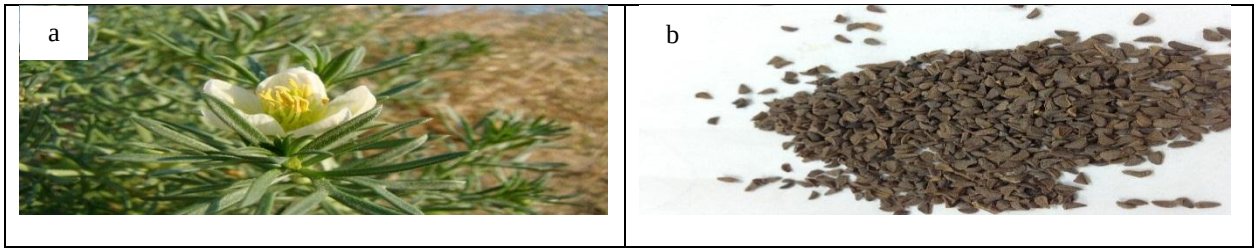


Figure 5 (a) Peganum harmala plant and its fruits (caliban.mpiz-koeln.mpg.de/mavica/index.html) (b) Peganum harmala seeds (2 X)

3.Results

Table 1 lists the used variables in our experiments and their ranges. Moreover, all previous sets produced total Harmala alkaloid yield range from 1.15% to 5.6% from the total plant material (1.15 – 2.6 low), (2.6 – 4.1 med.), and (4.1 – 5.6 high).

We applied our proposed intelligent system to three different run datasets of the harmla alkaloid extraction process. Waikato environment for knowledge analysis (Weka) version 3.7.2 [22-24] has been used to carry out experiments. For each run, we did the following:

- We prepare harmla alkaloid extraction process dataset and construct the information table, and use data normalization technique according to Scientific roles for total harmala alkaloid yield range (1.15 – 2.6 low), (2.6 – 4.1 med.), and (4.1 – 5.6 high).
- The fuzzy-rough reduction is calculated for all attributes to find the most important attributes that have the main effect on total harmala alkaloid yield range.
- Finally, a J48 classification algorithm is applied to the reduced feature set of harmla alkaloid extraction process. The accuracy is evaluated using 10-fold cross-validation. The performance of J48 classification algorithm is analyzed regarding the two most important parameters of the classification results which they are Accuracy and Confusion Matrix. *Table 1* shows the experimental results of our proposed intelligent system. The j48 tree view is a good understanding tool to present the scientific rule for increasing harmala alkaloid yield range, as shown in *Table 3*.

Then, we tried to eliminate the fuzzy-rough reduction step to demonstrate the effect of using the fuzzy-rough reduction on J48 classification accuracy and the complexity of the tree view. *Tables 2 and 3* show the results and the tree view of J48 classification technique for three different reducing run datasets.

Tables 4 and 5 show the results and the tree view of J48 classification technique without reducing features stage for various three runs datasets.

The results prove that Fuzzy-Rough reduction has improved the performance of J48classifier.

Table 1 The range of each variable in our experiments

Variables	From	To	The range
V1	20ml	75ml	20-39 low
			40-59 med.
			60-75 high
V2	50%	95%	50-69 low
			70-84 med.
			85-95 high
V3	30 ˚C	65 ˚C	30-44 low
			44-54 med.
			55-65 high
V4	30 min.	90 min.	30-49 low
			50-69 med.
			70-90 high
V5	25ml	75ml	25-44 low
			45-60 med.
			61-75 high
V6	1.1%	2.8%	1.1-1.7 low
			1.8-2.3 med.
			2.4-2.8 high
V7	10ml	45ml	10-24 low
			25-34 med.
			35-45 high
V8	2 times	4 times	2 low
			3 med.
			4 high
V9	9.1	11	9.1-9.7 low
			9.8-10.4 med.
			10.5-11 high
V10	2 times	4 times	2 low
			3 med.
			4 high
V11	25ml	75ml	25-44 low
			45-60 med.
			61-75 high

Table 2 The performance of the proposed intelligent system using fuzzy-rough with J48 classification technique for harmla alkaloid extraction process dataset

Run	Fuzzy-rough reduction	J48 classification accuracy	Confusion matrix			
Run 1	V2, V3, V5, V6, V11	91.25 %	**a**	**b**	**c**	**Classified as**
			27	3	0	**a = Low**
			2	31	2	**b = Med**
			0	0	15	**c = High**
Run 2	V2, V3, V5, V6, V11	86.25 %	**a**	**b**	**c**	**Classified as**
			35	3	0	**a = Low**
			5	20	2	**b = Med**
			0	1	14	**c = High**

Run	Fuzzy-rough reduction	J48 classification accuracy	Confusion matrix			
Run 3	V1, V2, V3, V5, V6	80 %	**a**	**b**	**c**	**classified as**
			31	7	0	**a = Low**
			4	20	3	**b = Med**
			0	2	13	**c = High**

Table 3 The tree view of J48 classification technique for three different reducing run datasets

