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PHYTOCHEMICAL SCREENING ETHANOL EXTRACT OF LEAVES IN *CAPPARIS ZEYLANICA* AND *WRIGHTIA TINCTORIA*

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ABSTRACT

Medicinal plants contain phytochemical compounds that are very useful as medicine in control of various diseases and disorders. In the present studies, ***Wrightia Tinctoria and Capparis Zeylanica,*** leaves extracts are tested for phytochemical, biochemical and GC-MS studies. Ethanolic extract shown good number of compounds compared to methanolic, ethyl acetate, aqueous and chloroform extracts. Phytochemical analysis of ethanolic leaves extract of ***Wrightia Tinctoria and Capparis Zeylanica,*** has shown the biological compounds like carbohydrates, cholesterol, Amino acids, Steroids, Alkaloids, Flavonoids, Cardiac glycosides, Saponins, Tannins, Terpenoids, Fattyacids, and Phenols. It was further to identify 10 different compounds of medicinal use in the ***Wrightia Tinctoria and Capparis Zeylanica,*** by GC-MS analysis. The result suggests that the phytochemicals present in ***Wrightia Tinctoria and Capparis Zeylanica,*** leaves extracts may show antimicrobial, anti-inflammatory and antioxidant properties.

Keywords: *Wrightia Tinctoria, Capparis Zeylanica, Phytochemical, Solvent extraction, Biochemical studies, GC-MS.*

1. INTRODUCTION

The developing countries mostly depend on traditional plants focusing towards healthcare applications. The traditional medicine involves the use of different plant extracts or the bioactive constitutions [2]. This study such as ethano medicine keenly represents one of the best avenues in searching new economic plants for medicine. Qualitative phytochemical analysis of *Momordica charantia* and *Nerium oleander* leaf extracts confirms the presence of various phytochemicals like carbohydrates, cholesterol, protein, aminoacid, alkaloids, flavinoids, tannins, saponins, cardiac glycosides, terpenoids, coumerins, anthocyanins, steroids, fatty acids, phlabotanins ,phenols and starch in their aqueous leaves extracts followed by ethanol, methanol, ethyl acetate and chloroform[4]. The results suggests that the phytochemical properties of the leaves curing various ailments and possess essential antioxidant and anti-cancer properties [5]. In keeping in this view, the present investigation is carried out in *Wrightia Tinctoria and Capparis Zeylanica*.

The world depends upon the herbal medicine for the largest source of the plant biodiversity still about 70% to 80% of world population which are being used since the ages as the traditional health care systems [6]. The medicinal plants contain bioactive phytochemical constituents that produce definite physiological effects on human body [7]. The natural compounds of various plants containing phytochemicals protect the human body from various diseases [8]. Generally the phytochemicals are protective property but non-nutritive plant chemicals which are divided into two groups that is primary and secondary metabolites according to their functions in plant metabolism [9].

Primary metabolites consist of carbohydrates, amino acids, proteins, chlorophyll while secondary metabolite consists of alkaloids, saponins, flavonoids etc. [10]. Plants always contains common source of medicaments, in traditional preparations or as pure active principles. Hence to identify plants or plant extracts that could be used to the drugs, or that could replace some pharmaceutical preparations need to be purchased and imported [11]. The Current research in drug discovery from medicinal plants involves a multifaceted approach combining the phytochemical, botanical, biological, and molecular techniques. The medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including inflammation, cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural products of plant origin recently introduced to the United States market, including art ether, galantamine, nitisinone, and tiotropium are currently involved in late-phase clinical trials. The drug discovery from medicinal plants to provide an source of new drug leads and numerous challenges are encountered including the procurement of plant parts, the selection and implementation of bioassays, and the scale-up of active compounds[12]. The Ethno pharmacologists, botanists, microbiologists, biochemists and natural-products chemists are combining the phytochemicals which could be developed for treatment of infectious diseases. Nearly 30% to 50% of current pharmaceuticals are derived from plants are used as antimicrobials. The traditional healers have long used plants to prevent or cure infectious conditions. The Plants rich in a wide variety of secondary metabolites like saponins, phenols, tannins, terpenoids, alkaloids, and flavonoids found *in vitro* show antimicrobial properties [13].

