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Phytochemical analysis of *Ceropegia juncea* (Roxb.): Traditionally used Medicinal plant

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Abstract:
Natural products had been indispensably used by many cultures and traditions from thousands of years. They are intensively explored for their bioactive pharmacophores by modern pharmaceutical companies. The potential bioactive phytocompounds like alkaloids, flavanoids, phenolic compounds and steroids are potential source for drug discovery. In the present study, an attempt has been made to explore the medicinal value of the plant, Ceropegia juncea (Roxb.) belongs to the family Asclepidaceae. Ceropegia juncea (Roxb.) recently has been claimed to be as Somalata and Sarcostemma acidum voigt is in as Soma plant currently. The plant is used in Somayaga (ritual practiced by sages from ages in India). The plant is used by various tribal communities in Kerala and Tamil Nadu and different other parts of India. The plant Ceropegia juncea (Roxb.) is screened for its phytochemical constituents. From the present study the plant is reported to have Alkaloids, Steroids, Terpenoids, Flavonoids, Saponins, Tanins, Glycosides, Anthocyanins, Anthracene glycosides and Coumarins were identified in the plant sample. The screening of the plant for phyto-chemicals is done by the method TLC and Coloumn chromatography.

1. Introduction

Since time immemorial man has been using herbs to cure common maladies and he virtually adored plants which provided him with vigor, vitality and wisdom. Ancient Science of Life is believed to be prevalent for the last 5000 years in India. Use of medicinal plants as crude drugs dates back to Vedic era. Ayurveda, extract of Atharvaveda is probably the oldest scientific system of medicine in the history of the world. It has been a part of ancient Vedic scripts and must have been practiced for thousands of years, since the Vedic period, about 3,500 years ago (Upadhyay, 1997).

Though thousands of medicinal plants mentioned in Ayurveda, only a few hundred plants are identified and approximately 300 to 600 plants were confirmed (Agarwal and Tivari, 2001). The Rasayana (Rejuvenation) and Vajikarana (Virilization) are the branches of Ayurveda and described the importance of Rasayanas and Vajikarana as health promoters. The rasayanas are the preparations from several plant extracts, which contain strong antioxidants and are used as rejuvenators or nutritional supplements (Sharma *et al.*, 1992; Thyagarajan *et al.*, 2002; Govindarajan *et al.*, 2005). The entire wealth of India is stored in the vast natural flora that has been gifted to her. Plants have been a source for the discovery of novel pharmacologically active compounds with many successful drugs. Traditional knowledge systems have given clues to the discovery of valuable drugs (Buenz *et al.*, 2004). Plant-human interrelation-ships embedded in dynamic ecosystems of natural and social components are denoted as Ethnobotany.

The potential bioactive compounds like alkaloids, flavanoids, and phenolic compounds, steroids are potential source for drug discovery. With the rapid development of Phytochemistry and Pharmacological testing methods in recent years, new plant drugs are finding their way into medicines as purified phytochemicals, rather in the form of traditional galenical preparations. In recent years, WHO has emphasized the importance of scientific investigations into indigenous herbal medicines (WHO, 1978). Different plant species belongs to different families and their phytochemical and biological properties were studied by many researchers. Nowadays traditional medicines and practices form an integral part of complementary and alternative medicine (CAM). Although, their efficacy and mechanisms of action have not been tested scientifically, in most cases, these simple medicinal preparations often mediate beneficial responses through their active chemical constituents.

Several scientific investigations have proved and high-lighted the importance and contributions of many plant families. Among different families studied, *Asclepidaceae*, now called *Asclepiadoideae* a subfamily belongs to the family *Apocyanaceae* having different species of plants with wide therapeutical properties. *Asclepiadoideae* is the largest cosmopolitan family having genera of 177 and nearly 3000 species (Meve, 2002b) and are well known for their ethnobotanical and ethnomedicinal importance (Jadaja, 2004).

