Abstract

Nakshatra plants are medicinally important and used from olden days onwards to treat various diseases. In the present study, a HPTLC method was developed for separation of glycosides in plants. The powdered drug of Nakshatra plant was extracted with ethanol and identification was done chemically by Molisch's, Fehling's, Bial's test and separation of glycosides was performed densitometrically at λ=254-700 nm using as a mobile phase, ethyl acetate: methanol: water (20:2.5:2.5). The results of qualitative analysis revealed the presence of carbohydrates, reducing sugars and pentose. HPTLC method achieved a good separation of glycosides at 254 nm, 304 nm, 354 nm, 404 nm with recorded peak areas and Rf values. The comparative common compound of sample glycosides was also done.

Keywords: Glycosides; HPTLC; Mobile phase; Nakshatra plants

Introduction

Nearly 80% of global population depends on plant derived medicines [1]. There has been a public demand for plant based health products in developed and developing countries [2] and 50% of modern clinical drugs originate from natural products [3]. Plant secondary metabolites (PSMs), the constituents of plant kingdom constituents, are the most widely distributed heterogenous group of substances. Among PSMs is, glycosides play numerous important roles in living organisms [4]. Glycosides are widely used in the manufacture of several products/ drugs by and also pharmaceutical industries for other purposes such as flavouring agents [5]. A large number of medicinal plants are now widely used all over the world for production of glycosidic compounds as both traditional and modern drugs and development of new drugs. These are formed by glycosylation of secondary metabolites. It is reported that species of Nakshatra plants containing different glycosidic compounds play an important role in ayurveda and are useful to different purposes in life [6]. Studies on Nakshatra plants for identification and classification of compounds having aldehyde groups present in plants as secondary metabolites revealed that these phytocompounds serve as building blocks in the synthesis of several commercially important products [7]. It is well known that human beings are always dominated by the presence of sun, moon and other planetary structures which play a vital role in Astrology and Nakshatra plants were named after respective planets and these plant species possess medicinal properties for treating various diseases [8,9]. Moreover, each plant of this group is believed to exert positive effect on the nature of human being thus increasing the positive energy level of that place [10]. These plant species were arranged according to Bentham and Hooker system of classification and reported from the flora of the Gujarat state in India [9].

Phytochemical evaluation was done in many of these plants to know moieties of flavonoids, terpenoids, phenolics and other alkaloids [11]. Available literature revealed that different glycosides from these plants may possess good therapeutic applications [12]. However, very limited information is available on glycosides in Nakshatra plants. In routine TLC (Thin layer chromatography) testing, the detection is done by spray method and the Rf value is not accurate [13]. Hence, this study is designed to identify glycosides present in these plants and to isolate glycosides from leaves of various Nakshatra groups of plants using HPTLC.

Materials and Methods

Materials

The leaves of Nakshatra plants growing in Anantapur district of Andhra Pradesh state in India were collected. The chemicals used for extraction, testing and chromatography were of analytical grade. The solutions and reagents were prepared using distilled water. TLC plates were purchased from MERCK (25 TLC aluminum sheets 20 × 20 cm Silica gel 60 F 254).

Methods

Collection of plant leaves and extraction: The freshly collected leaves were cleaned under water and dried for 4 to 8 days at room temperature and powdered using a mixer-grinder. The powdered sample (3 gm) or test was extracted with 40 mL of 70% EtOH on a rotary shaker (120 thaws / min) for 8 hours. Five mL of 70% lead acetate was added to the filtrate and centrifuged at 5000 rpm for 10 min. The supernatant was further centrifuged by adding 3 mL of 6.3% Na2CO3 at 10000 rpm for 10 min (approximately 3 mL of Na2CO3 for 20 mL of filtrate). The retained supernatant was dried and redissolved in 4 mL of chloroform. The residue dissolved in chloroform was tested for presence of carbohydrates. Testing for carbohydrates was carried out by Molisch’s, Fehling’s and Bial’s tests [14].

