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Research Article

Pharmacognostical and Phytochemical Investigations of *Harpullia arborea* (Blanco) Radlk. – A Sapindaceae Member

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ABSTRACT
Harpullia arborea is an important tree species with lots of medicinal importance and it belonging to the family Sapindaceae. It is commonly called as Soap Nut family which is found in most of the hilly regions in India. Each and every part of the plant is used traditionally in various ailments. The secondary metabolites present in *Harpullia arborea* were found to be alkaloids, flavanoids, glycosides, phenols, tannins, steroids, tri-terpenoids and resins. Gas chromatography (GC) is recognized as the most suitable technique to find out how many components and in what proportion there are in a complex mixture of volatile compounds. Isolation of individual components would however, help to find new drug.

Keywords: *Harpullia arborea*, physico-chemical, phytochemical, GC-MS.

INTRODUCTION

In modern years the uses of medicinal plants in treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. Many aromatic plants have been used traditionally in folk medicine as well as to extend the self life of foods, showing inhibition against bacteria, fungi and yeast[1]. Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been to possess an interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavours and fragrances[2].

Indian herbal drug industries generally face the problem of adulteration and substitution. There is an increase in domestic demand for raw materials used for perfumeries, pharmaceutical and biopesticidal units[3,4] In many instances research on crude drugs indicate that both macro and microscopic characters often help in correct identification of the drug. Several earlier workers have adopted macroscopical features as one of the effective parameters for the pharmacognostical identification of several plant derived crude drugs[5].

Standardization of herbal medicines and quality control of the plant raw materials are very important aspects of manufacture and supply of herbal drugs. Therefore the macroscopic characterization is used to establish pharmacognostic profile, which will help in crude drug identification as well as in standardization of the quality and purity. Column chromatography in chemistry is a method of process by which individual components of a mixture can be separated. For Column packing dry packing and the slurry method was generally followed. The slurry method normally achieves the best packing results, but there are several occasions when the dry packing method works just as well if not better. The slurry method is often used for macro scale separations[6].

Harpullia arborea (Blanco) Radlk. is one of the important genera of the family Sapindaceae distributed all over the India. The plant has got strong ethno-pharmacological background like useful in the treatment of inflammation, cardiovascular diseases, brain cancer, urinary infection etc. Although the plant is used in Ayurvedic medicine for the treatment of ailments there are no reports on the constituents that are responsible for the therapeutic effect. With this background the present study was aimed to identify the phytoconstituents present in *Harpullia arborea* using GC-MS analysis.

MATERIALS AND METHODS

Collection and preparation of plant material

Fresh leaves of *Harpullia arborea* were collected from, ABS Botanical Gardens, Kaaripatti, Salem. The plants were collected in their flowering and fruiting seasons from the natural habitat (Plate 1). While collecting the study plant, a thorough observation was made regarding the location, natural habitat, distribution pattern, habit, floral and fruit characteristics etc. The collected study plant was identified with the help of the existing Floras[7,8,9,10] and compared with type specimens available in the herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu.

Pharmacognostical studies

Macroscopical analysis

Macroscopic characters of *Harpullia arborea* were studied. The morphological characters of the stem and leaf

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Plate 1: The fruiting twig of *Harpullia arborea.*

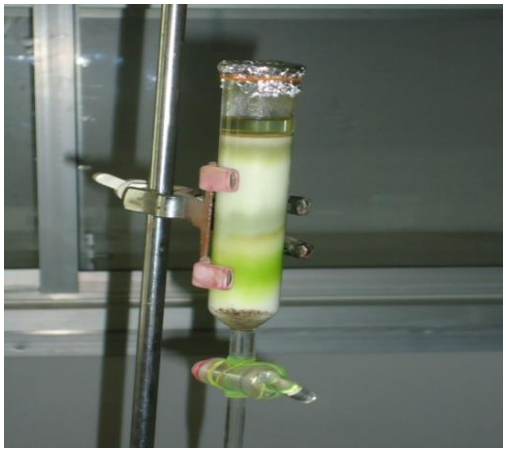


Plate 2: Pigments separation, Gas Chromatography – Mass Spectrometry (GC MS) studies.