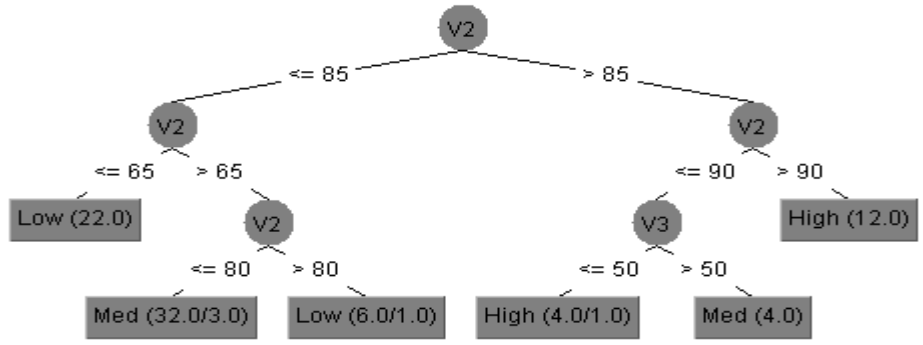
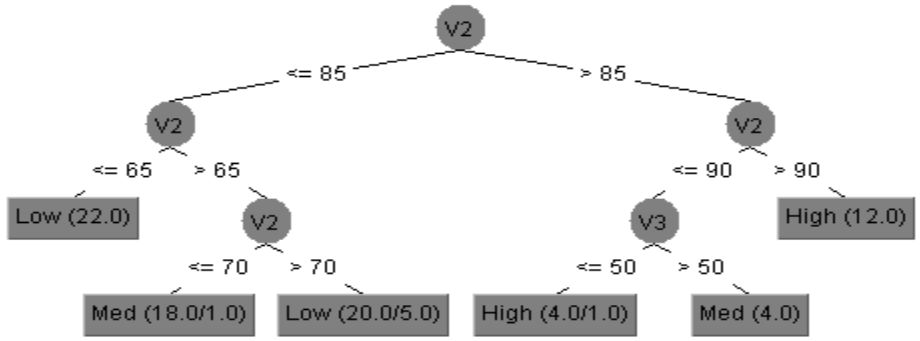
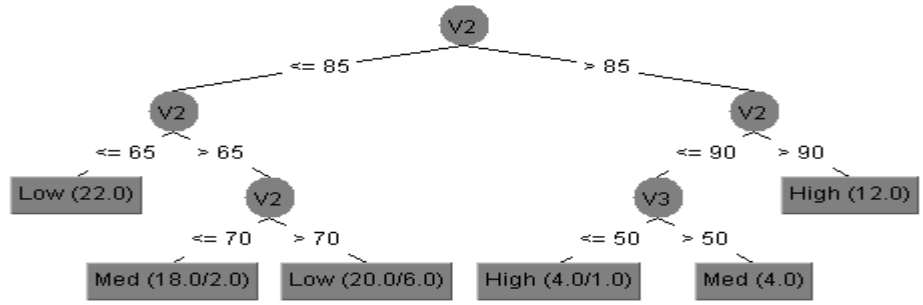
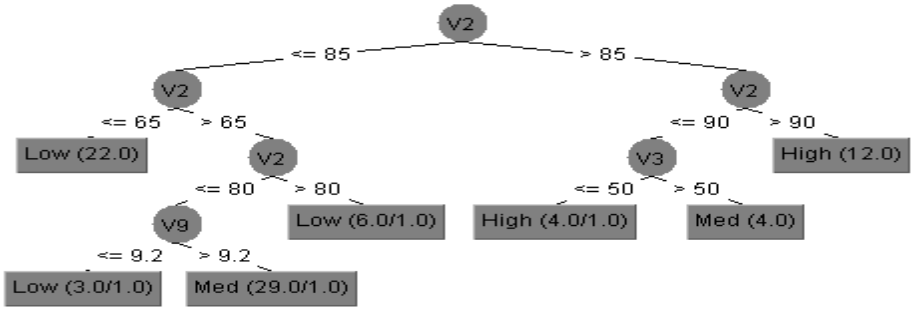
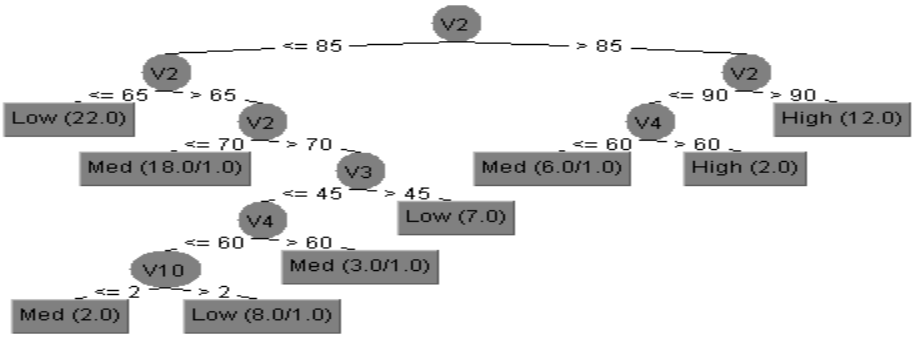
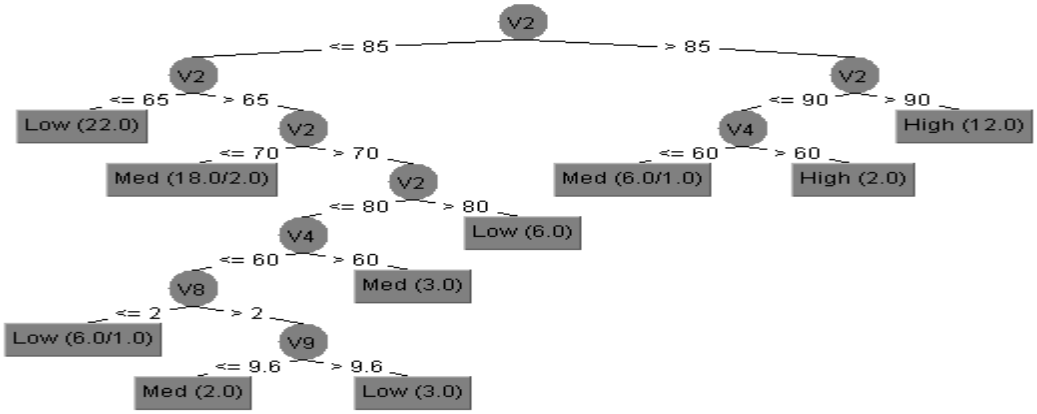
Run	Tree view
Run 1	
Run 2	
Run 3	

