



AN EVALUATION OF THE PHYTOCHEMICAL PROPERTIES AND ANTICANCER ACTIVITY OF SELECTED NATIVE MEDICINAL PLANTS

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ABSTRACT

In the present study, phytochemical constituents of medicinal plants were evaluated from their ethanolic extracts, such as *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum*. The extracts were subjected to a qualitative phytochemical screening using a standard procedure. Our study has identified phytochemicals present in leaf extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* using IR and GC-MS spectroscopy. An anticancer activity was performed on each of these extracts using two human cancer cell lines, a colon cancer cell line (HCT116) and a cervical cancer cell line (HeLa). Despite the four medicinal plants extracts having comparable anticancer activity, the ethanolic extracts of *Andrographis paniculata* and *Solanum trilobatum* show higher anticancer activity than *Centella asiatica* and *Psidium guajava*. The *Andrographis paniculata* (IC₅₀=27.54 µg/ml HeLa & 30.19 µg/ml HCT116) and *Solanum trilobatum* (IC₅₀=32.36 µg/ml HeLa & 35.48 µg/ml HCT116) shows equipotent activity against respective cell line than Doxorubicin (IC₅₀=20.89 µg/ml HeLa & 22.91 µg/ml HCT116).

Keywords: Anticancer activity, Phytochemicals, *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava*, *Solanum trilobatum*.

1. INTRODUCTION

One of the leading causes of death worldwide, cancer is a major public health problem. The prevalence of this disease is rising more rapidly in Africa, Asia, and Central and South America, which account for around 70% of all cancer deaths worldwide [1]. The development of cancer therapies has been the focus of many studies [2-4]. Cancer can be treated with chemotherapy, and advances in anticancer drugs have improved patient care. Unfortunately, conventional chemical drugs can also have adverse effects on normal cells/tissues, such as bone marrow inhibition, nausea, vomiting, and alopecia [5, 6]. Furthermore, many phytochemicals have been suggested as anticancer adjuvant therapies because of their pro-apoptotic and anti-proliferative properties [3, 4]. Since natural herbal medicines have many benefits, the continued search for anticancer agents and compounds from plants has played an essential role in finding the best way to decrease the side effects caused by chemotherapy since herbal medicines are completely natural and safe [7-10].

Bioactive components present in *Andrographis paniculata* include diterpenoids, flavonoids and polyphenols [11,12]. *Andrographis paniculata* has been reported to contain over 20 diterpenoids and over ten flavonoids when extracted with ethanol or methanol from the whole plant, leaf, and stem [13, 14]. The *Centella asiatica* contains triterpene acids such as Asiatic and madecassic acids [15]. Flavonoids like kaempferol and quercetin are also present in the plant [16]. Plants contain volatile and fatty oils. The fatty oils contain glycerides of palmitic, stearic, lignoceric, and oleic acids [17]. Traditions of its use have a long history and a number of them have been proven scientifically valid [18]. It has been used ethnomedicinally to treat wounds, boils, infections on the skin and soft tissues and to treat wounds and boils [19]. *Solanum trilobatum* leaves are used in *Ayurveda* and *Siddha* medicine systems to treat respiratory problems, including acute and chronic bronchitis, asthma, sinusitis, tonsillitis, common cold, cough, and pulmonary infections [20]. Among the main uses of the leaves are dyspepsia, spermatorrhoea, tuberculosis, ear problems

and bacterial infections [21]. Various pharmacological activities have been examined, including antibacterial, antifungal, anticancer, antioxidant, antidiabetic, hepatoprotective, antinociceptive, antiulcerogenic and anti-inflammatory [22, 23]. A leaf extract of *Psidium guajava* had the strongest anti-proliferative activity on P388 cell lines with an IC_{50} value of 0.379 mg/ml (four times stronger than vincristine), an effect mainly attributed to monoterpenes present in the essential oil [24, 25]. Another study found chemopreventive effects of a methanol leaf extract in mice inoculated with B16 melanoma cells. The incidence and average number of cancerous mice decreased significantly compared to the control group [26]. The findings suggest that *Psidium guajava* aqueous leaf extracts could control tumour development by decreasing Tr cells and shifting to Th1 cells [27]. Accordingly, we have evaluated the phytochemical properties and anticancer activity of selected native medicinal plants.