Table 1: Macroscopic analysis of *Harpullia arborea.*

S.No.		Macroscopic characters
1.	Stem:	Strong with secondary thickening Height: Up to 20 m in height Surface: Made up of bark Wood: White, soft Texture: Smooth bark Taste: Bitter Odour: Characteristic Colour: Greenish than become light brown
	Leaves:	Alternate, 10 to 30 cm long, pari-pinnate, exstipulate
	Leaflets:	Lanceolate, entire, acute, apex pointed and the base blunt and oblique, entire. Number of leaflets: 3-6 pairs of membranous
2.		Size: 3-8 cm in long, about 5 cm wide Surface: Quite glabrous except on the veins underneath Texture: Smooth Taste: Bitter Odour: Characteristic Colour: Dark green
3.	Flowers:	Small, greenish-yellow Inflorescence: Cymose panicles
4.	Fruit:	Brilliant orange scarlet inflated, bilobed capsule
	Size:	About 1.5 cm long and 2.5 cm wide
5.	Seeds:	Black, sub-globose

such as colour, surface texture, taste and odour were examined[11,12]

Shade drying and powdering of the collected plant material

Freshly collected leaves were cleaned to remove adhering dust and then shade dried. The shade dried plant materials were mechanically ground to coarse powder and passed through a Willy Mill to get 60-Mesh size and used for physicochemical, phytochemical and fluorescence analysis. Samples were stored in the good grade plastic containers which are maintained at room temperature until analysis[13]

Physico-chemical studies

Organoleptic characters of plant powder and the extract

The organoleptic evaluation of aerial plant powder and the extracts, such as colour, texture, odour and taste were carried out[11]

Behaviour of plant powder with different chemical reagents

The behaviour of powdered plant material treated with different chemical reagents such as concentrated HCl, concentrated H_2SO_4, acetic acid and ethanol was observed[14].

Qualitative phytochemical analysis

Phytochemical screening of different successive solvent extracts was carried out following the methods[13,15] Alkaloids, flavonoids, glycosides, phenols and tannins, saponins, steroids and terpenoids were qualitatively analyzed.

Column chromatography

Ethanolic leaf extract of Harpullia arborea (Cold extraction)

Ten grams of powdered leaves of *Harpullia arborea* were soaked within a glass percolator with ethanol and water (70:30) and allowed to stand at room temperature overnight for soaking so that alkaloids, terpenoids and other constituents if present will get dissolved. Then the next day percolate was collected. The extract was filtered, concentrated at 45°C under vacuum and then dried for future use. Then the ground liquefied plant material (*Harpullia arborea*) was placed in a 2 mL centrifuge tube for centrification (500-1000 rpm for 5 min.). Latter form pellet of plant solids plus liquid on top (supernatant). Then transfered the supernatant in a clean empty microcentrifuge tubes. This extract was preserved in a sealed sample tube and stored under refrigeration until analysis.

Column Packing

Stationary phase: Fifteen gram of Alumina

Solvent: Ethanol

Table 2: Organoleptic characters of plant powder of *Harpullia arborea.*

S.No.	Characters	Observations
1.	Colour	Greenish yellow
2.	Texture	Fine smooth powder
3.	Taste	Bitter
4.	Odour	Characteristic smell

Table 3: Organoleptic characters of plant extract of *Harpullia arborea*

S. No.	Extraction Medium (Cold Extraction)	Colour	Consistency	Odour
1.	Ethanol	Blackish green	Semi solid	Characteristic smell

Table 4: Behaviour of plant powder of *Harpullia arborea* with different chemical reagents

