The Pharmaceutical and Chemical Journal, 2018, 5(6):21-28

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

A Comprehensive Research on HPTLC Analysis and Interpretation of Three Plants, Mainly: *Commiphora myrrha, Operculina turpethum, and Pinus roxburghii* Sarg

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Abstract HPTLC analysis of plants and to analyse their phytoconstituents has been the modern practice of phytochemistry and plant medicine analysis which has accuracy around 90 %. For this present research, we studied and analysed three plants, mainly *Commiphora myrrha*, *Operculina turpethum, and Pinus roxburghii* Sarg. The results are highly interesting.

Keyword: Medicinal Plants, HPTLC Analysis and Interpretation, *Commiphora myrrha*, *Operculina turpethum, and Pinus roxburghii* Sarg

Introduction

Medicinal plants and Traditional medicinal plants are in use since ages in the history of mankind. In ancient Indian history vaids used to treat the ailment encountered by the human body with the use of aushadhi's. In present age, these are basicalls bhasms and extracts of selected medicinal plants in order to treat the specific ailment. In the present study and research, we analysed the selected medicinal plants using a high performance technique, i.e. HPTLC [1].

Plants

A. Myrrh

***Commiphora myrrha* (Nees) Engl.**
(Burseraceae)

Introduction

The genus *Commiphora* (Burseraceae) comprises over 150 species, most of which are confined to eastern Africa and few species occur in Saudi Arabia. Myrrh, a yellow nonvolatile gum resin secreted by plants of the genus *Commiphora* (Burseraceae). The genuine myrrh of commerce, also known as heerabol myrrh, is obtained from *Commiphora myrrha* (Nees) Engl. and is considered by some to be *C. myrrha* var. *molmol* Engl. Some other species, such as *C. abyssinica* Engl. and *C. schimperi* Engl. *Commiphora molmol* Engl. ex Tschirch also yield myrrh. *Heerabol myrrh* is often confused with *Bissabol myrrh* or sweet myrrh., an exudate from *C. erythraea* (Ehrh.) Engl. These species are native to Ethiopia, the Somali Republic and Saudi Arabia, and are not found in India, but the myrrh (gum resin) is imported into India since long. *Heerabol myrrh* is very often adulterated with the oleogum resin from *C. wightii* [1-2].

Common Names

Heerabol myrrh, Mulmul, Gummi myrrh, Morr, Mira, Didin.

Synonyms

Balsamodendron myrrha Nees; *C. abyssinica* Engl.; *C. molmol* Engl.

Vernacular names

Sanskrit: Bolah, Rasagandhah; **English:** Myrrh; **Hindi:** Bol, Hirabol; **Kannad:** Bola; **Malayalam:** Narumpasamaram, Narumpasa; **Tamil:** Vellaippapolam; **Telugu:** Balimtra-polam [3].

Morphology

The trees or shrubs may attain a height of 10 m. They are sturdy build, with knotted branches. *Commiphora myrrha* has a shrubby stem with spinescent branches, a very pale-grey bark, and a yellowish white wood. Its leaves are ternate on short petioles; leaflets obovate, obtuse, the lateral side smooth. The fruit is drupaceous, usually indehiscent surrounded at base by a 4-toothed calyx and supported on a very short stalk.

Myrrh occurs in somewhat irregular tears or masses weighing up to about 250 g. The surface is reddish-brown or reddish yellow in colour and powdery. The drug fractures and powders readily, the freshly exposed surface being of a rich brown colour and oily. Whitish marks are sometimes visible and thin splinters are translucent. Myrrh has an aromatic odour and an aromatic, bitter and acrid taste. Myrrh forms a yellowish emulsion when triturated with water. It may be distinguished from perfurmed bdellium and similar products by allowing an ethereal extract of the drug to evaporate to dryness and passing the vapour of bromine over the resinous film produced. A violet colour is given by genuine myrrh but not by bdellium. TLC and visualization with ultraviolet light at 365 nm are used by the BP as an identification test and also to establish the absence of *C. mukul*, an inferior bdellium product [4-5].