2. MATERIAL AND METHODS

2.1. Collection and authentication of plant material

The leaves of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava*, and *Solanum trilobatum* were collected from Agastheswaram Taluk, Kanyakumari district, Tamilnadu, India, during October and November of 2019. The plant specimen was identified and authenticated by Dr. C. Babu, Head of Botany and Associate Professor at Pioneer Kumaraswamy College, Nagercoil. A thorough washing process and thorough rinsing of the leaves was followed by a drying process at room temperature for 7-8 days, after which the dried plant leaves were powdered and stored in air tight containers.

2.2. Extract preparation

In order to prepare the extract, 50 grams each of dry *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava*, and *Solanum trilobatum* leaves were mounted on a Soxhlet extractor and dissolved in 250 ml of ethanol. In the Soxhlet loop, the extraction procedure was performed until the solvent became colourless [28]. After the extracts had been concentrated at room temperature so the solvent could evaporate, they were stored in an air-tight container, and the solvent was refrigerated at 4°C so that it could be used later [29].

2.3. Phytochemical analysis

Phytochemical analyses of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* leaves

were performed using a previously described standard method [30]. The individual extracts were subjected to numerous qualitative and quantitative chemical tests to establish their composition profiles. In standard procedures, the crude powder was obtained with different solvents and the phytoconstituents present in each were identified. The tests were generally performed to determine whether Protein, Carbohydrate, Phenol, Tannins, Flavonoid, Saponins, Glycosides, Steroids, Terpenoids, Alkaloids and Reducing Sugar are present.

2.4. Gas Chromatography Mass Spectrum (GC-MS) Analysis

GCMS analysis was performed on extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava*, and *Solanum trilobatum* plants from Heber Analytical Instrumentation Facility (HAIF), Bishop Heber College, Trichy-620017 to evaluate their phytochemical composition. The analyses were conducted using GC-MS equipment (GC MS QP2020; SHIMADZU), which includes an autosampler, an injector, a gas chromatograph (GC-2010) and a mass spectrometer. The GC-MS system was composed of SHRxi-5Sil-MS capillary standard non-polar column (dimensions: 30.0m, diameter: 0.25mm, film thickness: 0.25µm, which is composed of 100% Dimethyl polysiloxane). An electron ionization energy system was used, having an ionization energy of 70eV. Helium gas (99.99%) was used at a rate of 1.20ml/min and an injection volume of 5µl (split ratio: 10). A total of 21 minutes were spent running the GC with a temperature of 50°C (isothermal for 2 min.), increasing to 280°C for 10 min. Mass spectra were collected at 70eV at 0.3 seconds with a scan range of 50-500 m/z. Our calculation of the percentage of each component was based on the average peak area divided by the total peak area. To analyze mass spectra and chromatograms, we used Shimadzu's GC-MS real-time software [31].

2.5. Identification of components

We have interpreted GC-MS mass spectra by using data from the National Institute Standard and Technique (NIST14) [32] and WILEY8 [33] which contain more patterns. Molecular formulas, names, molecular weights, and structures of each component of the test material were determined by comparing the spectrum of the unknown component with the spectrum of the known components from NIST14 and WILEY8 libraries.