Powder + Reagents used	Colour of the powder
Powder as such	Pale green
Powder + Concentrated HCl	Dark green
Powder + Concentrated H_2SO_4	Greenish brown
Powder + Acetic acid	Greenish yellow
Powder + Ethanol	Blackish green

Table 5: Qualitative phytochemical screening of ethanolic leaf extract of *Harpullia arborea.*

Chemical constituents	Chemical tests	Ethanol
Alkaloids	Dragendorff's reagent	-
	Mayer's reagent	-
	Wagner's reagent	-
	Alkaline reagent test	-
Flavonoids	Zinc hydrochloride test	-
Glycosides	Borntrager's test	+
Phenols and tannins	Ferric chloride test	-
Saponins	Foam test	+
Steroids	Foam test	+
Triterpenoids	Libermann-Burchard test	+
Resins	Carbon tetrachloride test	+

Column chromatography was performed on a classic 20 cm long × 2 cm diameter glass column packed with 90 g alumina (slurry method) (Aluminiumoxid 60 HF_{254} basics, type E; Merck, Darmstadt, Germany) between two sterile cotton swabs and the excess of solvent mixture was allowed to run through the column (Plate – 2). The already prepared extract was drizzled gently on top along the side of the column so that the bed was not disturbed. After sometime, the filtrate is poured in to the column and pigments were run along with solvent in descending direction. Depending upon the differential stability the pigments was separated at different levels. Then the stopcock is opened and allowed the sample to flow into the bed. Finally 10-drops of sample were collected in each 1.5 mL of plastic microcentrifuge tubes.

GC MS conditions

The crude fractionated extract was subjected to GC-MS analysis on the instrument-THERMO MS DSQ II–TR, 5-MS capillary standard non - polar column and the GC-MS trace ultra version 5.0 software employing the following conditions: RT x 5 MS column (30 x 0.25 mm ID x 1 μM df, composed of 100% Dimethyl poly diloxane). Initially oven temperature was maintained at 70ºC for 2 minutes, and the temperature was gradually increased upto 250ºC at 10 and 1 μL of sample was injected for analysis. Helium gas 99.995% of purity was used as a carrier gas as well as a eluent. The flow rate of helium gas was set to 1 mL/min. The sample injector temperature was maintained at 250ºC and the split ratio is 10 throughout the experiment periods. The ionization mass spectroscopic analysis was done with 70 eV. The mass spectrum was recorded for the mass range 40-1000 m/z for about 35 minutes.

Identification of components was based on comparison of their mass spectra. As the compounds separated, on elustion through the column, were detected in electronic signals. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization detector where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments were actually charged ions with a certain mass. The m/z ratio obtained was calibrated from the graph obtained which was called as the mass spectrum graph which is the fingerprint of the molecule (Plate-4). The identification of compounds was based on the comparisons of their mass spectra with NIST Library 2008 WILEY8, FAME. Total GC running time is 37.51 min[16]

Mass spectrum of individual unknown compound was compared with the known compounds stored in the software database Libraries. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Macroscopical studies

The present macroscopical investigations of *Harpullia arborea* revealed that the species is a moderate sized tree with hard stem and smooth bark and it is hard, grows up to a height of 20 m (Plate 1). The leaves are alternate and pari-pinnate. Leaflets are alternate, lanceolate, acute, base blunt and oblique with glabrous and smooth texture. The size of the leaflet is 3-8 cm in long and about 5 cm wide. The stem and leaves showed characteristic odour and bitter taste. Fruits are brilliant orange scarlet inflated and bilobed capsule (Table 1).

