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Preliminary Phytochemical Analysis Of Illicium verum and Wedelia chinensis

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Abstract: The importance of plant is well known to us. We cannot imagine life and its growth without plants. Each and everything is depending directly or indirectly, on plants. Besides food, plants are primary source of material for other necessity of life. The traditional medicinal practices are important parts of the primary healthcare system in the developing as well as developed world. The herbal medicines are comparatively safer and cheaper than synthetic drugs. The plant based traditional knowledge has become a recognized tool in search for new sources of drugs and neutraceuticals. Ethnopharmacological knowledge can bring out many different tools for the developmemt of new drugs to treat various human diseases. The mode of action of the plant producing therapeutic effect can also be better investigated if the active principle or characterize. Hence, it is of interest to investigate the phytochemical analysis of acetone and ethyl acetate extract of *Illicium verum* and *Wedelia chinensis*.
Keywords: *Illicium verum, Wedelia chinensis*, acetone, ethyl acetate, TLC.

INTRODUCTION AND EXPERIMENTAL

Despite of tremendous progress in human health care system, the infectious diseases caused by microorganisms are still a major threat to the public health[1]. Nature has provided an important source of remedies to cure all the ointments of mankind. In the recent years, all the medicines used were from the nature source, especially from the plants. Plants contain hundreds or thousands of metabolites. Medicinal and aromatic plants, a gift of the nature, are being used against various infectious diseases in the world since the past history. The discovery, development and the use of modern medicines have a deep routed connection with the age old practice of folk and traditional medicinal background of the natives. Thus the ancient wisdom has been the basis of modern medicine and therapeutics[2].

Illicium is the sole genus in the family of schisandraceae. It comprises of forty two species of evergreen shrubs and small trees. The species of native is from the tropical and subtropical regions of Eastern and South Eastern Asia, South Eastern North America and the West Indies. The most frequently occurring species are *Illicium dunnianum, Illicium griffithii, Illicium verum* and *Illicium anisatum. Illicium verum* is commonly known as star anise or star aniseed or Chinese star anise[3]. It is a spice that closely resembles anise in flavours obtained from the star shaped pericarp of *Illicium verum.* It is a medium sized native evergreen small or medium sized tree of subtropical and temperate regions. *Illicium verum* has been used in a tea as the traditional remedy of rheumatism and the seeds are sometimes chewed after the meals to aid the digestion. The star anise is a major source of chemical compound, such as shikimic acid, a primary precursor in the pharmacological synthesis of anti influenza drugs namely Oseltamivir (Tamiflu). The shikimic acid is produced by most autotrophic organisms and whilst it can be obtained in a commercial quantity elsewhere[4]. There was a temporary shortage of star anise due to its use in the production of Tamiflu. Late in this year a wave was found of making shikimic acid

synthetically. Now the pharmaceutical industries derive some of the new materials, it needs from the fermentation of *E.coli* bacteria.

Wedelia is a flowering plant genus in the sunflower family, asteraceae. They are one of the genera, commonly called creeping-oxeoes. The name of the genus honours the German physician Dr. Georg Wolfgang Wedel. *Wedelia chinensis* (Osbeck) Merr is a procumbent perrineal herb upto 1m in height with stems rooting at the lower nodes; the leaves are simple, opposite, subsessile, linear-oblong, oblanceolate, scabrous with short white hairs; flowers are yellow in tetragonous rayed, axillary are terminal heads; fruits truncate, compressed or tubercled achenes without pappus or with ring of round wounds. The leaves are used for dyeing grey hair and for promoting the growth, the juice is used for tattooing, the color produced being a deindeliable bluish black, the route is pounded and used as a block dyeing with salts of Iron[5]. The leaves are regarded as tonic and alternative use in cough, cephalagia and diseases of the skin, especially alopecia[6]. The *wedelia* species are popularly known as margarids. This is a small herb almost ubiquitious, infusions of leaf or stem or largely used in the treatment of diseases of the respiratory systems as an expectorant or anti tussive. The additional ethnopharmacological uses of *W.chinensis* are analgesic, anti inflammatory, anti rheumatic, anti pyratic and anti anaemic[7]. It was found the use of this herb in the preclinical studies on the antinociceptive effect in the mice[8]. Several ethnopharmacological studies are carried out with plants of the genus *wedelia* for hepatprotection. Among them, *Wedelia calendulacea* and *Wedelia chinensis* provided protection from chemically induced liver injury both *in vitro* and *in vivo*[9,10].