Ceropegia juncea (Roxb) is one plant in this family having wide medicinal properties and is being used in different traditional medical systems and by tribal people for curing different ailments. *Ceropegia juncea* (Roxb) was also claimed as one of the *Soma* plant (Alam Muzaffer *et al*., 1982 BMER). Description of *Soma* plant (The plant which is used in the Soma Yaga, a ritual practiced by sages and Vedic pundits from ancient time in India) and its therapeutic properties were mentioned in ancient text called Rigveda and AtharvaVeda (Kocchar, 2001, SudhaKarayil *et al.*2011.). There were 24 plants denoted as Soma plants in the Susrutha Samhita, Padhy and Dash, (2004) reported that all the *Soma* plants shares a common feature having latex and bulb (tuberous roots). All the plants are considered as substitutes of original *Soma* plant having medicinal properties as described in the ancient literature (Kochhar, 2000, 2001; Sudha Karayil *et al*.,2011)

Based on the importance of the medicinal and therapeutical properties through ancient and ethno medicinal literature, the plant Ceropegia juncea (Roxb) was selected for the present study of phytochemicals and their biological activities. There was only limited literature regarding its phytochemicals. In the present study, the plant *Ceropegia juncea* (Roxb.) was screened for its phytochemical compounds by adopting the methods of TLC and HPLC and Column to isolate the compounds.

2. Materials & Methods

2.1. Collection of the plant

The plant selected for the present study was *Ceropegia juncea* (Roxb.) (*Somalata* in Sanskrit, *Moon creeper* or *Moon plant* in English) A divine herb (used in Yaga especially Somayagam ritual performed to please the Almighty for universal welfare practiced by Sages in India from ancient time). The plant is a herb with potential therapeutical properties for various ailments described in RigVeda, AtharvaVeda, and AyurVeda. It has been collected from the Western Ghats. Initially the whole plant was identified and procured from the foot hills of Kollengode, (Sahya Mountain ranges, Western Ghats), Palakkad district, Kerala, India with the help of local tribal people.

The plant *Ceropegia juncea* Roxb. (*Asclepidaceae*) was selected for the present study. The specimen was identified and authenticated by Plant Taxonomist (Natural Remedies, Bangalore with a specimen voucher (S 815) and by perusing through the literature (the Flora of British India by Sir.J.D. Hooker, Ayurvedic Pharmacopea, and Wealth of India and by google search, NCBI Taxonomy.

2.2. Preparation of Plant Material for analysis

The whole plant (without flowers and seeds) was collected and dried under shade for two weeks. The dried plant material was made pulverized by using electrical grinder into fine powder and was passed through 20 mesh sieve to obtain very fine particles and this was used for analysis.

2.3. Screening of Phytocompounds in the plant

Preliminary phytochemical screening for the detection of various phytocompounds was carried out by using standard procedures described by different authors (Heathcote and Hibbert, 1975; Harborne, 1998 and Khandelwal, 2008) with slight modification in optimized conditions. 100gms of Sample powder was extracted in Methanol and Hexane by Soxhlet extractor up to 48 hours. 48 hours continues heating applied for extraction at 67'c for Methanol, 70'c for Hexane. The extracts were used for further phytochemical analysis.

2.4. Extraction by Soxhlet

Use of commercially available Soxhlet was the convenient way to prepare crude extracts from plants. Soxhalet apparatus was used with pure solvents and were based on the polarity. Methanol and Hexane was used as polar and non polar solvents respectively for crude extraction. The extraction was carried up to 48hrs and the solvents were removed by distillation under reduced pressure and subjected to vacuum dry using rotary flash evaporator (Harborne, 1973; 1978). Further the extract was subjected to screening of phytoconstituents.