An effective collaboration between the traditional and western medical practitioners are due to the use of traditional and herbal medicines. Various nonscientific basis with renewed interest from western countries in herbal remedies can provide urgent need to develop new effective drugs from traditionally used medicinal plants. Recently phyto medicinal components received the attention of the pharmaceutical and scientific communities. It involves the isolation, differentiation and identification of the secondary

metabolites produced by the plants and used as the active principles in medical preparations [14]. Higher plants are solar-powered biochemical factories which manufacture their requirements to survive (both primary and secondary metabolites) from air, water, minerals, and energy from sunlight. Many species of higher plants synthesize and accumulate organic substances in quantities to be economically useful as chemical feed stocks or as raw materials for various scientific and commercial applications. The natural substances are applied, directly or indirectly, by a huge number of industries, and natural plant products. For example, phytochemicals are utilized to a large extent by the pharmaceutical, cosmetics, food and agrochemical industries. Economically important plants serve as irreplaceable sources of industrial oils (both volatile and fixed), flavors and fragrances, resins (e.g., rosin and tall oil), gums, natural rubber, waxes, saponins and other surfactants, dyes, pharmaceuticals, pesticides (e.g., insecticides and rodenticides), and many specialty products [15].The present investigation was carried out to determine the phyto-biochemical and to identify the chemical components by GC-MS analysis.

2. MATERIALS AND METHODS

Plant Materials: The leaves of *Wrightia Tinctoria and Capparis Zeylanica* are collected from Villupuram and Karur districts, Tamil Nadu. The leaves were washed thoroughly and dried in sunlight.

2.1 Extraction of Plant Materials

Nearly 30g of air dried powder were taken in 200ml of aqueous, methanol, ethanol, ethyl acetate and chloroform separately, plugged with cotton wool and then kept on orbital shaker for 48 hours with 150rpm at room temperature. The extracts were filtered with whatmann no 1 filter paper and collect the supernatant. Then solvent evaporated through rotavapour and make the final volume one-fourth of the original volume and stored at the 4^{o}C in air tight containers.

2.2 Preliminary Phytochemical Screening

The various extracts were used for preliminary screening for phytochemicals such as carbohydrates (molisch's test), cholesterol (libermanburchard test), protein (biuret test), amino acid (ninhydrin test), alkaloid (wagner and dragendroff's test), saponins, tannins, flavinoids, cardiac glycosides, terpenoids and phlobatanins.

2.3 Screening Procedures

Test for Carbohydrates: To 2ml 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 colour ring appeared at the junction of two layers immediately indicated the presence of observed.

Test for Steroids: To 2ml of acetic anhydride was added to 0.5gm of ethanolic extract added to 2ml of H_2S0_4. The colour change from violet to blue or green indicated the presence of steroids.

Test for Cholesterol: To 2ml of the extract 2ml of the chloroform was added in a dry test tube. Then 10 drop of acetic anhydride and 2 to 3 drops of conc H_2So_4 was added. A red rose colour changed to blue green colour.

Test for Proteins: To 2ml of the extract add the 2ml of biuret reagent. A violet colour ring indicated the presence of peptide linkages of the molecule.

Test for Amino Acids: To 2ml of the extract added the 2ml of ninhydrin reagent and kept in hot water bath for 20 minutes. Appearance of purple colour indicated the presence of amino acids in the sample.

Test for Alkaloids: To the extract added the 1%Hcl and 6drops of mayer's reagent and dragendroff's reagent. An organic precipitate indicated the presence of alkaloids in the sample.

Test for Flavonoids: 5ml of dilute ammonia solution were added to a portion of aqueous extract and add concH_2So_4. A yellow coloration is observed which confirms the presence of flovonoids and it disappears on standing.