Separation of glycosides by HPTLC

Sample application: Sample solutions for HPTLC analysis were applied by means of a CAMAG Linomat V automated with 6 mm. The glycosides were separated using EtOAc-MeOH-H2O (20:2.5:2.5) as the mobile phase. After chromatograph development, the zones were quantified by linear scanning at 254 to 700 nm by the increment of
50 nm with a CAMAG TLC scanner 3 with a deuterium source in the resolution mode 100 µm for step, slit dimension settings of length 5.00 and width 0.45 mm Micro and scanning speed 20 mm/s. The peak areas and Rf values were determined using CATS TLC software (version 4.x, CAMAG TLC, Software) [5,15].

Instrumentation

| Instrument      | CAMAG Company       |
|-----------------|---------------------|
| Sample Application Instrument | Linomat V           |
| Syringe Volume  | 100 µL              |
| HPTLC plate Size| 10 × 10 cm plate    |
| Sample application volume | 5 µL               |
| Band length     | 6 mm                |
| Distance between bands | 10 mm              |
| Chromatography development: Chamber | 20 × 10 cm         |
| Mobile Phase    | EtoAc-MeOH-H2O (20: 2.5: 2.5) |
| Saturation Period| 60 min             |
| Scanner         | Scanner III         |
| Wave length     | 254-700 nm          |
| Wave length increment | 50 nm             |
| Scanning Speed  | 20 mm/s             |
| Slit dimension  | 5.00 × 0.45 mm Micro|
| Resolution      | 100 um/step         |
| Determination   | CATS TLC software   |

Results and Discussion

The chemical structure of secondary plant product is glycosylated to form glycosides without exception more complex than that of primary products [16]. In nature glycosides are formed by interaction of nucleotide glycosides with alcohol, phenol, steroid, triterpenoid and flavonoids etc. and joined by glycosidic linkage [17]. These are present in flowers, fruit pigments and leaves show their action as anti-inflammatory, anti-diarrheal, anti-allergic, anti-thrombotic, anti-tumour [4]. HPTLC is the widely used technique for the identification of botanical glycosides from plants. HPTLC has many advantages like multiple separations and detection procedures [6]. Hence, it is useful in qualitative analysis of plant products. For making green society, our chromatographers now prefer to develop environmental friendly, useful chromatographic systems for analysis. HPTLC is a powerful technique used for detection and potential quantitation of drugs and compounds in clinical samples. Nowadays, the interest in study of natural product is growing rapidly, especially as a part of drug discovery programs [18]. The results of screening of the carbohydrate in Nakshatra plants are presented in Table 1. Different tests conducted with the samples revealed the presence of carbohydrates (all), reducing sugars (Amala, Jamun, Khair, Pimpal, Palas, Roal, Rui) and pentoses (Jamun, Palas, Arjun, Bakul, Savar, Kadamba, Neem) in moderate concentrations in Nakshatra plants. Further, glycosides were also noticed. It was concluded that the Nakshatra plants have secondary metabolite of glycosides of different groups. They are reported to be effective in reducing blood lipid, in assimilating cholesterol, inhibiting thrombosis, dilating the coronary artery, and as antioxidatives etc. Different groups of glycosides present in the plant extracts were separated by High Performance TLC. The pinkish violet colour confirmed the presence of glycosides in the given samples. From the results obtained after densitometric scanning, good separation was observed at wavelengths of 254 nm, 304 nm, 354 nm, 404 nm were shown in Table 2. Vet shows 9 peaks at 254 nm, similarly mango shows 6 peaks at 254 nm, savar and arjun shows 9 peaks at 404 nm. Payar, pimpal, umber, jai and palas shows 8 peaks at 404 nm phanas shows 4 peaks at 404 nm, only roal shows 8 peaks at 354 nm, kadam, nagakeshar and amla show 5 peaks at 304 nm while jamun, neem, beal and moha 4 peaks at 304 nm, khair, rui, vad and chandan show 3 peaks and velu and shami show 2 peaks at 304 nm in Table 3. The corresponding HPTLC densitograms of Nakshatra plants were presented in Figure 1a-1x. The most appropriate mobile phase used for the separation is EtoAc-MeOH-H2O (20: 2.5: 2.5). The concentration of glycosides was also assessed by determining the percent area and Rf values which are shown in Table 3. The peaks obtained from Nakshatra plant extracts when used for comparative analysis revealed that the Rf 0.83 is common for found in the extracts of prayer, pimple, jai, umber, neem and Velu as shown in Figure 2. Similarly, another glycoside with Rf value of 0.82 is common by foumnd in arjun, roal, mango, nagakesh and shami represented in Figure 3. Thus, present HPTLC studies confirmed the presence of active glycosides in the ethanolic extract of Nakshatra plant species. The active glycosides combine with proteins to form glycoproteins, with lipids to form glycolipids which involve are in membrane formations, transportation and signal transduction and hormonal regulation of several biological functions.

