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# Validating the properties of *Ranunculus sceleratus Linn*. by performing spectroscopic techniques and modern chromatography

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ABSTRACT

*Ranunculus sceleratus Linn.* is one of the well-known medicinal plants, being used from the ancient time in India and commonly called as "*Jal dhaniya*". It belongs to Ranunculaceae family derived from two Latin Words "Rana" means frog and "unculus" means little and referred together as "little frog" and also species sceleratus for cursed. It is an aquatic perennial herb. It consists of a herbaceous hollow stem, firm tap root, branched rhizome and leaves having a smooth upper side. The green plant is toxic for livestock and uncomfortable to human skin. This plant can grow up to 0.60 m tall, and also used as a food, medicine and possess other uses. Ranunculus sceleratus Linn. is commonly spread in the temperate and cold region in Global distribution (Indonesia, Malaysia, Nepal, Sri Lanka and India). According to Bentham and Hooker classification "*Genera Plantarum*", this plant belongs to the division Polypetale of Dicotyledones which processes more than 600 species. The Phytochemical screening was performed according to API norms, in addition to this UV VIS, FTIR, TLC and HPLC test were also carried out for further validation. The spectroscopy and chromatography examination revealed the presence of flavonoids, phenols and various other Phytoconstituents in *Ranunculus sceleratus Linn.* The spectroscopy and chromatography validation can help us it for medicinal and commercial purposes.

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## INTRODUCTION

*Ranunculus sceleratus Linn.* is also known as Kandira (Lucas, 2008), it is grown in ponds, lakes and water bodies, having modified Root, Stem and coriander leaf type leaves, branched rhizome, striated seed and globular fruit (Das, 2012). The whole plant is used as a medicine in Plague disease (Sharma, 2004).

*Rananculus sceleratus* such as it is a virulent poisonous plant, producing violent effect if taken internally and the bruised leaves applied to the skin act very efficaciously as a vesicant, it is used by beggers to keep open sores once caused by vesication or other means. In traditional medicine, *Ranunculus sceleratus* is used in malaria, scorpion bite, blood stasis, acute icteric hepatitis and internal abscess, *Ranunculus sceleratus Linn*. showed pharmacological effect such as antibiosis and relief of articular effusion.

Although it is one of the primitive types of species a very brief illustration is found in different botanical and Ayurvedic treatises. The scarcity over the information regarding the characteristics and its uses motivates the author to review it extensively in various research journals and other related literature. And to standardized the parameter after performing phytochemical, physiochemical, TLC, FTIR studies. Such exploration and validation help the society in the treatment of various disease and for medicinal and commercial purpose.

## MATERIALS AND METHODS

### Collection

*Ranunculus sceleratus Linn.* was collected from its natural habitat near Banaras Hindu University, Varanasi, in February 2018.

### Authentication of plants

Sample *(Voucher specimen no. Ranunculus.2018/3)* was authenticated by the experts from the Department of Botany, Institute of Science, Banaras Hindu University, Varanasi. Plant specimen was deposited in the museum of Department of Dravyaguna, Faculty of Ayurveda, for future reference.

### Chemicals

All analytical grade chemicals used in the study were purchased through Advanced Quality traders, E. Merk, Germany.

### Phytochemical screening

Plant was extracted in seven solvents (Petroleum ether, chloroform, Acetone, Benzene, Ethanol, Methanol and Distil water) are determined by their relevant chemical test with appropriate testing agents or reagents.

### Spectroscopic techniques

#### UV-VIS (ultraviolet, visible spectroscopy)

One gram of plant extracts was added in 10 ml of distilled water then filtered with the help of cartilage (0.2$\mu$m). Afterwards, it was scanned under ultraviolet, visible spectrophotometer ($\lambda$ 25 Perkin Elmer) at a range of 200-900nm to measure the size of biomolecules and uncertainty source that may arise from nature of the compound of plant extract.

#### FTIR (Fourier- transform infrared spectroscopy)

A pinch of powder drug was taken and placed over the crystal present on stage. The IR spectrum (Perkin Elmer, Spectrum-2) was scanned between 4000 to 400-1 and transmittance was recorded. Before scanning the sample, the background signal was also recorded. The peaks thus obtained were matched against IR interpretation chart, and the functional groups were noted.