Organoleptic characters of plant powder and the plant extract

Table 6: Phytocomponents identified from the ethanolic leaf extract of *Harpullia arborea* by GC-MS analysis

S. No.	Rt (min)	Name of the compound	Molecular formula	Molecular weight	Peak area %
1.	5.57	Hippuryl-L-histidyl-L-leucine	C21H27N5O5	429	2.05
2.	6.05	2-Benzoyl-8-octanelactam	C15H19NO2	245	1.86
3.	7.01	Hexadecanoic acid, phenylmethyl ester	C23H38O2	346	1.93
4.	7.67	Aspartame	C14H18N2O5	294	1.93
5.	7.97	Dodecane (CAS)	C12H26	170	1.93
6.	9.46	Cyclohexasiloxane, dodecamethyl - (CAS)	C12H36O6Si6	444	1.65
7.	11.11	Cyclohexanecarboxylic acid, hexyl ester	C13H24O2	212	1.61
8.	13.33	Oxiranedodecanoic acid, 3-octyl-, cis-	C22H42O3	354	1.95
9.	16.04	Hexadecamethylcyclooctasiloxane	C16H48O8Si8	592	1.93
10.	19.46	Neophytadiene	C20H38	278	3.57
11.	21.83	Dibutyl phthalate	C16H22O4	278	4.44
12.	23.75	Silicone oil	N/A	0	4.23
13.	24.13	1-Tetradecanol (CAS)	C14H30O	214	1.21
14.	25.19	Eicosamethylcyclodecasiloxane	C20H60O10Si10	740	1.26
15.	26.12	Oleic acid, eicosyl ester	C38H74O2	562	2.38
16.	30.98	(+-)-cis-3,4,6,9-tetrahydro-10-hydroxy-7-methoxy-1,3,8-trimethyl-1 H-naphtho[2,3-c]pyran-6,9-dione[(+-)-ventilagone 7-methyl ethyl]	C17H18O5	302	6.32
17.	31.09	Di-(2-ethylhexyl)phthalate	C24H38O4	390	17.67
18.	33.45	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	17.67
19.	35.37	Hexa-t-butylselenatrisiletane	C24H54SeSi3	506	3.09
20.	35.99	Squalene	C30H50	410	6.06
21.	40.25	5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl ester of (4R,5R)-p-Toluenesulfonic acid	C21H26O6S	406	3.28

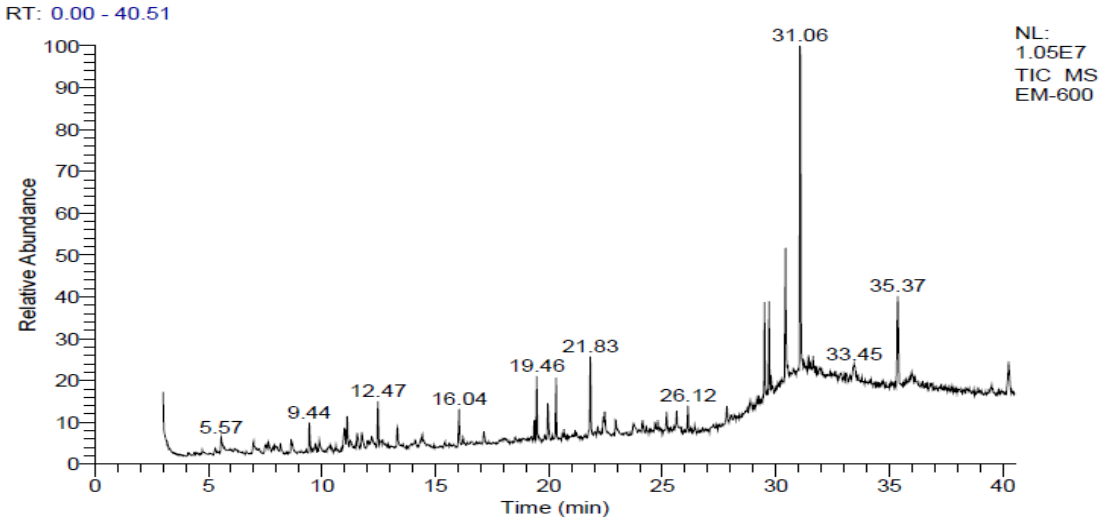


Figure 1: GC-MS Chromatogram of the ethanolic leaf extract of *Harpullia arborea*.

