Plant Archives
Journal home page: www.plantarchives.org
DOI Url: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.no1.006

PRELIMINARY PHYTOCHEMICAL AND FT-IR ANALYSIS OF METHANOLIC LEAVES EXTRACTS OF *HEMIDESMUS INDICUS* AND *TYLOPHORA INDICA*

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(Date of Receiving-15-09-2020 ; Date of Acceptance-30-11-2020)

ABSTRACT

Medicinal plants are of great importance for the health of individuals and communities. *Hemidesmus indicus* and *Tylophora indica* are used as traditional medicines. These plants contain a lot of bioactive compounds. The present investigation is carried out in *Hemidesmus indicus* and *Tylophora indica* leaves. In the present study, the methanol extract of *H. indicus* and *T. indica* were subjected for preliminary phytochemical analysis through FT-IR spectral analysis and the bioactive compounds were identified. The Preliminary Phytochemical analysis of methanolic leaves extract of *H.indicus* and *T.indica* revealed the presence of secondary metabolites. FT-IR analysis of methanolic leaf extract of *Hemidesmus indicus* and *Tylophora indica* revealed 16 and 13 major compounds respectively. The *H.indicus* has higher bioactive compounds compared to *T.indica.*

Keywords: FT-IR analysis; *Hemidesmus indicus;* Medicinal plants; Phytochemical analysis; *Tylophora indica*

INTRODUCTION

Nature has long been an important source of medicinal plants. Medicinal plants are a good source of economic value in the world. Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. About 20,000 plant species used for medicinal purposes are reported by WHO (Gullece *et al*., 2006). Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional methods practiced, such as Ayurveda, Unani and Siddha. Herbal medicines are still the mainstay of about 75-80 per cent of the whole population mainly in developing countries. Plant and plant products are being used as a source of medicine since long. According to World Health Organization (WHO), more than 80% of the world's population who are mostly in poor and less developed countries depend on traditional plant based medicines for their primary healthcare needs (WHO, 1993).

1.1. *Hemidesmus indicus*

Hemidesmus indicus is one of the important medicinal plants in the world. *H.indicus* belongs to Asclepiadaceae family and is popularly known as Indian Sarasaparilla, anantamool or nannari. It is found all over India growing under mesophytic or semi dry plains to an altitude up to 600 m (Satheesh *et al*., 2008; Austin, 2008). It is aromatic medicinal twinning shrub distributed in moist localities of India and Srilanka. *H.indicus* roots are woody, slender and aromatic in nature. Leaves are simple, petiolate, exstipulate, apiculate, acute or obtuse, dark green above but paler and sometime pubescent below. Flowers may be greenish purple to greenish yellow outside, dull yellow to light purplish inside with calyx deeply five lobed. Fruits are two straight slender, narrowly cylindrical, widely divergent follicles. This plant has many seeds that are flat, oblong, with a long tuft of white silky hairs. In South India it is a traditional and popular herb with a long history of use as a medicine and is one of the most wanted plant species in Ayurveda widely known as Nannari (Koppula and Kumar, 2013). It is a well known indigenous medicine for antioxidant and anti-inflammatory diseases. Most of the tribal people used this plant to treat skin, colorectal and liver cancer. The root decoction of the plant is used to treat in biliousness, respiratory disorders, eye diseases, epileptic fits in children, kidney and urinary disorders, loss of appetite and burning sensation (Boominathan *et al*., 2018a). It is also used in blood purifiers.

The phytochemical analysis of methanolic extracts of *H.indicus* showed the presence (+) of alkaloids, terpenoids, phenolic compounds root, flavonoids, glycosides and saponins (Boominathan *et al.,* 2018b). In plants, an FT-IR technique was used for evaluating the type of organic and inorganic complex. The analysis was carried out on drying and low acting temperature material of different parts of plants (Theivandran *et al.,* 2015).