Conclusion

A simple, precise, accurate and reproducible HPTLC method was successfully developed for analysis of medicinal products in Nakshatra plants containing glycosides. This method enabled us to detect and quantify the number of glycosides in various medicinal products such as extracts and formulations. The method can be applied by pharmacists to estimate glycosides in their products as a routine control method and also to keep a check on the variations from batch to batch. The ethanol extract of Nakshatra plants containing glycosides is found to be better when compared to extraction with other solvents and can be used for further formulations. From the HPTLC studies, it has been found that the mixture of ethyl acetate, methanol and water used as mobile phase contains not a single compound but a mixture of compounds confirming that the pharmacological activity shown by them is due to the cumulative effect of all the compounds in composite.
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| S. No. | Nakshatra | Botanical Name       | Common Name    | Family      | Molish’s Test | Fehling’s Test | Bial’s Test |
|--------|-----------|----------------------|---------------|-------------|---------------|---------------|-------------|
| 1      | Aswini    | Strychnus nuxvomica  | Kuchla, Kajara | Loganiaceae | +             | +             | Nil         |
| 2      | Bharani   | Emblica officinalis  | Aamla         | Euphorbiaceae | +             | ++            | Nil         |
| 3      | Krutika   | Ficus glomeata       | Umber         | Articaceae  | +             | +             | Nil         |
| 4      | Rohini    | Eugenia jambolana    | Jamun         | Myrtaceae   | +             | ++            | +           |
| 5      | Mruga Shirsha | Acacia catechu      | Khair         | Mimosaceae  | +             | ++            | Nil         |
| 6      | Andraa    | Santalum album       | Chandan       | Santalaceae | +             | +             | Nil         |
| 7      | Punvasu   | Bambus aerandniasia  | Veli          | Graminnae   | +             | +             | Nil         |
| 8      | Pushya    | Ficus religiosa      | Pinopal       | Articaceae  | +             | ++            | Nil         |
| 9      | Ashiesha  | Messu aferrea        | Naagkeshar    | Guttiferae  | +             | +             | Nil         |
| 10     | Magha     | Ficus bengalensis    | Vad           | Articaceae  | +             | +             | Nil         |
| 11     | Purvaphalguni | Butea frondosa     | Palas         | Fabaceae    | +             | ++            | +           |
| 12     | Uttaraphalguni | Ficus infectoria  | Payar         | Articaceae  | +             | +             | Nil         |
| 13     | Hasta     | Jasminum auriculatum | Jaai          | Oleaceae    | +             | +             | Nil         |
| 14     | Chitra    | Aegle marmalos       | Bael          | Rutaceae    | +             | +             | Nil         |
| 15     | Swati     | Terminalia arjuna    | Arjun         | Combretaceae| +             | +             | +           |
| 16     | Vishakha  | Adhatoda vasica      | Elephant apple| Guttiferae  | +             | +             | Nil         |
| 17     | Anuradha  | Minus opselengi      | Bakul         | Sapotaceae  | +             | +             | +           |
| 18     | Jeshta    | Salmalam albarica    | Saavar        | Malvaceae   | +             | +             | +           |
| 19     | Mul       | Vetera indica        | Roal          | Dipterocarpaceae | +       | ++            | Nil         |
| 20     | Purvaashadha | Calamus roteng      | Vet           | Palmae      | +             | +             | Nil         |
| 21     | Uttarashadha | Artocarpus integrifolia | Phanas    | Aricaceae   | +             | +             | Nil         |
| 22     | Shravan   | Calotrops gigantea   | Rui           | Asclepediaceae | +       | ++            | Nil         |
| 23     | Dhanishta | Prosopis spicgera    | Shami         | Mimosaceae  | +             | +             | Nil         |
| 24     | Shattaraka | Mitryna parvilolia  | Kadamba       | Rubiaceae   | +             | +             | +           |
| 25     | Purvabhadrapada | Mangifera indica    | Amba          | Anacardiaceae | +       | +             | Nil         |
| 26     | Uttarabhadrapad | Azadirachta indica | Neem          | Meliaeaceae | +             | +             | +           |
| 27     | Revati    | Madhuca indica       | Moha          | Sapotaceae  | +             | +             | Nil         |