The plant powder showed characteristic odour and bitter taste. Upon drying and powdering the colour of the powder changed from dark green to greenish black as shown in Table 2. The organoleptic characters such as colour, consistency and odour were noted in the ethanolic leaf extract of *Harpullia arborea* (Table 3).

Behaviour of plant powder with different chemical reagents

The behaviour of plant powder with various reagents were observed and presented in Table 4. Pale green to dark green was noted in the powder with different chemical reagents. The information collected from these test was useful for standardization and obtaining the quality standards[11,14,17]

Qualitative phytochemical evaluation

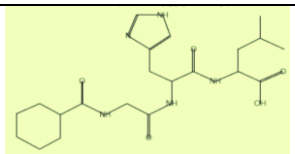
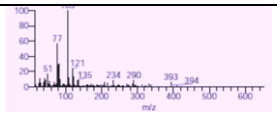
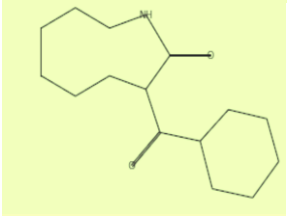
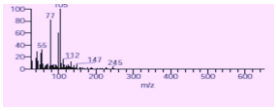
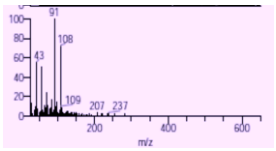
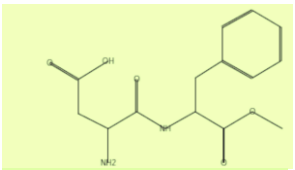
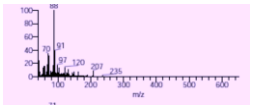
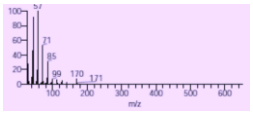
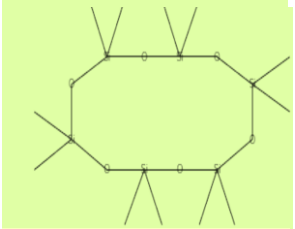
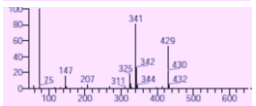
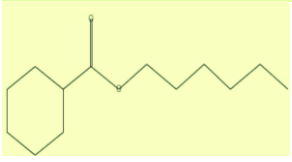
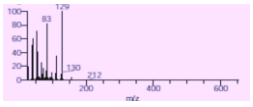
The results of the preliminary phytochemical screening of the study plant showed the presence of various phytochemicals (Table 5). The ethanolic leaf extract revealed the presence of glycosides, saponins, steroids, triterpenoids and resins and the same extracts showed negative response for alkaloids, flavonoids and phenols