2.6. Anticancer activity

The *in vitro* anticancer activity was analysed using MTT assay method. Monolayer cells were detached with

trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted with a hemocytometer and diluted with a medium containing 5% FBS. To give final density of 1×10^5 cells/mL, 96-well plates were seeded with 100 microlitres of cell suspension per well at a plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% air, and 100% relative humidity. The cells were treated with serial concentrations of the test samples after 24 h. The test samples were initially dissolved in neat dimethylsulfoxide (DMSO) to prepare the stock (200 mM) and were stored frozen prior to use. For the addition of drugs, an aliquot of frozen concentrate was thawed and diluted twice the desired final maximum concentration with serum-free medium. Three additional two-fold serial dilutions were performed to provide a total of four drug concentrations. The appropriate wells already containing 100 l of medium were added aliquots of 100 l of each of these different drug dilutions, resulting in the final drug concentrations required. After the addition of the drug, the plates were incubated for another 48 hours at 37°C, 5% CO₂, and 95% air with 100% relative humidity. A triplicate plate was maintained for each concentration [34].

The MTT is a yellow water-soluble tetrazolium salt. The succinate-dehydrogenase in the mitochondria cleaves the tetrazolium rings, converting the MTT into an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48h of incubation, 15µL of MTT (5mg/mL) in PBS was added to each well and incubated at 37°C for 4h. Once the medium with MTT was removed, the crystals of formazan were redissolved in 100µL of DMSO. The absorbance of the formed formazan was then detected at 570 nm using a microplate reader. The % inhibition of the cells was calculated according to the following formula:

$$\% \text{ Cell Inhibition} = 100 - \frac{\text{Abs (sample)}}{\text{Abs (control)}} \times 100$$

Nonlinear regression graph was plotted between % cell inhibition and log₁₀ concentration and IC₅₀ was determined using Graph Pad Prism software [35].

3. RESULTS AND DISCUSSION

In the present study, leaf extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava*, and *Solanum trilobatum* were analysed for anticancer properties. The use of phytochemical tests, which are cost-efficient and fast, is recommended for the quality control of anticancer

secondary metabolism. Our study found that phytochemicals were present in ethanol solvent extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava*, and *Solanum trilobatum*.

3.1. Qualitative phytochemical analysis of *Melia dubia* leaves extracts

Plants contain chemical substances that possess anticancer properties. The most important of these substances are alkaloids, terpenoids, steroids, fatty acids, and phenols. Table 1 shows qualitative phytochemical studies conducted on ethanolic extracts of leaves from *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum*. The phytochemical analysis data revealed that alkaloids, terpenoids, steroids, fatty acids and phenolic compounds were present. The ethanol extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* contained high concentrations of terpenoids. *Andrographis paniculata*, *Centella asiatica* and *Solanum trilobatum* extracts contained high concentrations of alkaloids. In extracts from *Andrographis paniculata*, steroids were found in high concentrations. Extracts of *Psidium Guajara* were found to contain very low levels of alkaloids. The phenolic compound was found in high concentrations in *Andrographis paniculata*, *Psidium Guajara*, and *Solanum trilobatum* extracts. Four extracts were found to contain saponin in medium amounts, while extracts from *Andrographis paniculata* and *Psidium Guajara* contained very small amounts of flavonoids.

Table 1: Phytochemical analysis of plant extracts

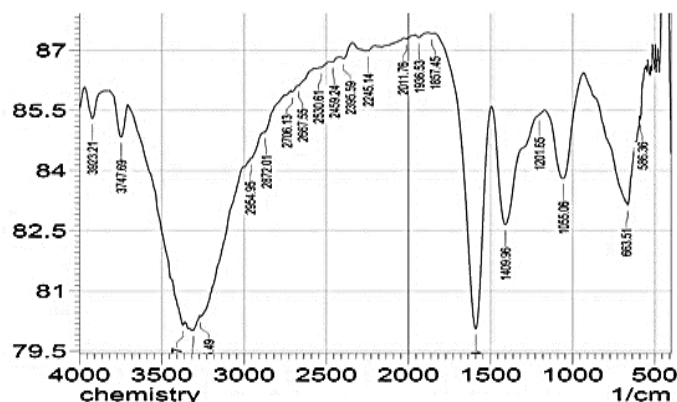
Phytochemical	Result for Ethanolic Extractants			
	<i>Psidium Guajava</i>	<i>Solanum Trilobatum</i>	<i>Centella Asiatica</i>	<i>Andrographis Paniculata</i>
Protein	++	+++	+++	++
Carbohydrate	+++	++	+++	+
Phenol	++	++	-	+++
Tannins	++	+++	+++	-
Flavonoid	+	+	-	++
Saponins	+	+	+	+
Glycosides	+	++	+	++
Steroids	+	+	+	++
Terpenoids	+++	+++	+++	+++
Alkaloids	+	++	++	+++
Reducing sugar	+	+++	++	-