Hence it is of interest to investigate the phytochemical analysis of acetone and ethyl acetate extracts of *Illicium verum* and *Wedelia chinensis.*

Collection of samples

The medicinal plants used for the experiment were leaves of *Illicium verum* and *Wedelia chinensis*. The plant parts were identified by Dr. Shashikala, Central Research Institute Anna Nagar, Chennai and the respective herbariums were submitted there.

Preparation of extracts

500 grams of dried powder of *Illicium verum* and *Wedelia chinensis* was packed in separate round bottom flask for sample extraction using two solvents namely acetone, ethyl acetate. The extraction was conducted with 750 ml of each solvent for a period of 24 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phytochemical analysis

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature[11,12,13].

Test for alkaloids

The extract of the crude dry powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrocholoric acid. The mixture was filtered and the filtrate was divided into three equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the third portion was treated with equal amount of Wagner's reagent respectively. The creamish precipitate, the orange precipitate and brown precipitate, indicated the presence of respective alkaloids[14].

Test for saponins

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration[15].

Test for steroids

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoides and orange colour for flavons[16].

Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxysugar characteristic of cardioids.

Test for Proteins

To 2ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

Test for Amino Acids

To 2ml of sample was added to 2ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

Test for Tri-Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the tri-terpenoids.

Test for Reducing Sugars

To 2 ml of extract 2drops of Molisch's reagent was added and shaken well. 2ml of conc. H_2SO_4 was added on the sides of the test tube. A reddish violet ring appeared at the junction of two layers immediately indicated the presence of carbohydrates.

TLC Analysis

A sample mixture was dissolved in solvent (ethylacetate) and spotted at one end of the silica gel TLC (MERK) plate. The plate was kept in the tank/beaker containing the mobile phase (Chloroform : Methanol – 19:1) in such a way that the end near the sample application should touch the mobile phase. The chromatogram was allowed to run about 30 min. The plate was dried at 30-40°C at hot air oven/RT. The compound was viewed under UV Transilluminator .The R_f value was calculated.

$$Rf = \frac{\text{Distance moved by the solute (b)}}{\text{Distance moved by the solvent (a)}}$$

RESULTS AND DISCUSSION

The history of natural products used in ancient times and folk medicine around the world is the basis for the use of many therapeutic drugs in modern day medicines. Traditionally, the natural plant products have been the source for searching the new drugs by Pharmaceutical companies[17]. Currently 25% of all the modern medicine is directly or indirectly derived from the higher plants. In the drug discovery, the major secondary metabolites terpenoids, alkaloids and phenolics are of potential medicinal in drugs. The secondary metabolites are synthesized by the plant during the development and are time, tissue and organ specific. They can be induced by biotic and abiotic factors. In contrast, the primary metabolites, they are not present in all plant cells and not essential to sustain the growth. The primary functions of secondary metabolite are deterrence against predators and pathogens, attraction and deterrence against pollinators, attraction of symbions and UV protectance[18].

Chromatography is an analytic method that is widely used for separation, isolation, identification and quantification of components in a mixture. Components of the mixture are carried through the stationary phase

by the flow of mobile phase. Separations are based on differences in migration rates among sample components[19]. Thin layer chromatography (TLC) was chosen over other chromatographic methods because it is a simple, quick and inexpensive procedure that can be used for the analysis of mixture. The TLC separation takes place in the open layer with each component having the same total migration time but different migration distances. The plates can be detected depending on the chemical structure of the compounds at the visible light, UV-254 nm and 365 nm or by using spray reagents[20]. The effectiveness of separation depends on the mixture to be separated, the choice of mobile phase and the adsorption layer[21].

The term Retention factor (R_f) that is commonly used to describe the chromatographic behaviour of sample solutes. The R_f value for each substance is the distance it has moved divided by the distance solvent front has moved. Usually, the centre of each spark is the point taken for measurement. The comparison of R_f values makes it possible to research the complex mixtures qualitatively. The extent of the surface of the spot is a measure for the quantity of the material present[21].

The selection of a solvent for application of the sample can be a critical factor in achieving reproducible chromatography with distortion free zones. In general, the application solvent should be a good solvent for the sample and should be as volatile as possible and more non-polar. The solvents chosen during this experiment for the extraction were methanol-water, methanol and methanol-chloroform. Silica-gel was chosen as the stationary phase. Since it is an efficient adsorbent for the TLC separation of most of the medicinal plant extracts and plant drug extracts[20].

In the present study we are investigating the phytochemical analysis of *Illicium verum* and *Wedelia chinensis.* In the table 1 shows the phytoconstituents of the acetonic extract of *Iliicium verum.* It shows that the presence of flavonoids, alkaloids, triterpenoids, tannins, steroids and cardiac glycosides. But the same extract shows saponins, antraquinones, reducing sugars, proteins and amino acids negative result. In case of ethyl acetate extract of *Illicium verum* shows the positive result for alkaloids, tannins and steroids whereas flavonoids, triterpenoids, saponins, reducing sugars, amino acids, anthraquinones, proteins nad cardiac glycosides indicates the negative result.

