Rapid extraction and quantitative screening of asiaticoside towards selecting elite chemotype of 'Thankuni' (*Centella asiatica* (L.) Urb.)

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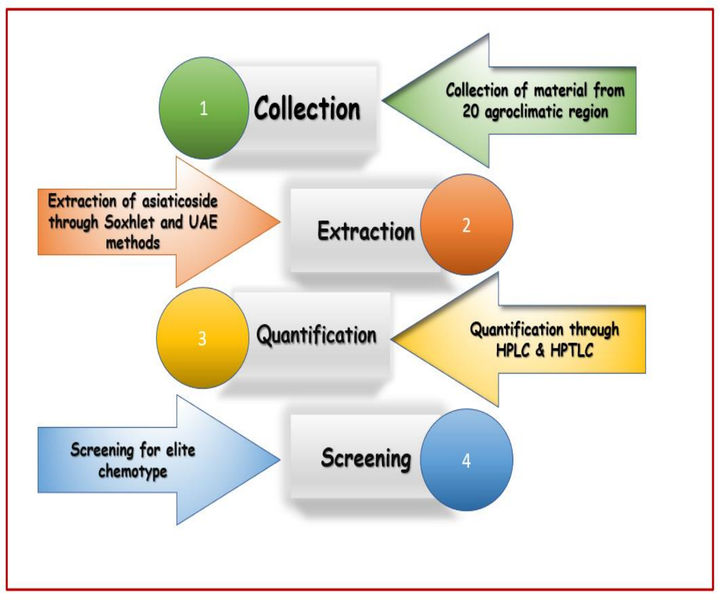
ABSTRACT

Medicinal plants that have been conventionally used in the pharmaceutical industries recently started attracting consumer interest because of their long history of being consumed and their ready acceptability- *Centella asiatica* (L.)Urb. is belong to this group of plants. Commonly known as 'thankuni', this valuable medicinal herb contains several triterpene saponins, asiaticoside, asiatic acid, madecassoside, madecassic acid, sapogenins, glycosides, and alkaloids accountable for several medicinal properties, including anti-inflammatory, antibacterial, antileprotic, antiulcer and wound-healing. In this study, twenty chemotypes of *C. asiatica* were collected from different regions of West Bengal to identify the elite one by extraction and analysis of asiaticoside through rapid extraction techniques- soxhlet and ultrasound-assisted extraction (UAE). Asiaticoside content was studied by High Performance Liquid Chromatography (HPLC) and High Performance Thin Layer Chromatography (HPTLC) systems. Extraction with Soxhlet apparatus results in 15.24- 36.92 mg of asiaticoside, while the UAE method shows 21.34-44.98 mg of asiaticoside from 1gm of dry leaf powder. The findings established here offer a fast way to extract and quantify bioactive compounds, which could be utilized by pharmaceutical industries.

Steps



INTRODUCTION

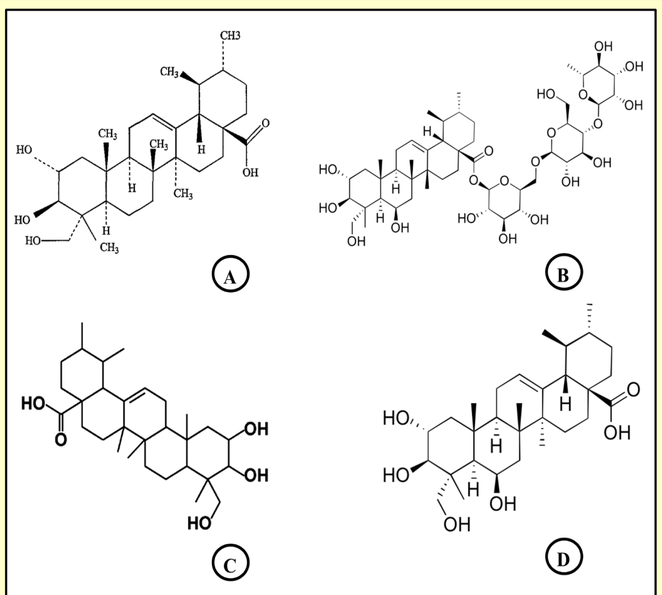
***Centella asiatica* (L.) Urb. is one of the most important indigenous medicinal plants.. Medicinal uses of this plant are against digestive disease and neurological diseases. Major bioactive compounds of this plant are asiaticoside, asiatic acid, madecassic acid, madecassoside etc. Therefore, raising the global demand for *Centella* requires great yielding variety. High-yielding variety or elite chemotype could be analyzed only by investigating crude extract. However, the proper evaluation of natural extract can meet elite chemotype's desired value. The evaluation could be done by several plant extraction techniques through conventional methods, such as Soxhlet, hot water extraction, ultrasound-assisted extraction etc., among which is the most reliable method (UAE) for the high yield of secondary compounds.**