2.5. Thin Layer Chromatography (TLC)

Screening of phytoconstituents in the plant material was carried out by TLC. (TLC-PLATE

Silica Gel 'MERCK'). Thin Layer Chromatographic technique was used to identify the presence of different phytochemicals such as alkaloids, phenolic compounds, flavaonoids, saponins, steroids, fatty acids, glycosides, carrotenoids, terpenoids, Tannins, Polyuronoids, Chlorogenic acid, Anthocyanin, Antracene glycosides, Coumarins present in the plant material. 5 ml of mobile phase poured in to mobile phase chamber and covered it with a watch glass for saturation of chamber. Here the chamber was saturated with mobile phase vapor. Plant extract and standards are prepared with mobile phase. The phytoconstituents were screened in Methanol and Hexane solvents. The Rf values of cleared spots were calculated. For Methanol extract: Mobile Phase MeOH: Water: Butanol 60:10:30 and the Solvent front is 16.2cms. For Hexane extract: Mobile Phase Water: Butanol: MeOH 35:20:45 and the Solvent front is 14.7 cms. Elution process carried out nearly 23 min. Total procedure carried out at room temperature and pressure. For spot identification subjected to U.V light chamber with 30 cm scale and measured the distance of spots from sample loading point. By calculating the distance of solvent front and distance of sample spots to get Rf value and the phytoconstituents identified were shown in tabulated form.

The compounds in the mixture were separated according to their relative mobilities. Merck Brand commercial analytical TLC plates (0.2mm) thickness were used and was coated with Silica gel 60 F254(0.2mm thickness of silica sorbent). 5µl of 100mg sample/ml solution was loaded on a pre coated Silica gel. The solvent front migrates up to the plate through the sorbent by capillary action and the migrating behaviour of the sample separated was given in the form of Rf values. Compounds were identified and visualized under UV light chamber (254 and 360nm UV lamp) and iodine vapours. The standards taken to run TLC for different compounds were mentioned.

3. Result

- Discription of the Plant: *Ceropegia*
- Family: *Apocynaceae*
- Sub family: *Asclepiadoideae*
- Genus: *Ceropegia* (L)
- Species: *Ceropegia juncea* (Roxb. Cor)

3.1. General characteristic features of the Plant

The plant is a twining perennial herb. Greenish cylindric twinner, leaves are scaly, opposite, lanceolate, acute, absent at the old stem, flowers axillary, corolla tube curved, purple dotted, corolla lobes lanceolate, pollen aggregated in the form of pollenia, fruit elongated follicle, seeds flat with tuft of whitish hairs. Stems succlent fleshy, Roots are tuberous. Godget flower errect, beautiful, light yellow green and purple colour and tuberous roots.

- Flowering Season: July to February.

The plant was known by different names as follows:

- Sanskrit : *Somalata, Somaraji, Soma valli*
- Telugu : *Pullakada, Bella-gada of the Telingas*
- Malayalam : *Somalata, Bhutumbi*
- Tamil : *Pulicha kodi*
- Hindi : *Somalata*

3.2. Distribution

Coast of Coromandel, Western Ghats (Sahya mountain ranges), Kerala, Karnataka, Andhra Pradesh, Tamil Nadu, Madhurai foot hills, Palani foot hills, Maharashtra, Srilanka.

3.3. TLC

Qulaitative analysis of phytocompounds was carried out by (Thin Layer Chromatography) TLC. The plant was screened for its phytoconstituents in Hexane and Methanol solvents. The presence of Alkaloids, Steroids, Terpenoids, Flavonoids, Phenolic Compounds, Carrotenoids, Fatty acids, Saponins, Tannins, Anthocyanins, Anthracene glycosides, Coumarins were identified in TLC analysis. The extract of the crude with methanol showed the presence of Steroids, Terpenoids, Flavonoids, Alkanoids, Fatty acids, Phenolic acids, Carrotinoids, Saponins, Tannins, Anthracene glycosides, Coumarins. The extract of the crude with hexane showed the presence of Steroids, Alkaloids, Carrotinoids, Saponins, Tanins, and Anthocyanins. Polyuronoids and Chlorogenic acids were not identified in the test plant in TLC analysis. By calculating the distance travelled by the solvent front and distance travelled by the sample spots revealed the Rf value. The Rf values of cleared spots were calculated. The phytoconstituents identified were tabulated. The identified phytoconstituents were separated by using coloum chromatography.

3.4. Column Chromatography

3.4.1. Column Conditions

The phytoconstituents (mixture of compounds) found in the plant sample is separated with the help of Column chromatography.