In table 2 shows that the phytochemical screening of the acetonic and ethyl acetate extract of *Wedelia chinensis*. Here the acetonic extract of *W. Chinensis* shows that positive result for flavonoids, alkaloids, saponins and tannins and negative result for triterpenoids, reducing sugars, amino acids, anthraquinones, steroids, proteins and cardiac glycosides. The ethyl acetate extract of *W. Chinensis* shows that steroids, tannins and alkaloids positive and flavonoids, triterpenoids, saponins, reducing sugars, amino acids, anthraquinones, proteins, cardiac glycosides implies the negative result.

The TLC plate (Fig 1) confirms the presence of the phytochemicals in the *Illlicium verum* and *Wedelia chinensis.* The R_f values for *I. verum* (Table 3) are 0.17cm, 0.26cm. 0.96cm and 1.0cm which confirm the result of preliminary phytochemical analysis. In case of *W. chinensis* the R_f values (Table 4) are 0.08cm and 1.0cm which also support the phytochemical results.

Table 1. The phytochemical constituents of acetonic and ethyl acetate extract of *Illicium verum*

Sl No.	Phytoconstituents	Acetone extract of *I. Verum*	Ethyl acetate extract of *I. Verum*
1	Flavonoids	++	--
2	Alkaloids	++	++
3	Tri-terpenoids	++	--
4	Saponins	--	--
5	Tanins	++	++
6	Reducing sugars	--	--
7	Amino acids	--	--
8	Anthraquinones	--	--
9	Steroids	++	++
10	Proteins	--	--
11	Cardiac glycosides	++	--

Positive: ++ Negative: --

Table 2. The phytochemical constituents of acetonic and ethyl acetate extract of *Wedelia chinensis*

Sl No.	Phytoconstituents	Acetone extract of *W.chinensis*	Ethyl acetate extract of *W.chinensis*
1	Flavonoids	++	--
2	Alkaloids	++	++
3	Tri-terpenoids	--	--
4	Saponins	++	--
5	Tanins	++	++
6	Reducing sugars	--	--
7	Amino acids	--	--
8	Anthraquinones	--	--
9	Steroids	--	++
10	Proteins	--	--
11	Cardiac glycosides	--	--

Positive: ++ Negative: --

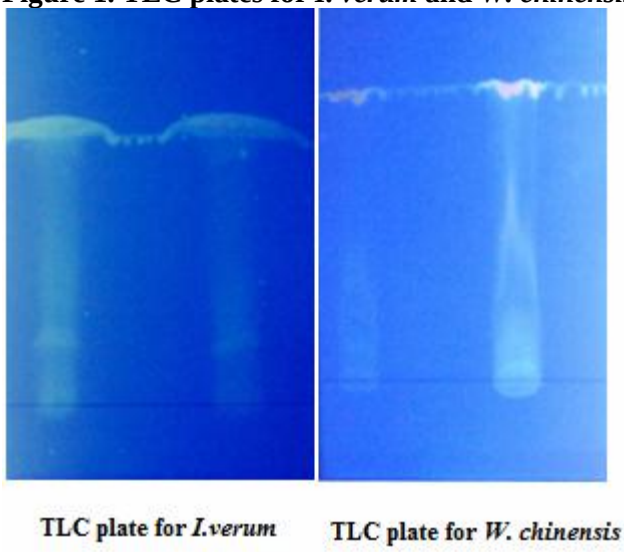
Table 3. R_f values for *I. verum*

Sample travelling (cm)	Solvent front(cm)	Rf value
1.0	5.8	0.17
1.5	5.8	0.26
5.6	5.8	0.96
5.8	5.8	1

Table 4. R_f values for *W.chinensis*

Sample travelling (cm)	Solvent front(cm)	Rf value
0.5	5.9	0.08
5.9	5.9	1

Figure 1. TLC plates for *I. verum* and *W. chinensis*



TLC plate for *I.verum* TLC plate for *W. chinensis*

CONCLUSION

Result of the study indicates that the *Illicium verum* and *Wedelia chinensis* are most useful medicinal plants which is containing various secondary metabolites. The plant shows the better result in the acetonic extract than ethyl acetate extract and the thin layer chromatography result also support the preliminary phytochemical analysis. Therefore these plants are can be used as useful drugs. In future, the structure of bioactive compound and the antibacterial activities can be investigated.

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