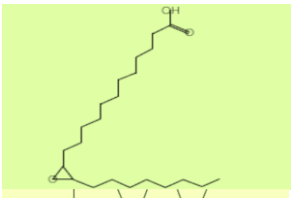
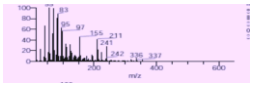
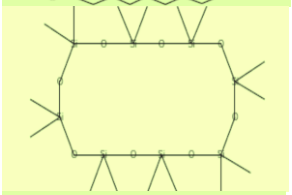
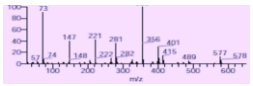
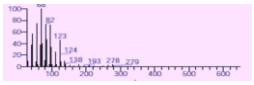
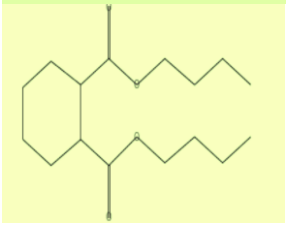
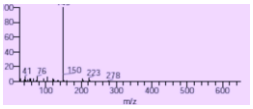
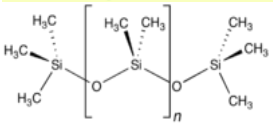
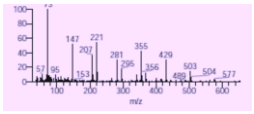
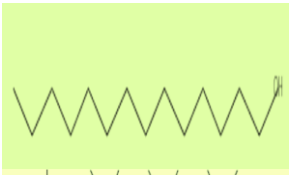
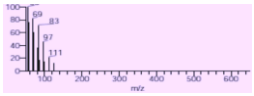
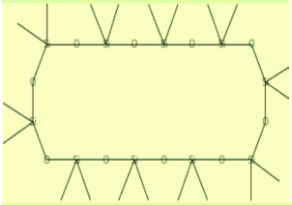
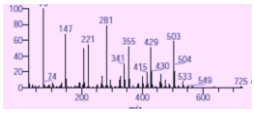
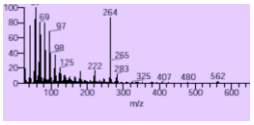
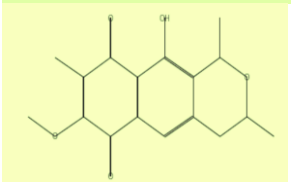
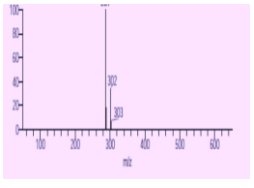
and tannins. The physicochemical constant values of *Harpullia arborea* leaves are helps in assessing the quality of the extract[18]

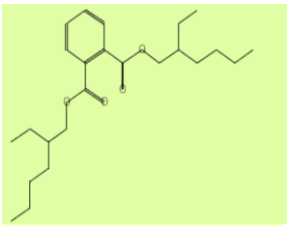
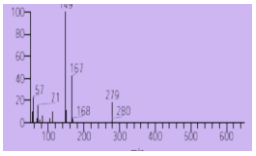
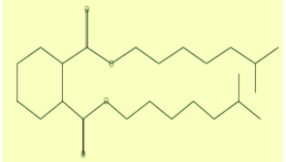
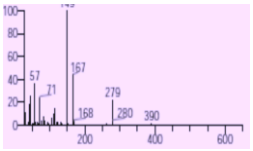
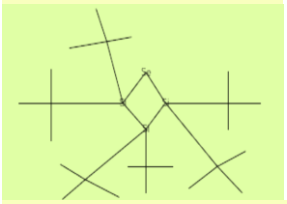
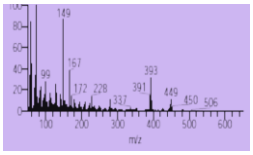
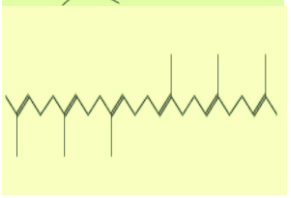
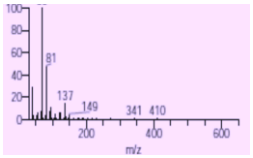
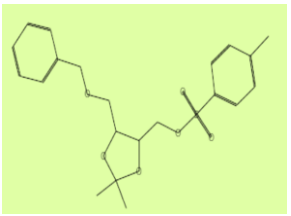
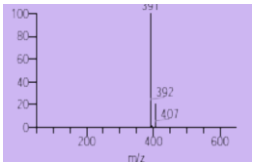
Gas Chromatography / Mass Spectrometry (GC / MS) analysis

The components present in the ethanolic leaf extract of *Harpullia arborea* were identified by GC-MS analysis. The GC- MS Chromatogram of the number of peaks of the compounds detected was shown in Figure.1. This analysis reveals the presence of phytoconstituents belonging to the type-acids, esters, alcohols, ethers, etc. The identified compounds of the ethanolic leaf extract of *Harpullia arborea* their retention indices, percentage composition, chemical structure and activities are given in Table 6 and 7.