Test for Terpenoids: To 2ml of extract add 2ml of chloroform and 3ml of conc H_2So_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

Test for Cardiac Glycosides: 5ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concH_2So_4. A brown ring of the interface indicated a deoxy sugar characteristic of cardenolides. A violet ring might appear below the brown ring whereas the acetic acid layer, a greenish ring might form just gradually throughout thin layer.

Test for Tannins: 5ml of extract was added to few drops of 1% of lead acetate. A yellow precipitate indicated the presence of tannins.

Test for Saponins: The extract with 20ml of distilled water agitated in a graduated cylinder for 15minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for Phlobatinins: When an aqueous extract was boiled with 1%aqueous HCl, red precipitate was deposited which was taken as evidence for the presence of phlobatinins.

Test for Fatty Acids: 0.5ml of extract was mixed with 5ml of ether. These extract was allow it for evaporation on filter paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids.

Test for Anthocyanins: 2ml of aqueous extract is added to 2ml of 2NHCl and ammonia. The appearance of pink-red turns blue violet indicates the presence of anthocyanins.

Test for Leucoanthocyanins 5ml of aqueous exctract added to 5ml of isoamyl alcohol. Upper layer appears red in colour indicates for the presence of leucoanthocyanins.

Test for Phenols: Take 2ml of extract to add 3ml of ethanol and a pinch of $FeCl_3$ to form greenish yellow colour indicates the presence of phenols

2.4. Gas Chromatography-Mass Spectrometry Analysis

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the etanolic extract of leaves was performed using aclarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column. Elite wax (Polyethylene glycol) wasthe polar column used in the estimation. An inert gas such as Hydrogen or Nitrogen or Helium was used as a carrier gas at a flow rate 1 ml/min, split 10:1. The test sample was evaporated in the injection port of the GC equipment and segregated in the column by adsorption and desorption technique with suitable temperature programmes which is controlled by software.

Different components were eluted based on the boiling point of the individual components [17]. The GC column was heated in the oven between 110 ºC to 280 ºC. The time at which each component eluted from the GC column is termed as retention time (RT). The total GC running time was 36 min. The eluted component was detected in the mass detector. The spectrum of the known components stored in the NIST library ascertained the name, molecular weight and structure of the components of the test material in GC-MS study. Identification of components was based on comparison of their mass spectra with NIST Libraries as well as on comparison of their retention indices with literature [18].

3. RESULTS

The present study carried out on the plant leavess that revealed the presence of medicinally active metabolites. The phytochemical characters of *Wrightia Tinctoria*, *Capparis Zeylanica* leaves extracts are showed in table.1The aqueous extract of this plant contains metabolites like carbohydrates, aminoacids, alkaloids, cardiac glycosides, tannins, phlobatinins, fattyacids and coumarins.

Table1. Phytochemical screening of *Wrightia Tinctoria, Capparis Zeylanica* leaves

Name of the compound	Ethanol extract	Methanol extract	Ethyl acetate extract	Chloroform extract	Aqueous extract
Carbohydrates	+	+	-	+	+
Cholesterol	+	+	-	+	-
Protein	-	-	-	-	+
Amino acids	+	+	+	+	+
Steroids	+	+	-	-	-
Alkaloids	+	+	-	+	+
Flavonoids	+	-	-	-	-
Cardiac glycosides	+	+	+	+	+
Saponins	+	-	-	+	-
Tannins	+	+	+	+	+
Terpenoids	+	+	-	-	-
Phlobatinins	+	+	-	-	+
Fatty acids	+	+	+	+	-
Anthocyanins	-	-	-	-	-
Leucoanthocyanins	-	-	-	-	-
Coumarins	+	+	-	-	+
Phenols	+	+	-	-	+
Quinones	-	-	-	-	-
Emodins	-	-	-	-	-

When compared with tested extracts, ethanolic extract found to be containing more bio- and phyto-compounds like carbohydrates, cholesterol, aminoacids, steroids, alkaloids, cardiac glycosides, saponins, tannins, terpenoids, phlobatanins, fatty acids, coumarins and phenols. The methanolic extract shown the various metabolites like carbohydrates, steroids, aminoacids, steroids, alkaloids, cardiac glycosides, tannins, terpenoids, phlobatanins, fattyacids, and phenols. The ethyl acetate had shown very less metabolites in their extract like amino acids, cardiac glycosides, tannins and fatty acids. The choloroform extract found to be containing carbohydrates, cholesterol, aminoacids, alkaloids, cardiac glycosides, tannins, saponins and fatty acids.