MATERIALS AND METHODS

Twenty chemotypes of *C. asiatica* were collected from different agroclimatic regions of West Bengal (Table 1). The bioactive compound (asiaticoside) was extracted through Soxhlet and ultrasound-assisted extraction process in the presence of methanol. Soxhlet process was carried out for 8-10 hours at 50-60° C. UAE process was performed by sonicating sample-solvent mixture at **40kHz** for 40-50 mints. After extraction, quantitative and qualitative estimation of asiaticoside from different chemotypes were performed through HPLC in reverse phase C-18 column at 210 nm.

Table 1: Collection of *Centella asiatica* from different Agroclimatic region of West Bengal

Sl. no.	Locations	District	Latitude and Longitude
1	Durlavpur	Howrah	22°2737N 87°57'3026E
2	Kalna	Bardhaman	23.4497° N,88.3066° E
3	Baaruipur	Kolkata	22.35525° N, 88.40243° E
4	Nayachak	Howrah	22°29235N 88°02316E
5	Maadanpur	North 24 Parganas	22.7248° N, 88.4789° E
6	Banamalipur	Hooghly	22.7617° N, 88.1950° E
7	Chakdaha	Nadia	23.0765° N, 88.5293° E
8	Krishnanagar	Nadia	23.4009° N, 88.5014° E
9	Bhandartikuri	Bardhaman	23.4316° N, 88.3372° E
10	Haripur	Birbhum	23.6773° N, 87.1935° E
11	Katwa	Bardhaman	23.2164° N, 88.3529° E
12	Sainthiya	Birbhum	24.0763° N, 87.7099° E
13	Ramkrishnapur	Dakshin Dinajpur	25.2757° N, 88.9713° E
14	Rampara	Howrah	22°43303N 880791E
15	Bandipur	North 24 Pargana	22.7312° N, 88.3953° E
16	Khardah	North 24 Pargana	22.7240° N, 88.3868° E
17	Uluberia	Howrah	22.4679° N, 87.9315° E
18	Kalyani	Nadia	23.0114° N, 88.4598° E
19	Kakdwip	South 24 Pargana	21.8401° N,88.2470° E
20	Tarakeswar	Hooghly	22.53037° N,88.0043° E



Bioactive compound found in *Centella asiatica* : A) Asiaticoside, B) Madecassoside, C) Madecassic acid, D) Asiatic acid