### Modern chromatography

#### TLC (Thin layer Chromatography)

The extract was applied 2 cm on the lower edge of the plate by the help of a microcapillary tube. And then extracts were loaded in small-volume spot on each plate, the plate was taken out, the solvent front was marked, and the plate was dried at room temperature. Thin-layer chromatography was detected by observation of spots for identical Rf value and to determine the purity of a sample.

#### HPLC (High-performance liquid chromatography)

1g of plant extract was added in 10ml of methanol then sonicated in the sonicator machine (Labman), afterwards filtered with the help of cartilage (0.2$\mu$m) and injected with a microsyringe (20 $\mu$l) and finally scanned with HPLC machine to detect the flavonoids and Phenolic. Standard- (flavonoids and Phenolic) Catechin hydrate, Myricetin, Rutin, Quercetin, Caffeic acid, Kaempferol and Gallic acid, all solutions are prepared in methanol (1mg/ml). Mobile phase A- methanol: acetonitrile: water: acetic acid (50ml:25ml:425ml:5ml) For 0-20 min and mobile phase B- methanol: acetonitrile: acetic acid (300ml:200ml:5ml) for 20- 25 min.

## RESULTS AND DISCUSSION

Directly or indirectly, herbal plants are used upon their characteristics, and their character features are detected through different parameters like spectroscopy and chromatography.

### Phytochemical screening

Phytochemical Screening of *Ranunculus sceleratus Linn*. in a different solvent.

According to Table 1, Some more phytoconstituents are found, i.e. Amino acid, Proteins, Alkaloids, Phytosterols, Flavonoids, Steroids, Fatty acid, Terpenoids, Phenols and Saponin, but in the previous study, terpenoids, tannins, flavonoids, saponins, alkaloids, Protein and resins have been reported (Zayat *et al.*, 2015).

### Spectroscopic techniques

#### UV-VIS (Ultraviolet, visible spectroscopy)

The Ultraviolet-visible spectroscopy profile of Figure 1 of the extract was observed at 200-900 nm wavelength range and 265 nm recorded band. In the previous study, Phenolic and flavonoids components generally absorb at 230-290 nm (Mishra *et al.*, 2015).

**Table 1: Phytochemical screening of R. sceleratus Linn**

| | TLC PROFIL | | | | | | |
|---|---|---|---|---|---|---|---|
| Phytocon-stituents | Petroleum ether | chloro-form | Ace-tone | Ben-zene | Ethanol | Methanol | Distil water |
| Amino acid | + | + | + | + | - | + | + |
| Proteins | - | + | - | - | - | - | - |
| Carbohydrate | - | - | - | - | - | - | - |
| Alkaloid | - | - | + | - | + | - | - |
| Phytosterol | + | + | + | + | + | + | + |
| Tannin | - | - | - | - | - | - | - |
| Flavonoid | - | - | + | - | + | + | + |
| Steroids | - | + | + | - | + | + | - |
| Fatty acid | + | + | - | + | - | - | - |
| Terpenoid | - | - | + | - | + | + | - |
| Coumarin | - | - | - | - | - | - | - |
| Emodin | - | - | - | - | - | - | - |
| Phenol | - | - | - | - | - | + | - |
| Phlobatannin | – | - | - | - | - | - | - |
| Saponin | - | - | - | - | - | - | + |

**Table 2: R. sceleratus was evaluated by the help of FTIR profile**

| | FTIR PROFILE | | |
|---|---|---|---|
| Peak Number | Peak | Group | Compound Class |
| 1 | 3288 | O-H Stretching | Alcohol, Carboxylic acid |
| | | C-H Stretching | Alkyne |
| 2 | 2920 | O-H Stretching | Alcohol, Carboxylic acid |
| | | N-H Stretching | Amine salt |
| | | C-H Stretching | Alkane |
| 3 | 1594 | N-H Bending | Amine |
| | | C=C Stretching | Cyclic alkene |
| 4 | 1403 | O-H Bending | Alcohol, Carboxylic acid |
| | | S=O Stretching | Sulfate, Sulfonyl Chloride |
| 5 | 1314 | C-F Stretching | Fluro compound |
| | | O-H Bending | Phenol |
| | | S=O Stretching | Sulfone |
| | | C-N Stretching | Aromatic Amine |
| 6 | 1237 | C-F Stretching | Fluro compound |
| | | C-O Stretching | Alkyle arile ether |
| | | C-N Stretching | Amine |
| 7 | 1026 | C-N Stretching | Amine |
| 8 | 631 -509 | C-X (X=Cl or Br) | Halo compound |