1.2. *Tylophora indica*

Tylophora indica is a valuable medicinal plant belongs to the family Asclepiadaceae. It is a perennial woody climber, distributed throughout Southern and Eastern part of India in plains, forests and hilly places. It grows widely in plains and hilly areas of India up to an altitude of 1000 m in Bengal, Assam, Orissa and Southern India (Halliwell *et al*., 2002). It gives off numerous long fleshy roots. Stems are slender, twinning, tortuous,

Table 1: Preliminary Phytochemical analysis of methanolic leaves extract of *H.indicus.*

Present (+); Absent (-)

Sl. No.	Chemical compounds	Methanol
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Steroids	+
6	Terpenoids	+
7	Phenols	+
8	Tannins	+
9	Carbohydrates	+
10	Amino acids	+

Table 2: Preliminary Phytochemical analysis of methanolic leaves extract of *T.indica*

Present (+); Absent (-)

Sl. No.	Chemical compounds	Methanol
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Steroids	+
6	Terpenoids	+
7	Phenols	+
8	Tannins	-
9	Carbohydrates	+
10	Amino acids	+

Table 3: FT-IR analysis of methanolic leaf extract of *H.indicus*

Sl. No.	Wave No	Molecular Motion	Functional group	Absorption Intensity
1	3253.480	O-H Bending	Alcohol	Strong
2	2918.780	C-H Stretching	Alkane	Medium
3	2853.818	C-H Stretching	Alkane	Medium
4	2647.723	C-H Stretching	Aldehyde	Medium
5	2475.668	S-H Stretching	Thiol	Weak
6	2320.044	O=C=O Stretching	Carbon dioxide	Strong
7	1730.803	C=O Stretching	Carboxylic acid	Strong
8	1598.191	N-H Bending	Amine	Medium
9	1507.786	C=C Stretching	Aromatic	Weak
10	1439.956	C-H Bending	Alkane	Medium
11	1361.275	O-H Bending	Phenol	Medium
12	1238.208	C-O Stretching	Alkyl aryl ether	Strong
13	1196.625	C-O Stretching	Tertiary alcohol	Strong
14	1032.887	S=O Stretching	Sulfoxide	Strong
15	985.260	C=C Bending	Allene	Strong
16	717.059	C-Cl Stretching	Chloro compound	Strong

terete, reaching 10 -12 feet in length. The leaves are broadly ovate, rounded or cordate. The roots are fairly sweet and subsequently acrid taste, aromatic odour and a brittle fracture (Manish *et al*.). *Tylophora indica* is used for the treatment of asthma bronchitis, whooping cough, inflammation, allergies dermatitis, dysentery, diarrhoea and rheumatic gouty pains. It is used for its antitumor, anti-inflammatory, anti-anaphylactic properties and is also used to treat jaundice in certain parts of India (Chitnis *et al*., 1972 and Bhutani *et al.,* 1987).

T.indica contains certain bioactive compounds like alkaloids, flavonoids, tannins and saponins, (Rao, 1971; Benjamine, 1973). The root and leaves contain 0.2 to 0.46% therapeutically important alkaloids, tylophorine, tylophorinine and tylophorinidine. Major alkaloids tylophorine has immunosuppressive anti inflammatory (Gopalakrishnan *et al.,* 1979), antitumor (Kaur *et al.,* 2012), stimulant of adrenal cortex (Udupu *et al.,* 1991) and anti amoebic (Bhutani *et al.,* 1985) properties. The plant extracts have been reported scientifically for their biological activities. Many phytochemical drugs could protect humans against certain diseases.

MATERIALS AND METHODS

2.1. Plant material

Hemidesmus indicus and *Tylophora indica* leaves were collected from Mannampandal, Mayiladuthurai during the month of March 2018. The aerial parts of the plants were separated, and first washed under running water to remove the soil particles, then washed with distilled water, cut into small pieces, and shade dried. The dried parts were ground to a fine powder using a mixture grinder.