Medicinal Uses

Myrrh is widely used in India though it is a rare and costly product. It is very often adulterated with gum of *Balsamodendron mukul* which, on account of its close resemblance to myrrh, is known as 'false myrrh'. It is mixed with equal parts of honey and rectified spirit and dissolved in rose water or infusion of rose petals (50 parts) and the solution is good for mouth-wash and also for internal administration in stomatitis. With borax it makes an application for parasitic-stomatitis or thrush. It is useful in dyspepsia and mixed with molasses or preferably with iron and vegetable bitters it is given to treat amenorrhea, chlorosis, other atonic uterine infections, and as a stimulating expectorant in chest infections, especially in chronic bronchitis, asthma and phthisis. Externally it is prescribed as an astringent, stimulating application in ulcerated conditions and a gargle for spongy gums and in ulcerated sore throat. Myrrh is dissolved in human or ass's milk and dropped into the eye to cure purulent ophthalmia. It is useful as a dentifrice in caries of the teeth, either alone or mixed with other drugs; and is applied on the head to prevent hair from falling off. A tincture of myrrh combined with glycerine is given internally every one or two hours to cure diphtheria. The tincture of myrrh is useful in menstrual disorders and chlorosis of young girls. Three grains each of powdered myrrh and rhubarb with five grains of *Ipomoea hederacea* are a good stomachic and laxative. Myrrrh is also used in incense and perfume [4,6].

B. Jalap [*Operculina turpethum* (Linn.)], Silva Manso, (Convolvulaceae)

Introduction

It is a small genus of herbaceous twiners distributed in the tropics of both hemispheres. One species occurs in India. *Operculina turpethum* is the source of the drug known as turpeth or Indian Jalap and used as purgative. The drug occurs in two forms, white turpeth (Safed nisoth) and black turpeth (Krishna nisoth) [7].

Synonyms

Ipomoea turpethum R. Br.; *Convolvulus turpethum* Linn.

Vernacular names
Sanskrit: Trivrit; **Hindi:** Nisoth, Nisotar, Pitohri; **Bengali:** Dudhkalmi; **Marathi:** Nishottar; **Gujarati:** Nashotar; **Oriya:** Dudholomo; **Tamil:** Shivadai, Kumbam, **Telugu:** Tellategada, **Malayalam:** Chivaka, Trikolpakonna; **Kannad:** Bilitigade, Bangada balli

Common names
False Jalap, Indian Jalap, Indian Rhubarb, Black nishoth.

Distribution
It grows throughout India up to an altitude of 900 m in Bangladesh, Sri Lanka, Malay Islands, tropical America, Mauritius, Philippines, tropical Africa and Australia [5].

Cultivation
It is occasionally grown in gardens for ornament and sometimes cultivated.

Morphology
A large, climbing herb and perennial twiner with milky juice and fleshy branches. The leaves are very variable in shape, 5-10 by 1.3-7 cm., ovate or oblong; the flowers are tubular-campanulate, white, in few flowered cymes; the capsules globose with 4 or less, dull black, glabrous seeds. The roots are long, slender, fleshy, much branched and the stems are very long, twisted together, angled and winged, pubescent, tough and brown when old. The drug consists of cylindrical pieces of root and stem, 1.5-15 cm long with 1-5 cm diameter, often with central woody portion removed by splitting the bark on one side; external surface longitudinally furrowed giving the drug a rope-like appearance; fracture short in bark and fibrous in wood; odour distinct but unpleasant or musty; taste somewhat nauseating or bland at first, then slightly acrid [6].

Medicinal Uses
It is almost as effective as true Jalap (*Exogonium purga*), superior to rhubarb (*Rheum emodi* Wall ex Meissin), and useful in all affections where Jalap or rhubarb is indicated. The drug is administered in the form of powder; it may also be given in combination with cream of tartar in equal proportion. White turpeth is preferred to black turpeth as cathartic; the latter produces drastic purgation and causes vomiting, fainting and giddiness. The black variety is a powerful drastic; beneficial in loss of consciousness, burning sensation and intoxication. The root is bitter with a sharp taste and is prescribed as anthelmintic, febrifuge and purgative. It is also very useful to relieve ascites, leucoderma, itch, ulcers, abdominal troubles, anaemia, fevers, piles, tumors, jaundice, ophthalmia and is good in diseases of the liver, heart and eye. The root is efficacious in biliousness, tremors of the body, diseases of the brain, paralysis, pains in the muscles, bronchitis and pains in the joints and in the chest. The root is prescribed in the treatment of snake-bite and scorpion sting but it is not an antidote to either snake-venon or scorpion venom. In western India, a flower paste is applied to the head to mitigate hemicrania. Young leaves and tender stems are consumed as vegetable in Philippines. Stems are used for tying purposes [5].