- Length: 1 mtr
- Radius: 5mm diameter
- Stationary Phase: Silica gel 100-200 mesh
- Flow rate: 0.2 ml/min
- Mobile phase: Meoh: But-ol: Water 45:20:35

3.4.2. Column Packing

One meter column was selected for separation of compounds. Two different columns were prepared for Methanol extraction and Hexane Extraction. Column length is 1 meter and inner diameter is 5 mm. Column was packed with Silica gel powder without air gaps. Sample extraction was sprayed on silica powder. Mobile phase: MeOH:But-ol:water 45:20:35. The sample gets absorbed on Silica powder. Neutral alumina is poured on silica gel to control chlorophyll interference. Flow rate was adjusted to 0.2 ml/min and Column was fixed on retard stand and kept constantly.

3.5. Standards for Phytoconstituents

Steroid	Progestrone
Terpenoid	Lanosterol
Flavanoid	Apigenin
Alkaloid	Nicotinic acid
Fati acid	Oleic acid
Phenolic compound	Cresol
Carotinoids	Beta carotinone
Saponins	Solanine
Tanins	Phloroglucinol
Polyuronids	Galacturonic acid
Chlorogenic acid	Chlorogenic acid
Anthocyanin	Cynadine
Anthracene glycoside	Anthra quinine

Table 1

- Phytocompounds in *Ceropegia juncea* (Roxb) in Methanol and Hexane extract

Compound	**Methanol**	**Hexane**
Steroids	P	P
Terpenoid	P	N
Flavoniods	P	N
Alkaloids	P	P
Fatty Acids	P	N
Phenolic Compounds	P	N
Carrotinoids	P	P
Saponins	P	P
Tannins	P	P
Polyuronoids	N	N
Chlorogenic acid	N	N
Anthocyanin	N	P
Anthracene glycosides	P	N

Coumarins	P	N

Table 2: Screening of Ceropegia juncea (Roxb.) for phytochemicals

- Phytoconstituents in *Ceropegia juncea* (Roxb.)
- Mobile phase MeoH, Water 80:20
- Solvent Front 15.8 cm
- METHANOL:

Compound	**Sample traveled distance (std)**	**Sample traveled distance (sample)**	**$R_{f\ std}$**	**$R_{f\ sample}$**
Steroids	12.2	11.7	0.772	0.7405
Terpinoids	1.3	1	0.0822	0.0769
Flavoniods	4.7	4.5	0.297	0.284
Alkaloids	3.1	2.9	0.1962	0.1835
Fatty Acids	9	9.2	0.569	0.5822
Phenolic Compounds	6.5	6.9	0.4113	0.4367
Carrotinoids	11	10.8	0.6962	0.6835

Table 3

- Mobile phase Water, Butanol, MeoH 35:20:45
- Solvent Front 13.6 cm
- HEXANE:

Compound	**Sample traveled distance (std)**	**Sample traveled distance (sample)**	**$R_{f\ std}$**	**$R_{f\ sample}$**
Steroids	8.0	8.1	0.61	0.595
Terpinoids	----------	----------	----------	----------
Flavoniods	----------	----------	----------	----------
Alkaloids	4.7	4.6	0.345	0.338
Fatty Acids	----------	----------	----------	----------
Phenolic Compounds	----------	----------	----------	----------
Carrotenoids	2.1	2.5	0.154	0.183

Table 4

- T.L.C PLATE SILICAGEL 'MERCK'
- MOBILE PHASE MEOH, WATER : Butanol 60:10:30
- Solvent Front 16.2 cm
- METHANOL:

Compound	Sample traveled distance (std)	Sample traveled distance (sample)	$R_{f\ std}$	$R_{f\ sample}$
Saponins	2.3	2.2	0.141	0.135
Tannins	10.55	10.6	0.648	0.654
Polyuronoids	----------	----------	----------	----------
Chlorogenic acid	----------	----------	----------	----------
Anthocyanin	----------	----------	----------	----------
Anthracene glycosides	8.6	8.5	0.53	0.524
Coumarins	5.9	5.8	0.364	0.358