2.2. Preparation of crude extracts

One hundred grams of powdered leaf material was successively extracted with methanol by using

Table 3: FT-IR analysis of methanolic leaf extract of *H.indicus*

Sl. No.	Wave No.	Molecular motion	Functional group	Absorption Intensity
1	3334.542	N-H stretching	Amine	Medium
2	3305.728	C-H Stretching	Alkyne	Strong
3	2923.161	C-H Stretching	Alkane	Medium
4	2875.187	C-H Stretching	Alkane	Medium
5	2321.495	O=C=O Stretching	Carbon dioxide	Strong
6	2096.148	N=C=S stretching	Isothiocyanate	Strong
7	1684.471	C=O Stretching	Primary amide	Strong
8	1609.222	C=C stretching	a-6 unsaturated ketone	Strong
9	1446.579	C-H bending	Alkane	Medium
10	1350.738	S=O stretching	Sulfonate	Strong
11	1240.663	C-O stretching	Alkyl aryl ether	Strong
12	1201.907	C-O stretching	Vinyl ether	Strong
13	1032.868	S=O stretching	Sulfoxide	Strong

Soxhlet apparatus for 8 hours (Vogel, 1978) .The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporation (Heidolph, Germany) under reduced pressure at 40ºC and the crude extracts were kept at 4ºC in refrigerator for antimicrobial assay.

2.3. Phytochemical Analysis:

The methanolic leaves extract of *Hemidesmus indicus* and *Tylophora indica* were used for qualitative phytochemical studies like alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, glycosides, tannins, carbohydrate and amino acids.

2.4. Fourier Transform Infra-Red spectra:

IR spectrum was recorded in spectrophotometer (Thermo scientific NICOLED-iS5). The active principle was mixed with KBr and pellet technique was adopted to record the spectra.

RESULTS AND DISCUSSION

The Preliminary Phytochemical analysis of methanolic leaves extract of *H.indicus* revealed the presence of secondary metabolites: alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, phenols, tannins, carbohydrates and amino acids (Table 1). The Preliminary Phytochemical analysis of methanolic leaves extract of *T.indica* revealed the presence of following secondary metabolites: alkaloids, flavonoids, glycosides, saponins, steroids, terpenoids, phenols, carbohydrates and amino acids (Table 2). All Phytochemicals except tannin were present in the *Tylophora indica* methanol extract. The *Hemidesmus indicus* plant had highest secondary metabolites compared to *T.indica* plant.

The compounds present in the methanolic leaf extract of *Hemidesmus indicus* were identified by FT-IR analysis and presented in Table 3. A total of 16 major compounds were identified. Absorbance and functional groups were interpreted as follows, 3253.48cm^{-1} indicated O-H stretching; 2918.78, 2853.81 2647.72cm^{-1} indicated C-H stretching; 2475.66cm^{-1} indicated S-H stretching; 2320.04cm^{-1} indicated the O=C=O stretching; 1730.80cm^{-1} indicated C=O stretching; 1598.19cm^{-1} indicated N-H bending; 1507.78cm^{-1} indicated C=C stretching; 1439.95cm^{-1} indicated C-H bending; 1361.27cm^{-1} indicated O-H bending; 1238.20, 1196.62cm^{-1} indicated C-O stretching; 1032.88cm^{-1} indicated S=O stretching; 985.26cm-1 indicated C=C bending and 717.05cm^{-1} indicated C-Cl stretching. Some major compounds were alcohol, alkane, aldehyde, thiol, carbon dioxide, carboxylic acid, amine, aromatic, phenol, alkyl aryl ether, tertiary alcohol, sulfoxide, allene and chloro compounds. The FT-IR spectrum of *H.indicus* is shown in (Figure 1).

The compounds present in the methanolic leaf extract of *T.indica* were identified by FT-IR analysis and presented in Table 4. A total of 13 major compounds were identified. Absorbance and functional groups were interpreted as follows; 3334.54cm^{-1} indicated the N-H stretching; 3305.72, 2923.16, 2875.18cm^{-1} indicated the C-H stretching; 2321.49cm^{-1} indicated the O=C=O stretching; 2096.14 cm^{-1} indicated N=C=S stretching; 1684.47cm^{-1} indicated C=O stretching; 1609.22cm^{-1} indicated C=C stretching; 1446.57cm^{-1} indicated C-H bending; 1350.73cm^{-1} indicated S=O stretching; 1240.66, 1201.90cm^{-1} indicated C-O stretching and 1032.86cm^{-1} indicated S=O stretching. Some major compounds were amine, alkyne, alkane, carbon dioxide, isothiocyanate, primary amide, a-6-unsaturated ketone, alkane, sulfonate, alkyl aryl ether, vinyl ether and solfoxide compounds. The FT-IR spectrum of *T.indica* is shown in Figure 2.