C. Colophony

***Pinus roxburghii* Sarg.**
(Pinaceae)

Introduction
Pinus, a large genus of monoecious, evergreen, resiniferous trees, commonly known as Pines, is distributed in the northern hemisphere, extending to south across the equator in Indonesia. There are around 70 species found in northern temperate and on mountains in the northern tropics. Pines occur widely in the temperate regions; in the warm temperature and sub-tropical countries they are found chiefly in the hills. Five species occur wild in India, *P.*

gerardiana Wall., *P. insularis*, Endl., *P. wallichiana* A.B. Jackson, *P. armandii* Franch. and *P. merkusii*, Jungh and De Vriese.

In India *P. wallichiana* (Kail), a soft-pine, and *P. roxburghii* (Chir) a hard pine, yield commercial timber. Equally important are the oleoresins exuded by several species of pines. On distillation, the resins yield an essential oil, commonly known as Turpentine oil or Turpentine, and a non volatile product, the Rosin or Colophony. India produces large quantities of turpentine oil and rosin, mainly from *P. roxburghii*, some of the pines yield edible seeds. *P. gerardiana*, found in the north-western Himalayas and Afghanistan, is the source of the much relished Chilgoza seeds [5].

Common names

Chir, pine, chil, Himalaya long-leaved pine.

Synonyms

Pinus longifolia Roxb.

Vernacular names

Sanskrit: Bhadradaru, Manojna; Hindi: Chil, Chir, Salla; Bengali: Saralgachha; Gujarati: Saraladeodara; Kashmir: Salla, Sarl; Malayalam: Charalam; Tamil: Simaidevadari; Telugu: Devadaru [5].

Distribution

A tall tree with a spreading crown is found in the Himalayas from Kashmir to Bhutan and in the Siwalik hills at altitudes of 450-2,400 m. It comes up tolerably well in the plains also and is sometimes planted in gardens for ornamental purposes. The chir pine occurs in the Himalayas almost exclusively in the outer hills and valleys, which receive the bulk of the rainfall during the monsoon.

Cultivation

Natural regeneration

It takes places through seeds. Under ordinary forest conditions, trees less than 30-year old seldom bear cones. The cones begin to open during April-May of the third year, i.e., about -24 months after their appearance and the seeds get dispersed during April-July. Under natural conditions, the seeds germinate as soon as sufficient moisture is available. The germination commences at the beginning of the monsoon. A number of factors such as light, drought, tonography and soil have considerable influence upon the extent and quality of natural regeneration.

Artificial regeneration

It is required transplanting nursery-raised seedlings or by direct sowing. The mature cones are collected from the trees during March-April and are placed in the hot sun to loosen the scales, and thereafter the seeds are threshed out. The seeds are sown in the nursery during March-April in shallow drills 15 cm. apart. The seedlings are picked out in July. One-or two-year old seedlings are usually transplanted at the beginning of the rains.

Morphology

A large tree, branches more or less whorled; bark dark grey, often reddish, deeply fissured, rough, exfoliating in longitudinally elongated plates; leaves in clusters of three, 20-30 cm, long, triquetrous, finely toothed, light, green, persisting on an average for a year and a half; male flowers about 1.5 cm long, arranged in the form of cones; female cones, solitary or 2-5 together, ovoid, 10-20 cm. × 7.5 ×-13.0 cm. when ripe, brown, woody, seeds winged; without wing 7.5-130.0 mm. × 5.0×6.5 mm.; wings long, membranous.

Colophony or Rosin

The colophony is obtained as the solid residue in the distillation of the turpentine oil from the oleoresin. It is recovered from the still, after melting, by passing high pressure steam through the jacket. The molten mass is strained through filtering trays and filled into wooden casks, where it is allowed to cool for 1-2 days before

marketing. The yield of colophony is about 75 per cent of the quantity of oleoresin distilled. There are different grades of the rosin available in the market.

The colophony is faintly aromatic and occurs in the form of transparent or slightly translucent brittle lumps with glassy structures. It is classified according to its colour into three types, viz., pale, medium and dark which are further divided into eight colour grades. It is soluble in ether, chloroform, light petroleum, alcohol, acetone, and most volatile and fixed oils. The colophony is not usually adulterated as it is probably one of the cheapest resins available in the market.

Medicinal Uses

The plant resin is sweet, bitter, pungent; thermogenic; oleagenous and intestinal antiseptic. Internally, the colophony is used as a stomachic and externally as a plaster, and is applied to buboes and abscesses for suppuration. The wood is considered stimulant, diaphoretic and useful in burning of the body, cough, fainting and ulceration. The resin is stimulant both externally and internally. Internally, it acts chiefly on the mucous membrane of the genito-urinary organs, and is, therefore, a very good remedy for gonnorhoea. The gum has a bad smell and taste. It has shown diuretic, emmenagogue, purgative and expectorant actions. It is also useful in inflammations, asthma, chronic bronchitis, piles, diseases of the liver and spleen, urinary discharges, earache, toothache, lumbago, tuberculosis, scabies and epilepsy. The oil lessens inflammation. The gum has shown good effect in diseases of the vagina and uterus.