Table 5

- MOBILE PHASE WATER, BUTANO, MeoH 35:20:45
- Solvent front 14.7 cm
- HEXANE:

Compound	Sample traveled distance (std)	Sample traveled distance (sample)	$R_{f\ std}$	$R_{f\ sample}$
Saponins	4.5	4.7	0.306	0.319
Tannins	1.8	1.9	0.122	0.129
Polyuronoids	----------	----------		
Chlorogenic acid	----------	----------		
Anthocyanin	9.3	9.2	0.632	0.625
Anthracene glycosides	----------	----------		
Coumarins	----------	----------	----------	----------

Table 6

4. Discussion

Many drugs are derived from plants only. The naturally occurring compounds provide the starting point for development of analogues. The selection and screening of plant for its therapeutic properties and different biological activities depends on the ethno medicine used for different ailments. Isolation of ethno-pharmacologically and pharmacognostically active phytoconstituents from plants viewed as potential sources of new natural drugs, antibiotics, foods and supplements etc. Having basic information on the medicinal

use of the plant *Ceropegia juncea* (Roxb.) is selected for the study of its phytoconstituents to establish the ethno medicinal information of the plant. From the methanol extract of the sample the compounds of Steroids, Terpenoids, Flavonoids, Fatty acids, Phenolic compounds, Alkaloids, Carotenoids were identified. In hexane extract the compounds of Steroids, Alkaloids, Carotenoids were identified. In the crude extract of the hexane showed the presence of Anthocyanins was identified. Anthracene glycosides and Coumarins were present in methanol extract. Presence of these compounds in the plant showed its therapeutical values. Gupta and Kohli (2010) reported the phytochemicals in *Sarcostemma acidum* (*Asclepiadacea*) one of the *Soma* plant, and these results were in accordance with them. Polyuronoids, Chlorogenic acid, Anthracene glycosides and Coumarins were not traced in Hexane extract. The mixture of these compounds were isolated and separated by using Coloumn chromatography.The separated compounds were subjected to spectral studies. Adibatti, *et al.* (1991) reported new pyridine alkaloid Cerpegin from *Ceropegia juncea*. There were several reports on preliminary screening of phytochemicals in medicinal plants. Sharma paras *et al.*, (2011) reported the phytoconstituents in *Ceropegia juncea* Roxb.and the findings were relevant with the present findings. Phenolic compounds and Coumarins were screened from *Ceropegia juncea*. Coumarins were also reported in tonka beans, seed of *Dipteryx odourata* and *D. oppositifolia*, and were used pharmaceutically as flavouring agents. The Phytocompounds in several plants exhibit wide range of medicinal and therapeutical properties and were reported by many authors. Terpenoids by virtue of their pleasant flavour have tremendous importance in perfumery, cosmetics, soaps, pharmaceutical and food. They exert wide spectrum of activities like antiseptic, stimulant, carminative anti-helmenthic, and analgesic, antirheumatic. Tannins are one of the most widely occurring groups of natural substances in different families of higher plants. They are used as mild antiseptics, to check haemorrhages, Tannins are also used in leather industry. Tannins acts as astringent and are used for treating intestinal disorders (Dharmananda, 2003) and they possess antimicrobial and antioxidant drugs (Riviere *et al.*, 2009; Shokunbi Odetola, 2008). Anthracene glycosides constitute a major class of glycosides. They are mainly puragative and anti-depressant etc. Flavonoids possess numerous pharmacological activities. They are well known for their anti-inflammatory, antioxidant, cytotoxic and reduce in hypertension (Aguinaldo *et al.*, 2005; Wu *et al.*, 2008). Phenolic compounds exhibit anti-oxidative and hepatoprotective properties (Wang *et al.*, 1995). Alkaloids, steroids have anti-inflammatory properties (Chawla *et al.*, 1987). Saponins, anthocyanins have healing properties (Okonkwo& Nnaemeka, 2010). Fatty acids and coumarins are regarded as therapeutically dynamic compounds. They act as anticancer agents, anti-inflammatory, anti-depressant, and anti-oxidative in nature. (Kokate, Pharmacognosy, 39th Edition).

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