The strong antimicrobial activities of the plant can be due to the bioactive compounds present in the roots, the ethanolic extract of root is reported to contain triterpenes, flavonoids, tannins, cumarins and glycosides (Alam *et al.,* 1998). In this study it is clear that methanolic leaves extract contains all chemical compounds. The aqueous ethanolic root extract is also reported to contains alkaloids, saponins, phenols and tannins (Anoop and Jegadeesan, 2003). Similarly Suganya *et al.,* reported the

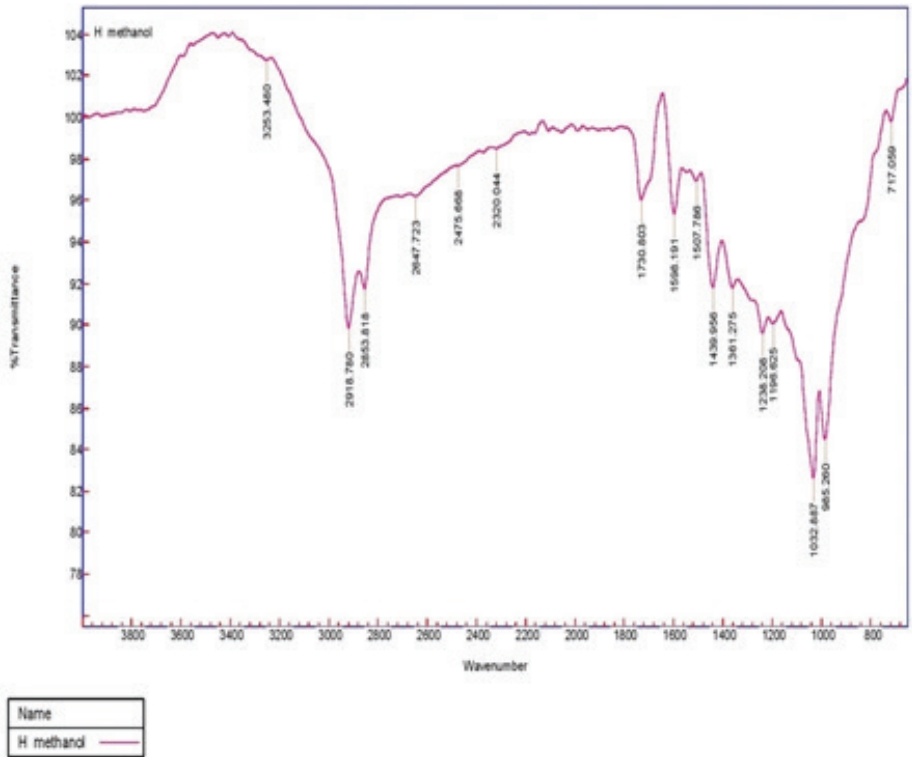

Figure 1: FT-IR Analysis of methanolic leaves extract of *Hemidesmus indicus*

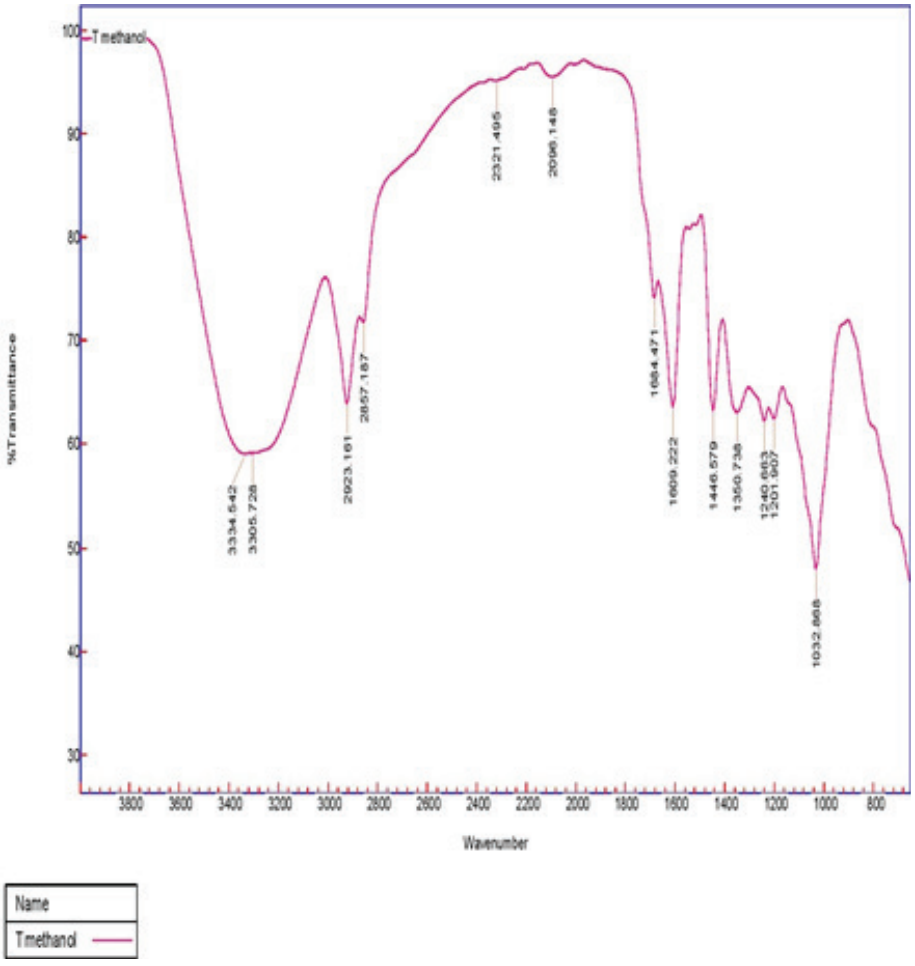

Figure 1: FT-IR Analysis of methanolic leaves extract of *Tylophora indica*

presence of alkaloids, carbohydrates, glycosides, phenolic compounds, tannins, saponins, glycoside protein, amino acids and flavonoids constituents in *H.indicus*. (Suganya Venkatesan *et al.,* 2016). In recent past, FT-IR technique had been performed to analyze the difference in the chemical composition of prokaryotes and eukaryotes (Schmitt and Flemming, 1998). The FT-IR technique was not so common used in phytochemical researches of *H.indicus* and *T.indica*.

The preliminary phytochemical analysis of methanolic leaves extract of *T.indica* revealed the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides, saponins, steroids, phenols, carbohydrate and amino acids. Similarly (Ranemma *et al.,* 2017) reported that alkaloids, flavonoids phenols, saponins, steroids tannins and terpenoids are present in the plant. In this study it is found that all chemical compounds were present in methanolic extract except tannins. These secondary