The colophony is principally used in paper, soap, cosmetics, paint, varnish, rubber and polish industries. It is employed as an ingredient of printing inks, casein glues, and as a binder in plastics, dry battery and insulating compositions. It is utilized in the manufacture of fire works, shell explosives, insecticides and disinfectants and enters into certain lubricating composition. It is employed in brewing and in mineral benefication as a frothing agent and also applied to reduce slipping. The turpentine oil is one of the most important basic raw materials for the synthesis of terpene chemicals which are used in a wide variety of industries, such as adhesives, lubrication additives, synthetic resins, solvents, plasticizers, paints, varnishes, soaps, perfumery, cosmetics and paper and rubber chemicals. The pine oil is used as an adjunct in the scenting of soaps, in bath preparations, room sprays, deodorants and similar products [5].

Materials & Methods

For this research, a HPTLC was used for the analysis of the three plants.

High performance thin layer chromatography (HPTLC) is an invaluable quality assessment tool for the evaluation of botanical materials. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. Additionally, numerous samples can be run in a single analysis thereby dramatically reducing analytical time. With HPTLC, the same analysis can be viewed using different wavelengths of light thereby providing a more complete profile of the plant than is typically observed with more specific types of analyses. TLC and HPTLC are methods commonly applied for the identification, the assay and the testing for purity, stability, dissolution or content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmetics, nutrients). These flexible and cost-effective techniques present the advantage of the simultaneous processing of standards and samples with versatile detection possibilities, including a great variety of post-chromatographic derivatization reagents. The validation of analytical methods is largely recognized as the best safeguard against the generation of unreliable data and is becoming an absolute requirement in many fields. Validation is the process by which it is established, by laboratory studies, that the performance characteristics of an analytical method meet the requirements for the intended applications. Depending on the objective of the analytical procedure, the typical validation characteristics which can be considered through a statistical approach are accuracy, precision, specificity or selectivity, detection limit, quantification limit, linearity and ruggedness [8-11].

Advantages of HPTLC

The analysis of herbals and herbal preparations is challenging for several reasons:

- As analytes, herbs are extremely complex. Even herbal preparations such as extracts contain numerous compounds in concentrations that can cover several orders of magnitude.
- In many instances, chemical composition of the herb is not completely known, there are no established methods of analysis available.
- The requirement for a fingerprint analysis can be completely different from those for a quantitative determination of marker or key compounds, although the herbal preparation is the same in both instances. For example, as many components as possible should be separated for fingerprints, but for quantitative determination of marker compounds, it is necessary to fully separate those compounds from all others.
- Constituents of herbals that belong to very different classes of chemical compounds can often create difficulties in detection. With this in mind, TLC and HPTLC can offer many advantages.

Steps involved in HPTLC

- Selection of chromatographic layer
- Sample and standard preparation
- Application of the sample and standard
- Chromatographic development
- Detection of the spots
- Scanning
- Documentation of chromatoplates [10-11]

Experimental

HPTLC of plant extracts

Collection of materials

Drug samples were procured from the different commercial sources and identified by Dr. H.B. Singh, Taxonomist, National Institute of Science Communication and Information Resources (NISCAIR), CSIR, New Delhi.

The selected plant drugs are,

1. *Commiphora myrrha* (Myrrh)
2. *Operculina turpethum* (Jalap)
3. *Pinus roxburghii* (Colophony)

Preparation of plant material

Commiphora myrrha (oleo-gum-resin)

The powdered drug material (100 g) was extracted with 250 ml of methanol at room temperature for 24 hours and filtered. The extract was concentrated under reduced pressure and used for TLC fingerprinting.

Operculina turpethum (roots)

The powdered drug material (100 g) was extracted with 250 ml of methanol in a Soxhlet apparatus for 8 hours and filtered. The extract was concentrated under reduced pressure and used for TLC fingerprinting.

Pinus roxburghii (oleo-resin)

Air dried powdered drug (20 g) was kept in contact with methanol for extraction at room temperature for 5 hours and filtered. The extract was concentrated under reduced pressure and used for TLC fingerprinting.