FIG 1: Leaves of *Centella asiatica*

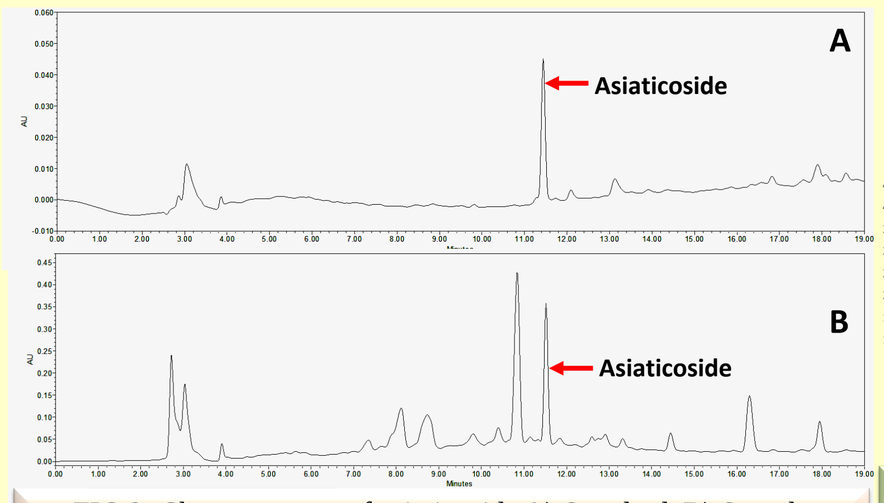


FIG 2: Chromatogram of asiaticoside A) Standard, B) Sample

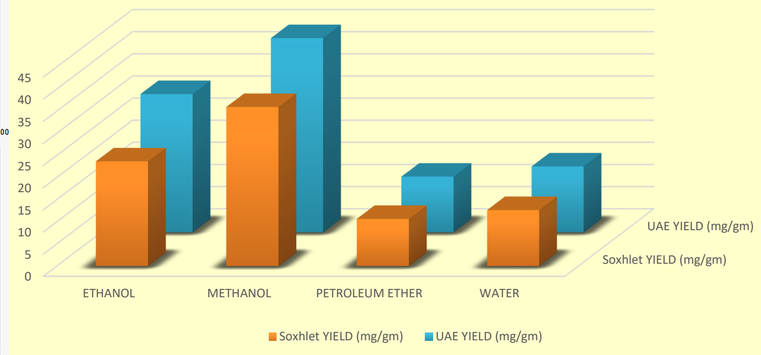


FIG 3:Graphical representation of the yield of asiaticoside through different extraction procedure and solvent system

FIG.4: HPTLC plate with asiaticoside and sample after derivatization with anisaldehyde spraying reagent

RESULT & DISCUSSION

Extraction with Soxhlet apparatus results 14.56- 37.84 mg of asiaticoside while with UAE method 20.37-45.26 mg of asiaticoside from 1gm of dry leaf powder. Screening and estimation of active metabolite "asiaticoside" was performed in High Performance Thin Layer Chromatography (HPTLC) at 600 nm and High Performance Liquid Chromatography (HPLC) system at 210 nm. The observed data has shown that UAE method gives much better result than Soxhlet extraction both qualitatively and quantitatively.

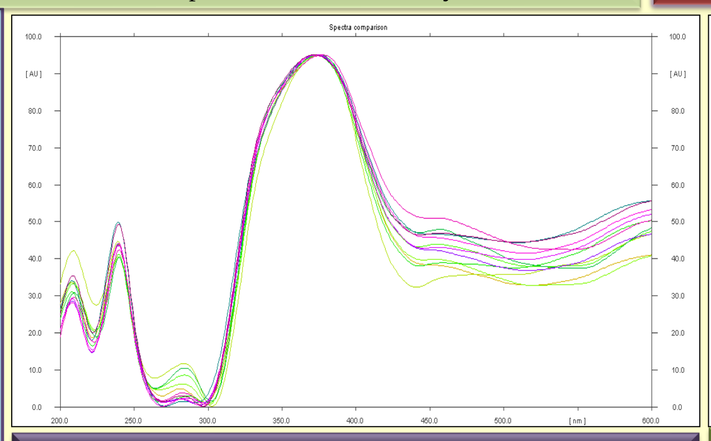


FIG. 5:The overlaid UV spectra of standard bacoside A and sample.

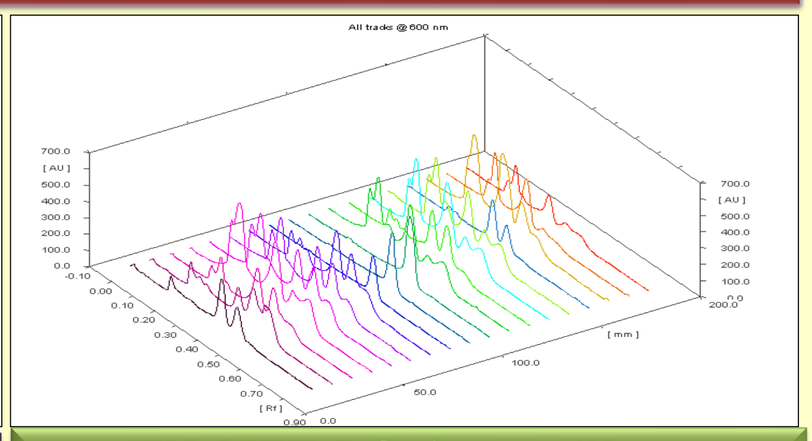


FIG. 6: HPTLC– densitogram at 600 nm.

CONCLUSION

The selection of the elite chemotype of *C. asiatica* was screened through the higher bioactive compound content that could be used for large-scale cultivation and utilization in pharmaceutical industries. As UAE has the unique capacity to enhance extraction from substrates both qualitatively and quantitatively, it could be a handy tool for industrial production.

REFERENCES

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