Table-7: Mass spectrum and structure of phytocomponents identified by GC-MS in ethanolic leaf extract of *Harpullia arborea*

S. NO	NAME OF THE COMPOUND	STRUCTURE	HIT SPECTRUM	NATURE OF COMPOUND	ACTIVITY
1.	Hippuryl-L-histidyl-L-leucine			Tripeptide	Lung disease (bronchial asthma)
2.	2-Benzoyl-8-octanelactam			Oligomers	Antihistaminic *activity*
3.	Hexadecanoic acid, phenylmethyl ester			Fatty acid	Cardiovascular diseases, Antioxidant, Central nervous system, Control of insulin secretion, Antioxidant, Hypocholesterolemic, Nematicide, Hemolytic
4.	Aspartame			Amino acids	Anti-ulcer, Brain cancer, Neurotoxic effects, Headaches
5.	Dodecane (CAS)			Alkane hydrocarbon	Carcinogens
6.	Cyclohexasiloxane, dodecamethyl - (CAS)			Silicon *compound*	Increase the risk of neoplasms in humans
7.	Cyclohexanecarboxylic acid, hexyl ester			*Esters*	Defoaming agent, Lubricant

8.	Oxiranedodecanoic acid, 3-octyl-, cis-			*Esters*	Antimicrobial agents, Cytotoxicity
9.	Hexadecamethylcyclooctasiloxane			Aldehyde	Antimicrobial activity
10.	Neophytadiene			Hydrocarbon	Antioxidants, antibacterial
11.	Dibutyl phthalate			Plasticizer	Urinary infection, Antioxidants Antimicrobial, Antifouling
12.	Silicone oil			Polymerized siloxane	Antiflatulents, Vitreous complaints, Scar treatment
13.	1-Tetradecanol (CAS)			Primary aliphatic alcohols	Reduction of myristic acid, Antimicrobial activity
14.	Eicosamethylcyclodecasiloxane			Chloroalkyl *groups*	Antimicrobial *activity*, Anticorrosion efficiency
15.	Oleic acid, eicosyl ester			*Eicosyl ester*	Antifungal *activity*
16.	(+-)-cis-3,4,6,9-tetrahydro-10-hydroxy-7-methoxy-1,3,8-trimethyl-1 H-naphtho[2,3-c]pyran-6,9-dione[(+-)-ventilagone 7-methyl ethyl]			O-*methyl groups*	Antiviral *activity*

17.	Di-(2-ethylhexyl)phthalate			Phthalate plasticizers	Hemophiliacs, Kidney disorders, Antimicrobial and Cytotoxic Activity
18.	1,2-Benzenedicarboxylic acid, diisooctyl ester			Ester	Endocrine disruptors
19.	Hexa-t-butylselenatrisiletane			Ether	Anthelmintic
20.	Squalene			Triterpene	Neutralize different xenobiotics, anti inflammatory, anti-atherosclerotic, anti-neoplastic, role in skin aging and pathology, adjuvant activities, antioxidant, chemo preventive, anti-cancer, pesticide
21.	5-Benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl ester of (4R,5R)-p-Toluenesulfonic acid			*Ester*	Antibacterial *activity*

Twenty one compounds were detected from the ethanolic leaf extract of *Harpullia arborea*. The results showed the presence of di-(2-ethylhexyl)phthalate (17.67 %), 1,2-benzenedicarboxylic acid, diisooctyl ester (17.67 %), (+-)-cis-3,4,6,9-tetrahydro-10-hydroxy-7-methoxy-1,3,8- (6.32), squalene (6.06), dibutyl phthalate (4.44), silicone oil (4.23), neophytadiene (3.57), 5-benzyloxymethyl-2,2-dimethyl-1,3-dioxolan-4-yl ester of (4R,5R)-p-toluenesulfonic acid (3.28), oleic acid, eicosyl ester (2.38) and hippuryl-L-histidyl-L-leucine (2.05). The spectrum profile of GC – MS confirmed the presence of 10 major components with retention time 31.09, 33.45, 30.98, 21.83, 19.46, 40.25, 26.12 and 5.57 respectively (Table 6). The spectra of the compounds were matched with Wiley 9.0 and National Institute of Standards and Technology libraries. Table 7 lists the major phytocomponents and their chemical structure and biological activities obtained through the GC – MS study of *Harpullia arborea.*