Table 4 The performance of J48 classification technique without reducing features stage for three different runs datasets

Run	Classification accuracy	Confusion matrix			
Run 1	81.25 %	**a**	**b**	**c**	**Classified as**
		26	4	0	**a = Low**
		5	26	4	**b = Med**

Run	Classification accuracy	Confusion matrix			
		0	2	13	**c = High**
Run 2	83.75 %	**a**	**b**	**c**	**classified as**
		33	5	0	**a = Low**
		4	21	2	**b = Med**
		0	2	13	**c = High**
Run 3	78.75 %	**a**	**b**	**c**	**classified as**
		31	7	0	**a = Low**
		5	19	3	**b = Med**
		0	2	13	**c = High**

Table 5 The tree view of J48 classification technique without reducing features stage for various three runs dataset

Run	Tree view
Run 1	
Run 2	
Run 3	

4.Discussion

In this study, we want to extract the relationship between factors participated in the extraction process to reach the most optimum factors giving high yield amount of harmala alkaloids. The data obtained from 80 times the extraction process of harmala alkaloids were analyzed for 3 runs using a fuzzy rough algorithm with 11 attributes. After preparing the dataset and construct the information, the fuzzy rough reduction is calculated for all attributes. We found that V1, V2, V3, V5, V6, and V11 are the most important factors that have a main effect on the harmala alkaloid extraction process. All runs confirm that the concentration of methanol used is the major attribute affects the extraction process. If the concentration is above 85% and less than 90%, it gives the highest yield amount and using methanol concentration above 90% by using temperature of water bath about 50C^{o} produce high yield comparable to temperature. Using methanol for extraction process with a concentration less than 65% produced a low yield of alkaloids. Therefore, it may be due to the high-water content, but ranging from 65% to 80% produce yield classified to be medium.

Classification technique without reducing the reducing stage for the three runs with an accuracy 81%, 83%, and 78.7% respectively. It indicated that V2 is the most effective attribute (factor) in the extraction process. Similar to the reduced classification technique, the technique without reduction stage confirms that the concentration of used Methanol should be within the range of 80% to 90% to give a high number of alkaloids. Otherwise, if it is used less than 65% Methanol concentration, it provides a very low amount. The time of extraction process on the water bath also is an effective factor in the extraction. Extraction lasts on a water bath for 60 min gives a high number of alkaloids. However, increasing the time not effective in raising the amount extracted. Using base with high PH above 9.5 for neutralizing the acid in the medium is effective in the extraction process which shown in the first run. We found using more than two times in defatting step with increasing the PH of the base to be above 9.6 may have a helpful tool in increasing the alkaloid yield extracted.

5.Conclusion and future directions

We proposed an intelligent system that integrates the fuzzy-rough sets feature selection with J48 classification. It is implemented to study the factors influencing the harmala alkaloid extraction process to conclude a simple, rapid, and economical method for extraction. We performed the method of extraction for 80, and three runs, then applied to the intelligent model using fuzzy-rough with J48 classification technique for determination of the most important factors, which achieved high accuracy of 91.25%, 86.25% and 80% for the three runs. We found that the amount and concentration of methanol, the temperature of the water bath, the amount and concentration of used HCl, and the amount of the used methylene chloride are the major factors that are affecting the extraction process. Using methanol with concentration from 85% to 90% with heating in a water bath around 50C^{o} for 60 minutes with defatting more than 2 times with petroleum ether and using a high amount of methylene chloride for fractionation of alkaloids base from aqueous phase is the most optimum technique to obtain a high harmala alkaloids yield.

Acknowledgment

None.

Conflicts of interest

The authors have no conflicts of interest to declare.

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