TLC fingerprinting studies

Solvent systems were developed for establishing the TLC patterns for the methanolic extracts of the selected drugs. Various visualization techniques were used to come up with the best TLC fingerprint, like UV 254, UV 366, iodinisation and spray reagents like anisaldehyde, ninhydrin, aniline phthalate, Folin's reagent, vanillin and

sulphuric acid were also tried. The developed plates were dried in air, visualized in UV at wavelengths 254 and 366 nm and photographed. Then they are derivatized with anisaldehyde reagent, sulphuric acid and vanillin reagent and visualized in UV at wavelengths 254, 366 nm and in daylight and photographs were taken thereof [12].

HPTLC scanning

The developed plates were taken to the Camag HPTLC scanner IV for the densitometric scanning. The plates were scanned with UV 366 nm.

Results

Table 1: HPTLC fingerprinting profiles of methanolic extract of *C. myrrha* (oleo-gum-resin), *O. turpethum* (roots) and *P. roxburghii* (oleo-resin)

Plants	Solvent System	Visualizing agent	Fig.	No. of spots (R_f values) at 366 nm
C. myrrha	Toluene - ethyl acetate - formic acid (9.5: 0.5 : 0.1)	Vanillin - Sulphuric acid	1	10 (0.27, 0.32, 0.36, 0.42, 0.44, 0.52, 0.54, 0.64, 0.68, 0.72)
O. turpethum	Toluene - ethyl acetate - formic acid (9.3:1.0:0.1)	–	2	10 (0.07, 0.10 0.15, 0.20, 0.23, 0.26, 0.35, 0.51, 0.61, 0.74)
P. roxburghii	Toluene-ethyl acetate (9.5 : 0.7)	–	3	9 (0.18, 0.21, 0.25, 0.28, 0.33, 0.37, 0.53, 0.56, 0.59)

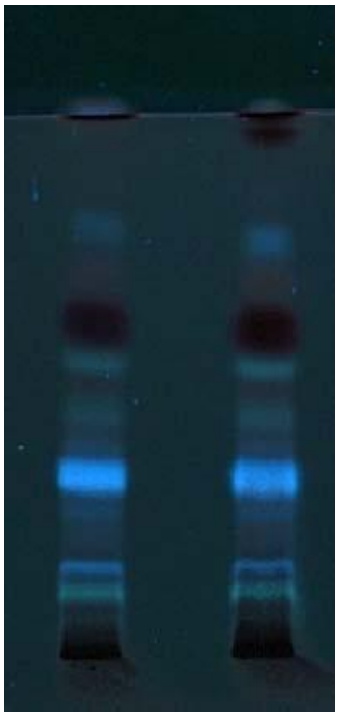

Figure 1: HPTLC fingerprinting of methanolic extract of C. myrrha

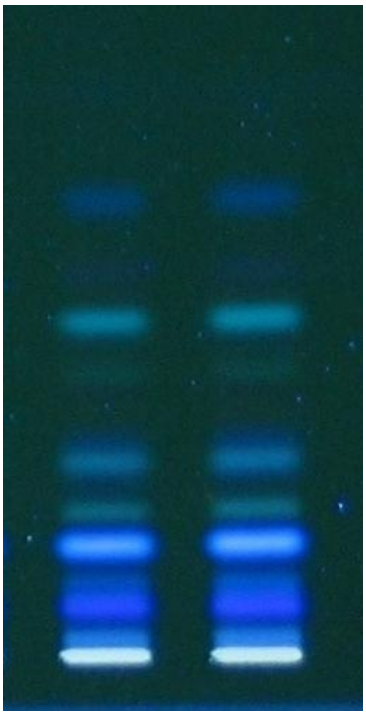

Figure 2: HPTLC fingerprinting of methanolic extract of O. turpethum

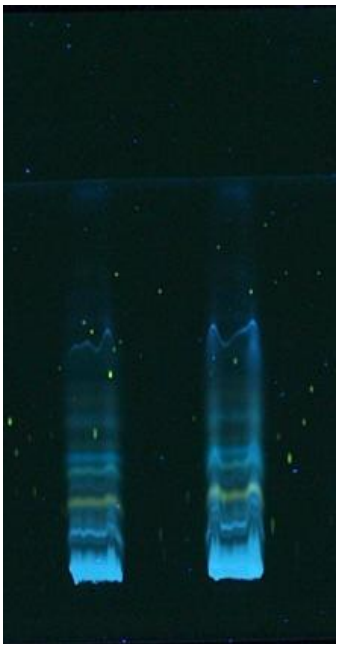

Figure 3: HPTLC fingerprinting of methanolic extract of P. roxburghii

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