In the present study, the GC-MS analysis of the ethanolic leaf extract of *Harpullia arborea* showed the presence of ten major compounds. In terms of percentage amounts Di-(2-ethylhexyl) phthalate, 1,2-Benzenedicarboxylic acid, diisooctyl ester, (+-)-cis-3,4,6,9-tetrahydro-10-hydroxy-7-methoxy-1,3,8- and Squalene were predominant in the extract and have the property of antioxidant, hemophiliacs, kidney disorders, endocrine disruptors, chemo preventive and anti cancer activity. Among the other identified phytochemicals, the fatty acid esters namely, hexadecanoic acid phenylmethyl ester and oleic acid eicosyl ester have the property of antioxidant, hypocholesterolemic, cardiovascular diseases, central nervous system, control of insulin secretion, nematicide, hemolytic, emulsifying agent, hypotensive, nimaticide and pesticide activities. Similar observations were made by Sharmila *et al.,* 2017. Among the identified phytochemicals, squalene has antioxidant activity and also used in cosmetics, and more recently as an immunologic adjuvant in vaccines. The biological activities listed are based on Dr. Duke's phytochemical and ethnobotanical databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The

mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *Harpullia arborea* using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The presence of various bioactive compounds confirms the application of *Harpullia arborea* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

Plant-based drugs have been used worldwide in traditional medicine for the treatment of various diseases. Approximately 60% of world's population still relies on medicinal plants for their primary healthcare[18,19] Phytochemical constituents such as alkaloids, flavanoids, glycosides and several other aromatic compounds are secondary metabolites in plants that have alleviated the pathogenic and environmental stress[20,21] In accordance with this fact the preliminary phytochemical screening of *Harpullia arborea* showed the presence of various phytochemicals like glycosides, saponins, steroids, triterpenoids and resins and the same extracts showed negative response for alkaloids, flavonoids, phenols and tannins (Table 5). These data confirms similarity with earlier reports[22,23] The need of the hour is to screen a number of plants that are traditionally used and also to evaluate their probable phytoconstituents[24,25]

Dodecanoic acid has antimicrobial activity against methicillin - resistant *Staphylococcus aureus*[26]. He reported that silicone oil control flatus (antiflatulents) [27] Oleic acid as its sodium salt was a major component of soap as an emulsifying agent and also used as emollient. Small amounts of oleic acid are used as an excipient in pharmaceuticals[28] Rizzo was reported that oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil[29] Adverse effects also have been documented, however, since both oleic and monounsaturated fatty acid levels in the membranes of red blood cells have been associated with increased risk of breast cancer, although the consumption of oleate in olive oil has been associated with a decreased risk of breast cancer[30]. Squalene is used in cosmetics, cancer treatment and more recently as an immunologic adjuvant in vaccines[31].

Harpullia arborea is a plant used in traditional medicine however there are no reports for phytochemical analysis of the plant. We report the presence of some of the important components resolved by GC – MS analysis and their biological activities also identified. Thus, this type of GC – MS analysis is the first step towards understanding the nature of active principles in this medicinal plants and this type of study will be helpful for further detailed study.

CONCLUSION

The information obtained from pharmacognostical studies will be of used for supplementary pharmacological and therapeutical evaluation of the species and will assist in standardization for quality, purity and authentication with the help of which adulteration and substitution can be prevented. Our systematic investigation reveals the potential of *Harpullia arborea* leaves as a good source of bioactive compounds such as fatty acid esters, alcohols, hydrocarbons, aldehydes, fatty acids and amides that justify the use of this plant for its various ailments. Isolation of individual components would however, help to find new drugs.

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