**Table 3: Ranunculus sceleratus was evaluated by the help of TLC**

| Extract | Solvent Front (cm) | Peaks Obtained (cm) | $R_f$ Value (cm) | Mean Rf Value |
|---|---|---|---|---|
| Petroleum Ether | 6.5 | S1 2.7 | 0.41 | 0.617 |
| | | S2 3.3 | 0.50 | |
| | | S3 4 | 0.61 | |
| | | S4 6.2 | 0.95 | |
| Chloroform | 6.5 | S1 2.1 | 0.32 | 0.641 |
| | | S2 3.4 | 0.52 | |
| | | S3 3.8 | 0.58 | |
| | | S4 4.2 | 0.64 | |
| | | S5 5.5 | 0.84 | |
| | | S6 6.2 | 0.95 | |
| Acetone | 6.8 | S1 0.8 | 0.11 | 0.53 |
| | | S2 2.9 | 0.42 | |
| | | S3 4.4 | 0.64 | |
| | | S4 6.5 | 0.95 | |
| Benzene | 6.8 | S1 2.5 | 0.36 | 0.636 |
| | | S2 4 | 0.58 | |
| | | S3 6.6 | 0.97 | |
| Ethanol | 6.8 | S1 0.6 | 0.11 | 0.475 |
| | | S2 1.3 | 0.19 | |
| | | S3 2.1 | 0.30 | |
| | | S4 3.5 | 0.51 | |
| | | S5 5.6 | 0.80 | |
| | | S6 6.4 | 0.94 | |
| Methanol | 6.9 | S1 0.4 | 0.5 | 0.575 |
| | | S2 0.9 | 0.9 | |
| | | S3 2.3 | 0.33 | |
| | | S4 4 | 0.57 | |
| Distil Water | 6.8 | - | - | 0 |

**Table 4: Analytical condition**

| Analytical Condition | |
|---|---|
| Column | Shim-pack GIST/GISS C 18 |
| Mobile Phase | Phase A- Methanol 10: Acetonitrile 5: Water 85: Acetic acid 1.<br>Phase B- Methanol 60: Acetonitrile 40: Acetic acid 1. |
| Time Program | 40 min |
| Flow rate | 1ml/min |
| Column Temp. | 32°C |
| Injection Vol. | 20$\mu$L |
| Detection | Ch2 254 nm |

**Table 5: Retention time (RT), wavelength(nm), Area and Height of Methanol and distil water extract of R.scelerates and standard of Phenolic acid and flanvonoids for HPLC method validation**

| | HPLC PROFILE | | | |
|---|---|---|---|---|
| Name of Extract and Standard | λmax (nm) | RT (min) | Area % | Height % |
| Extract of R. sceleratus in Methanol | 254 | 3.572 | 51.073 | 45.458 |
| Extract of R. sceleratus in distil water | 254 | 3.431 | 99.601 | 99.767 |
| Caffeic acid | 254 | 3.536 | 85.449 | 86.704 |
| Kaempferol | 254 | 4.971 | 34.333 | 22.269 |
| Gallic acid | 254 | 3.575 | 34.899 | 35.153 |
| Catechin hydrate | 254 | 4.944 | 88.978 | 91.905 |
| Quercetin | 254 | 4.218 | 90.996 | 86.989 |
| Rutin | 254 | 3.567 | 99.536 | 99.837 |
| Myricetin | 254 | 3.946 | 99.024 | 99.333 |