Note: ++ indicates the presence

**Table 1:** Qualitative tests for carbohydrates in Nakshatra plants.

![Figure 1b: Densitogram obtained from Vet.](image-url)
Figure 1c: Densitogram obtained from Moha.

Figure 1d: Densitogram obtained from Amla.

Figure 1e: Densitogram obtained from Chandan.

Figure 1f: Densitogram obtained from Beal.
| Botanical Name     | Hindi/Marathi Name | UV-Visible at 254-700 nm |
|-------------------|--------------------|-------------------------|
| Mangifera indica  | Amba               | 254                     |
| Calamus rotung    | Vet                | 254                     |
| Madhuca indica    | Moha               | 304                     |
| Bambusa erandinas | Velu               | 304                     |
| Santalum album    | Chandan            | 304                     |
| Messua ferrea     | Naagkeshar         | 304                     |
| Ficus bengalensis | Vad                | 304                     |
| Azadirachta indica| Neem               | 304                     |
| Calotropis gigantea| Rui               | 304                     |
| Aegle marmalos    | Beal               | 304                     |
| Eugenia jambolana | Jamun              | 304                     |
| Emblica officinalis| Aamla             | 304                     |
| Prospis spicigera | Shami              | 304                     |
| Acacia catechu    | Khair              | 304                     |
| Mitrigyna parvifolia| Kadamba         | 304                     |
| Minusops elengi   | Bakul              | 304                     |
| Vetteria indica   | Roal               | 354                     |
| Artocarpus integrifolia| Phanas         | 404                     |
| Terminalia arjuna | Arjun              | 404                     |
| Ficus infectoria  | Payar              | 404                     |
| Butea frondosa    | Palas              | 404                     |
| Ficus religiosa   | Pimpal             | 404                     |
| Strychnus nuxvomica| Kuchlia, Kajara   | 404                     |
| Salmalia malbarica| Saavar             | 404                     |
| Jasminum auriculatum| Jaai             | 404                     |
| Ficus glomeata    | Umber              | 404                     |

**Table 2:** Optimum UV-Vis ranges (254 to 700 nm) for separation of glycosides in Nakshatra plants.
Figure 1j: Densitogram obtained from Neem.

Figure 1k: Densitogram obtained from Nag.

Figure 1l: Densitogram obtained from Velu.

Figure 1m: Densitogram obtained from Khair.
Figure 1n: Densitogram obtained from Kadamba.

Figure 1o: Densitogram obtained from Bakul.

Figure 1p: Densitogram obtained from Roal.

Figure 1q: Densitogram obtained from Panas.

Figure 1r: Densitogram obtained from Arjun.
Figure 1s: Densitogram obtained from Payar.

Figure 1t: Densitogram obtained from Pimpal.

Figure 1u: Densitogram obtained from Palas.

Figure 1v: Densitogram obtained from Jai.

Figure 1w: Densitogram obtained from Savar.