3.2. FT-IR Analysis

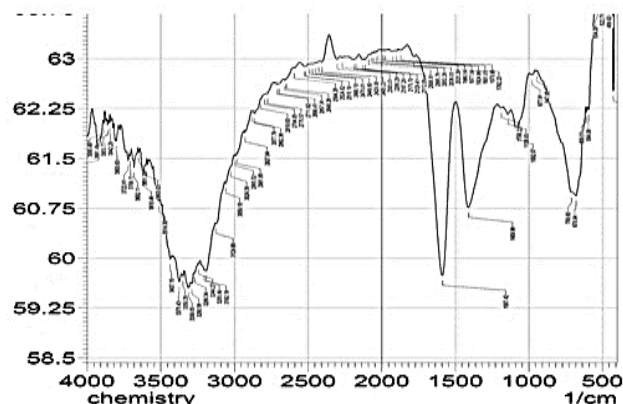
A spectroscopic analysis of infrared spectra may identify functional groups of active principles. Fig. 1 shows the

FT-IR spectroscopic analysis of the aqueous extract of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* leaf using the KBr disc method. The FTIR spectrum of *Andrographis paniculata* leaves extract exhibit in the characteristic absorption band were exhibited a 3323 cm^{-1} (for NH stretching), 3304 cm^{-1} (for OH stretching), 3242 cm^{-1} (for NH asymmetric stretching), 3194 cm^{-1} (for NH symmetric stretching), 1597 cm^{-1} (for C=O stretching), 1409 cm^{-1} (for CH stretching), 1201 cm^{-1} (for -C-O-C- stretching), 1041 cm^{-1} (for OH 1° alcohol) and 586 cm^{-1} (for C-Cl stretching) were exhibited by aqueous plant extract. FTIR spectrum of *Centella asiatica* leaves extract showed characteristic absorption bands at 3323 cm^{-1} (for NH stretching), 3345 cm^{-1} (for OH stretching), 3242 cm^{-1} (for NH asymmetric stretching), 3194 cm^{-1} (for NH symmetric stretching), 1594 cm^{-1} (for C=O stretching),

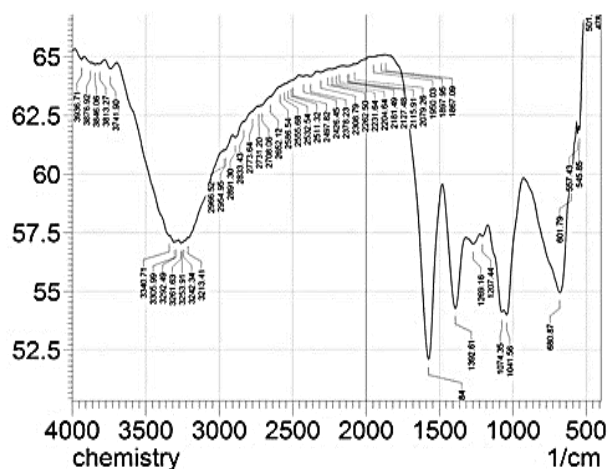
1404 cm^{-1} (for CH bending), 1298 cm^{-1} (for CH stretching), 1044 (for OH 1° alcohol) and 565 cm^{-1} (for C-Cl stretching). FTIR spectrum of *Psidium guajava* leaves extract showed the characteristic absorption bands at 3340 cm^{-1} (for NH stretching), 3305 cm^{-1} (for OH stretching), 3252 cm^{-1} (for NH asymmetric stretching), 2954 cm^{-1} (for CH- CO stretching), 1584 cm^{-1} (for C=O stretching), 1392 cm^{-1} (for CH stretching), 1207 cm^{-1} (for -CH stretching), 1041 cm^{-1} (for OH 1° alcohol) and 557 cm^{-1} (for C-Cl stretching). FTIR spectrum of *Solanum trilobatum* leaves extract showed the characteristic absorption band at 3345 cm^{-1} (for OH stretching), 3259 cm^{-1} (for NH asymmetric stretching), 3197 cm^{-1} (for NH symmetric stretching), 1594 cm^{-1} (for C=O stretching), 1404 cm^{-1} (for CH bending), 1265 cm^{-1} (for CN stretching), 1074 (for CH stretching) and 678 cm^{-1} (for C-Br stretching).