metabolites contribute significantly towards the biological activities of medicinal plants such as hypoglycemia, antidiabetic, antioxidant, antimicrobial, anti inflammatory, anti carcinogenic, anti malarial, anticholinergic and anti leprosy activities (Negi *et al.,* 2011). Through the FT-IR spectrum we can confirm the presence of functional group in the plant part extracts, identify the medicinal materials from the adulterate and can evaluate the quantity of medicinal materials (Kumar and Prasad, 2011). The present results of FT-IR spectroscopic revealed the functional constituent present in the wild and tissue culture plant samples of *T.indica*. It also showed the similarly and variation between these two samples based on the functional group with the help of absorption spectrum. Many researchers used the FT-IR spectrum as a tool for classifying and discriminating closely related plants (Helm *et al.,* 1991; Ellis *et al.,* 2002).

CONCLUSION

In the present study it is concluded that the leaves of *H.indicus* and *T.indica* have numerous medicinal properties and isolation of individual phytochemical constituents may proceed to find out novel drugs. *Hemidesmus indicus* contains all the phytochemicals and *Tylophora indica* contains all except tannins. 16 and 13 compounds were identified by the FT-IR analysis of methanolic leaf extract of *H.indicus* and *T.indica* respectively. The compounds have to isolated and tested separately for antimicrobial activities since the

therapeutic potential of both the plants are high. Overall it is understood that *H.indicus* contains more chemical compounds compared to *T.indica.*

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