Figure 1x: Densitogram obtained from Umber.
| Plant name and Wave length (nm) | Peak | Start Rf | End Rf | Area | Area% |
|--------------------------------|------|----------|--------|------|-------|
| **Mango at 254**              |      |          |        |      |       |
| 1                             | 0.01 | 0.11     | 6882.2 | 14.74|
| 2                             | 0.30 | 0.38     | 710.6  | 1.57 |
| 3                             | 0.60 | 0.62     | 163.4  | 0.36 |
| 4                             | 0.67 | 0.74     | 510.4  | 1.13 |
| 5                             | 0.78 | 0.82     | 437.0  | 0.96 |
| 6                             | 0.82 | 0.99     | 36820.6| 81.24|
| **Vet at 254**                |      |          |        |      |       |
| 1                             | 0.12 | 0.20     | 806.9  | 2.63 |
| 2                             | 0.24 | 0.30     | 1214.8 | 3.97 |
| 3                             | 0.31 | 0.36     | 1929.3 | 6.30 |
| 4                             | 0.36 | 0.42     | 1927.1 | 6.29 |
| 5                             | 0.44 | 0.48     | 748.5  | 2.44 |
| 6                             | 0.51 | 0.58     | 770.3  | 2.52 |
| 7                             | 0.68 | 0.76     | 1408.6 | 4.60 |
| 8                             | 0.76 | 0.92     | 12886.3| 42.08|
| 9                             | 0.92 | 1.00     | 8933.1 | 29.17|
| **Moha at 304**               |      |          |        |      |       |
| 1                             | 0.28 | 0.34     | 528.8  | 3.41 |
| 2                             | 0.34 | 0.41     | 978.8  | 6.32 |
| 3                             | 0.75 | 0.79     | 239.5  | 1.55 |
| 4                             | 0.79 | 0.99     | 13743.4| 88.72|
| **Amala at 304**              |      |          |        |      |       |
| 1                             | 0.04 | 0.12     | 3439.6 | 11.74|
| 2                             | 0.57 | 0.64     | 424.8  | 1.45 |
| 3                             | 0.76 | 0.80     | 258.7  | 0.86 |
| 4                             | 0.81 | 0.93     | 17205.8| 58.70|
| 5                             | 0.93 | 1.00     | 7987.3 | 27.25|
| **Chandan at 304**            |      |          |        |      |       |
| 1                             | 0.74 | 0.85     | 3281.1 | 14.39|
| 2                             | 0.85 | 0.92     | 8392.2 | 36.81|
| 3                             | 0.92 | 1.00     | 11126.8| 48.80|
| **Beal at 304**               |      |          |        |      |       |
| 1                             | 0.00 | 0.07     | 7279.0 | 27.71|
| 2                             | 0.09 | 0.18     | 3205.2 | 12.20|
| 3                             | 0.81 | 0.94     | 9046.8 | 34.44|
| 4                             | 0.94 | 1.00     | 6740.2 | 25.66|
| **Vad at 304**                |      |          |        |      |       |
| 1                             | 0.03 | 0.1     | 2938.6 | 9.87 |
| 2                             | 0.35 | 0.39     | 350.9  | 1.18 |
| 3                             | 0.77 | 0.99     | 26475.9| 88.96|
| **Shami at 304**              |      |          |        |      |       |
| 1                             | 0.05 | 0.07     | 300.9  | 1.1  |
| 2                             | 0.82 | 0.99     | 27171.9| 98.9 |
| **Rui at 304**                |      |          |        |      |       |
| 1                             | 0.01 | 0.10     | 4517.4 | 12.05|
| 2                             | 0.79 | 0.90     | 11842.2| 31.58|
| 3                             | 0.90 | 1.00     | 21144.5| 56.38|
| **Neem at 304**               |      |          |        |      |       |
| 1                             | 0.02 | 0.06     | 1095.2 | 4.22 |
| 2                             | 0.11 | 0.20     | 2095.5 | 8.08 |
| 3                             | 0.72 | 0.83     | 2696.8 | 10.40|
| 4                             | 0.83 | 1.00     | 20037.9| 77.29|
| **Naagkesar at 304**          |      |          |        |      |       |
| 1                             | 0.03 | 0.09     | 2508.9 | 6.3  |
| 2                             | 0.04 | 0.19     | 12209.3| 3.09 |
| 3                             | 0.27 | 0.81     | 1084.8 | 2.72 |
| 4                             | 0.37 | 0.42     | 1184.4 | 2.96 |
| 5                             | 0.82 | 1.0      | 33841.2| 84.93|