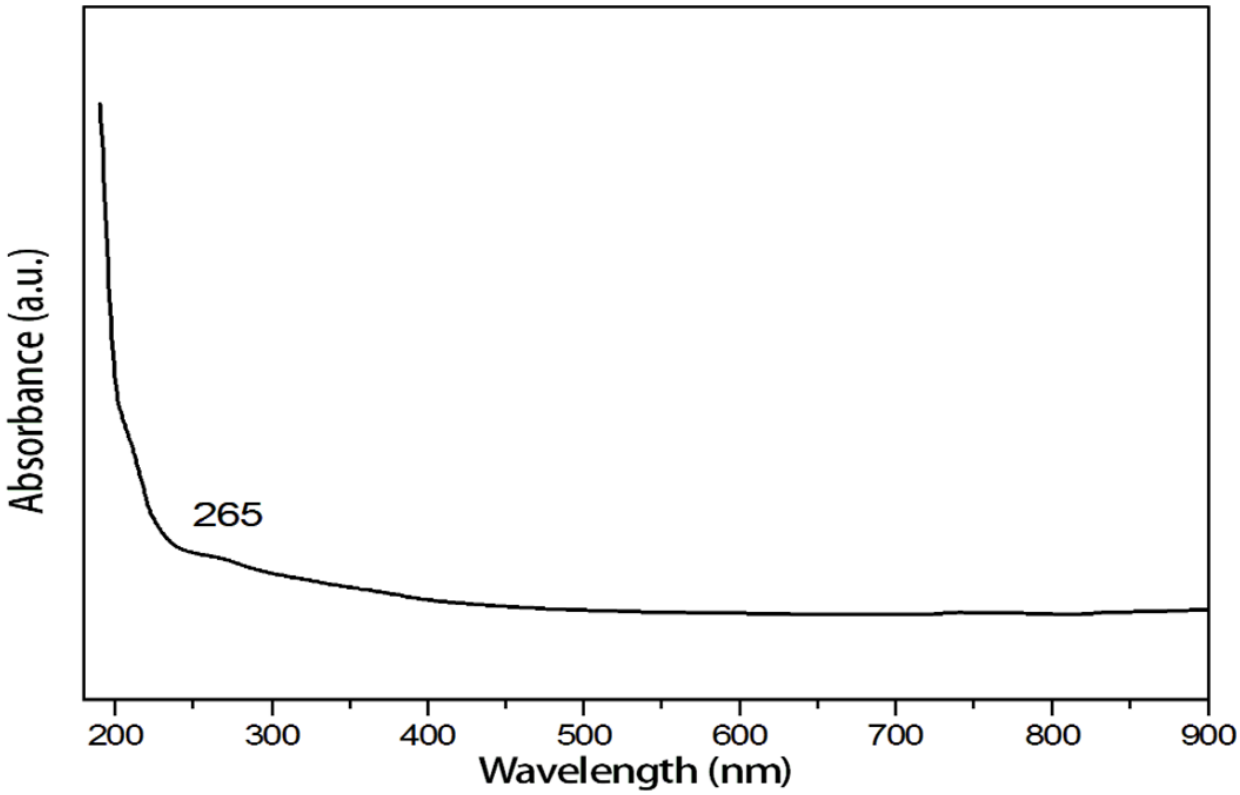


**Figure 1: UV- VIS of distilwater extract of Ranunculussceleratus Linn**

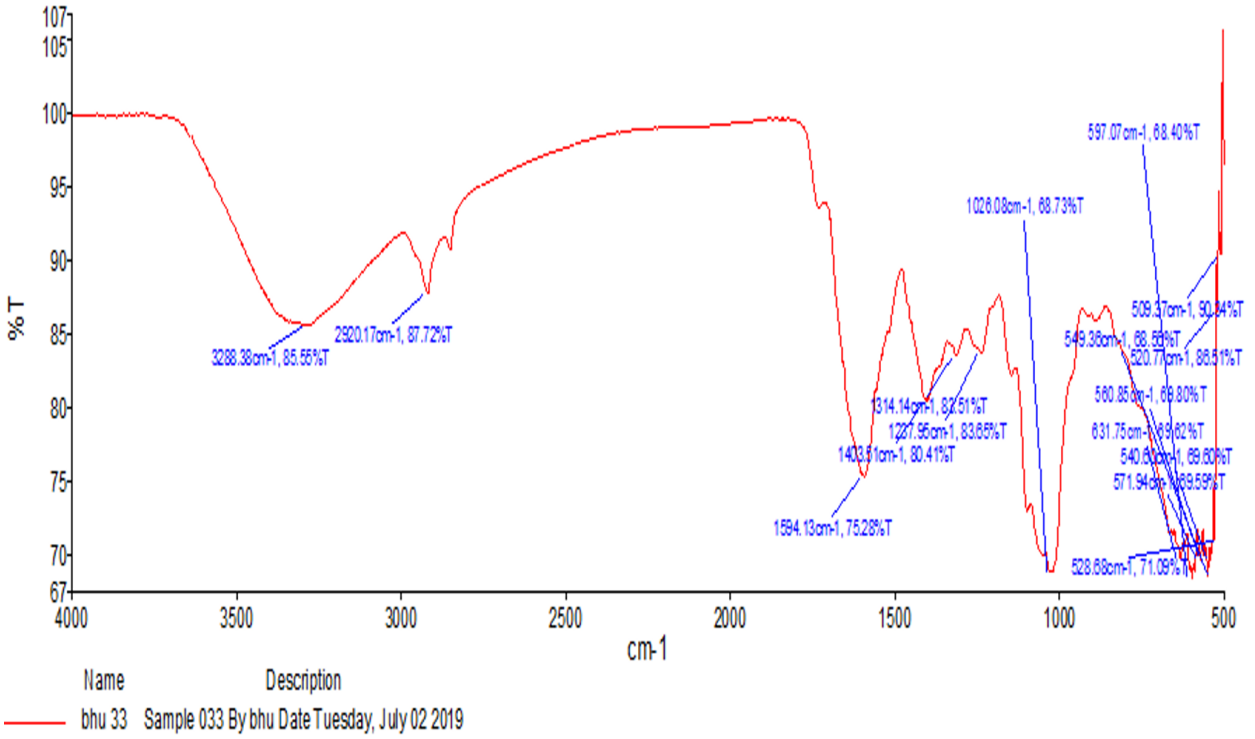


**Figure 2: Ranunculus sceleratus was evaluated by the help of FTIR**

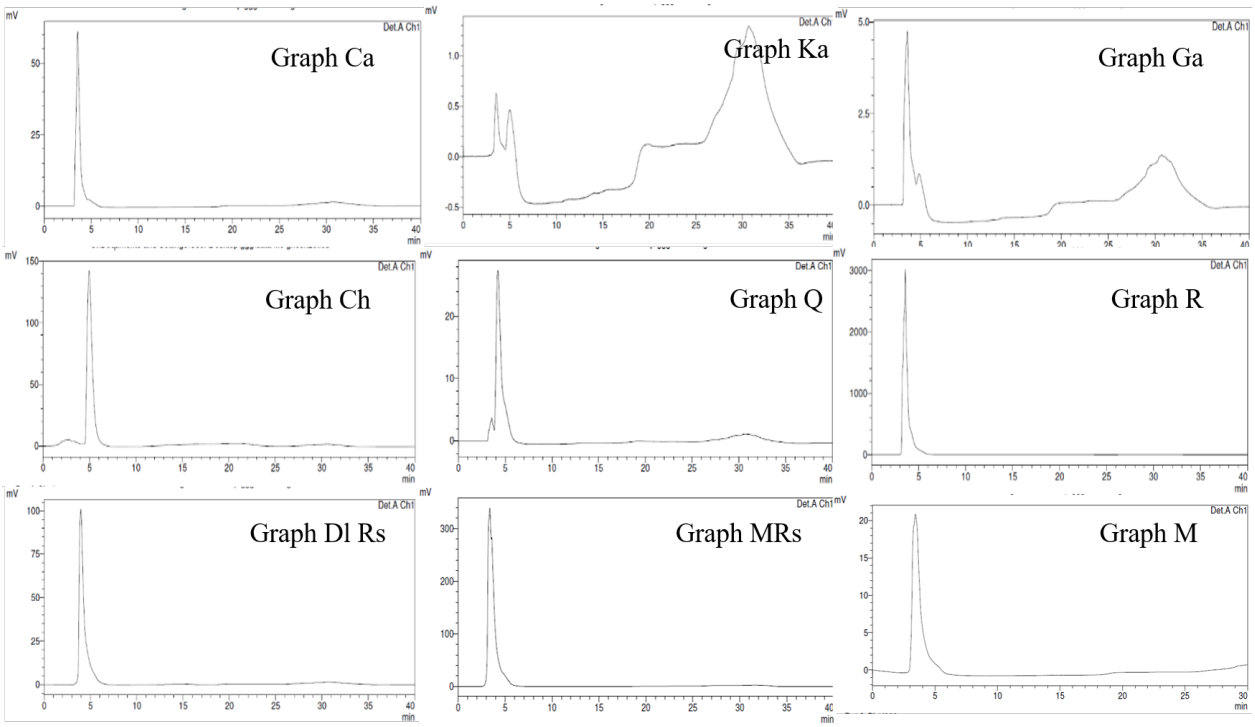


**Figure 3: HPLC Chromatogram of standard phenolic acid(Ca=Caffeic acid, K=Kaempferol, Ga= Gallic acid), Flavonoids (Ch= Catechinhydrate, Q= Quercetin, R= Rutin and M= Myricetin), Rs= methanol extract ofRanunculus sceleratus and Dl Rs = Extract of R. sceleratus in distil water**

Hence, it confirms the presence of pheno and flavonoids in the extract of *Ranunculus sceleratus*.

**FTIR (Fourier- transform infrared spectroscopy)**

FTIR is a characterisation method which gives the vibration energy based on peak value (Kumar and Ramaswamy, 2014; Mishra *et al.*, 2015). The compressing act of the functional group that are available on the extract of *Ranunculus sceleratus*. The major bands were observed at 3288,2920,1594,1403,1314,1237 and 1026 $cm^{-1}$ in Figure 2.

According to Table 2, The peak indicates OH stretching might be alcohol, carboxylic acid. OH bending show phenol. CH stretching is alkyne and alkane. NH stretching is amine salt, and NH bending is an amine. C=C Stretching is