The GC-MS combines the gas chromatography and mass spectrometry techniques. The result carried out by this technique revealed that the each peak represents the different compounds present in the ethanolic extract of plant (figure 1).

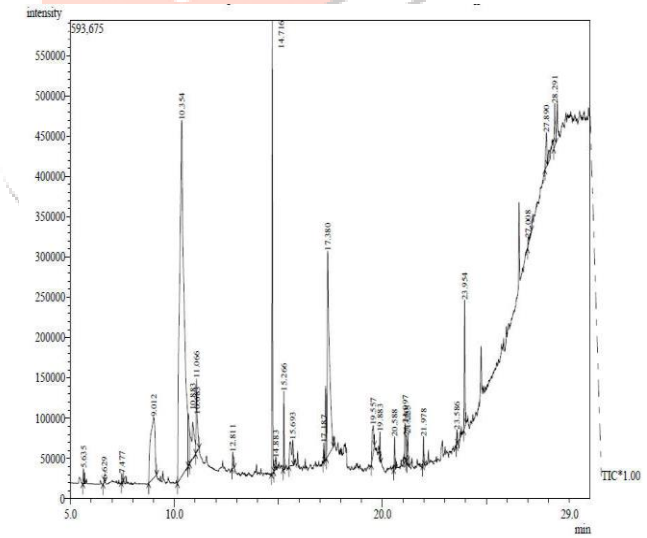

Figure 1. Chromatogram of *Wrightia Tinctoria and Capparis Zeylanica*.

The compounds are separated according to its RT value. Following table gives the list of compounds identified by GC-MS study. GC-MS analysis showed the presence of 10(table 2) different compounds of pharmacological value.

Table 2. List of compounds present in *Wrightia Tinctoria and Capparis Zeylanica* plant carried out by GC-MS analysis

S. No.	RT	Compounds	Formula	M.W
1.	10.88	Propoxur-M	C27H30 Cl3NO5	554.8
2.	11.06	Benzofuran	C8H6O	118.13
3.	14.71	Caryophyllene	C15H24	204.35
4.	14.88	Norpinene	C15H24	204.35
5.	15.69	Cycloheptasiloxane	O7Si7	308.59
6.	20.58	4-Hexen-1-ol	C6H12 O	100.15
7.	21.09	OctadecylTrimethyls	C27H50 ClNO3S	504.2
8.	21.97	Benzenepropanoic acid	C9H10 O3	166.17
9.	27.89	9-Octadecenoic acid	C18H34 O2	282.46
10.	28.29 2	Bromopropionic acid	C3H5BrO2	C3H5BrO2

These compounds are separated based on their Retention time.

4. DISCUSSION AND CONCLUSION

The phytochemical screening and qualitative estimation of the plants studied showed that the leaves were rich in aminoacids, cardiac glycosides, and fatty acids in all the extracts. Methanolic and ethanolic extracts shown some common metabolites like carbohydrates, aminoacids, cholesterol, steroids, alkaloids, cardiac glycosides, tannins, terpenoids, phlobatanins, fatty acis and phenols. The presence of cardiac glycosides in medicinal plants is used in the Indian medicinal system [16, 17]. The plant studies can be seen as potential source of useful drugs and GC-MS analysis showed the presence of 10 different compounds.. Further studies are going on *Wrightia Tinctoria and Capparis Zeylanica are* leaves extracts to identify benefits and uses in the field of medicine.

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