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Table 3: HPTLC profile of the Nakshatra plants for glycosides.

|   | Rf values | % of Area |
|---|-----------|-----------|
| 6 | 0.79      | 0.83      | 1280.4 | 4.09 |
| 7 | 0.83      | 0.90      | 5845.4 | 18.04|
| 8 | 0.90      | 0.99      | 16159.3| 51.65|

**Palas at 404**

|   | Rf values | % of Area |
|---|-----------|-----------|
| 1 | 0.11      | 0.13      | 3674.1  | 13.10|
| 2 | 0.14      | 0.20      | 719.9   | 2.57 |
| 3 | 0.30      | 0.38      | 1088.8  | 3.88 |
| 4 | 0.39      | 0.42      | 486.6   | 1.72 |
| 5 | 0.73      | 0.78      | 1207.6  | 4.31 |
| 6 | 0.79      | 0.84      | 1866.4  | 6.65 |
| 7 | 0.84      | 0.95      | 1345.3  | 48.55|
| 8 | 0.95      | 1.00      | 5389.8  | 19.22|

**Jai at 404**

|   | Rf values | % of Area |
|---|-----------|-----------|
| 1 | 0.11      | 0.15      | 354.7   | 19.70|
| 2 | 0.16      | 0.21      | 1030.0  | 5.73 |
| 3 | 0.34      | 0.37      | 605.7   | 3.73 |
| 4 | 0.39      | 0.43      | 573.7   | 3.14 |
| 5 | 0.47      | 0.49      | 89.8    | 0.50 |
| 6 | 0.76      | 0.78      | 184.9   | 1.03 |
| 7 | 0.83      | 0.93      | 5068.2  | 28.20|
| 8 | 0.93      | 1.00      | 6876.9  | 38.27|

**Savar at 404**

|   | Rf values | % of Area |
|---|-----------|-----------|
| 1 | 0.11      | 0.15      | 5594.0  | 17.96|
| 2 | 0.15      | 0.18      | 1166.4  | 3.75 |
| 3 | 0.24      | 0.29      | 1574.3  | 5.06 |
| 4 | 0.33      | 0.36      | 1190.6  | 5.82 |
| 5 | 0.36      | 0.41      | 1100.4  | 3.71 |
| 6 | 0.61      | 0.67      | 444.1   | 1.43 |
| 7 | 0.79      | 0.84      | 16667.8 | 5.36 |
| 8 | 0.84      | 0.90      | 4413.9  | 14.17|
| 9 | 0.90      | 0.97      | 14089.7 | 45.24|

**Umber at 404**

|   | Rf values | % of Area |
|---|-----------|-----------|
| 1 | 0.02      | 0.07      | 261.9   | 0.85 |
| 2 | 0.11      | 0.17      | 709.6   | 2.30 |
| 3 | 0.21      | 0.29      | 2300.4  | 7.47 |
| 4 | 0.30      | 0.41      | 4898.2  | 15.91|
| 5 | 0.41      | 0.44      | 1008.2  | 3.27 |
| 6 | 0.44      | 0.51      | 1594.0  | 5.18 |
| 7 | 0.76      | 0.83      | 2666.2  | 8.66 |
| 8 | 0.83      | 1.00      | 17350.2 | 56.35|

**Figure 2:** Percentage of Common glycosides present in Payar, Pimpal, Jai, Umber, Neem, Velu plant extracts.

**Figure 3a:** HPTLC Spectrum of glycosides from Nagkesher, Vad, Jamun, Mango, Shami, Adulsa, Amla, Rui, Velu overlaid with the corresponding peaks at 254 nm.

**Figure 3b:** HPTLC Spectrum of glycosides from Palas, Kadamba, Pimpal, Kair, Savar, Bakul, Jai overlaid with the corresponding peaks at 254 nm.
A comparative HPTLC analysis of Nakshatra plant extracts provides a comparative account of the amount of glycosides present in the particular plants. For further study, pure common glycoside compound should be isolated and identified on the basis of reference standards from leaves of Nakshatra plants for assessing its potent pharmacological properties.

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