Cyclic alkene. S=O Stretching is Sulfone, Sulfate and Sulfonyl Chloride. C-F Stretching is Fluro compound. C-N Stretching is Amine and Aromatic Amine. C-O Stretching is Alkyle arile ether, and C-X (X=Cl or Br) is halo compound.

**Modern chromatography**

**TLC (Thin layer Chromatography)**

According to the Table 3, Chloroform extract showed maximum mean $R_f$ value which is 0.641 and Distil Water could not detect any peak that's, why the mean $R_f$ value is 0. In the previous study, Maximum $R_f$ value shows highly pure compound and less $R_f$ value indicates impurity of the compound (Kanoujiya *et al.*, 2016).

**HPLC (High-performance liquid chromatography)**

A typical HPLC chromatogram of all standard recorded at 368 nm is present in different figure (Figure 3), and a brief summary of HPLC instrument working is shown in analytical condition (Table 4).

According to Table 5, The plant extract was evaluated with seven standards (gallic acid,quereotin, catechin, rutin, caffic acid, myricetin) of phenolic and flavonoids phytoconstituents to detect there capability. Caffeic acid (3.536 RT), Gallic acid (3.575) and Rutin (3.567) are present in both extract of methanol and distilled water of *R. sceleratus*.

In the previous study, Rutin and Caffeic acid both were present in another species of *R. arvensis* (Bhatti *et al.*, 2015).

## CONCLUSION

The present study evaluates phytochemical, spectroscopy and chromatography of the whole plant of *Ranunculus sceleratus Linn.* for correct identification and standardisation and also indirect developing for further research. In Phytochemical study, found three new phytochemicals, i.e. Amino acid, Phytosterol & fatty acid and carbohydrates result is differing from the previous study. The TLC result indicates that the Chloroform extract successfully separates the compound and the Rf value (0.641) shows the purity of *Ranunculus sceleratus Linn.* FTIR shows the presence of different functional groups such as Carboxylic acid, Alcohol, Alkene, Amine, Sulfone, Aromatic Amine, Alkyl aryl ether, Fluoro compound and Halo compound. UV VIS and HPLC indicate the presence of phenolic and flavonoids in the extract. According to all above parameters *Ranunculus sceleratus Linn*. possesses a large number of Phytoconstituents. That's why they have a tremendous medicinal impact on herbal drugs.

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**Conflict of Interest**

The corresponding author declare no conflict of interest.

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