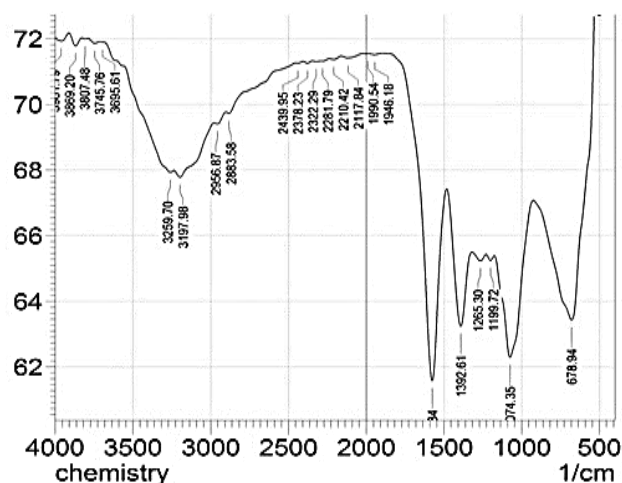
Andrographis paniculata



Centella asiatica



Psidium guajava



Solanum trilobatum

Fig. 1: FTIR spectrum of ethanolic plant extract

3.3. GC-MS analysis of Plant extract

GC-MS is the best tool for determining the functional groups that are involved in the bioactivity of Terpenoids, Steroids, Fatty Acids, Phenolic Compounds, Alkaloids, Saponins and Flavonoids. We analysed the ethanolic extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* using Gas Chromatography - Mass Spectroscopy, as shown in Tables 2-5 and Fig 2. Among seventy-five compounds identified in the *Psidium guajava* extract, six showed to be anticancer in nature. The GC-MS analysis of *Psidium guajava* extract revealed the presence of anticancer compounds like Caryophyllene, Curcuphenol, caryophyllene oxide, phytol, Hexadecanoic acid and Octadecanoic acid (Table 2 and Fig. 3). In the *Solanum trilobatum* extracts, thirteen compounds were identified and ten of those compounds

appeared anticancer. The anticancer compounds in benzene extracts, such as Dodecanoic acid, Tetradecanoic acid, Loliolide, Phytol, n-Hexadecanoic acid, Stearic acid, Linoleic acid, Oleic acid, Cynaropicrin and dl-alpha-Tocopherol (Table 3 and Fig. 4). Among the 40 compounds identified in the *Centella Asiatica* extracts, eight were anticancer such as Dihydroactinidiolide, Myristinic acid, Palmitic acid, Phytol, Linoleic acid, Stearic acid, Andrographolide and Squalene (Table 4 and Fig. 5). The *Andrographis paniculata* extracts identified forty compounds out of which twelve were anticancer. Anticancer compound such as Dodecanoic acid, Caryophyllene oxide, Tetradecanoic acid, Loliolide, Globulol, Stearic acid, Phytol, n-Hexadecanoic acid, Linoleic acid, Andrographolide, Lupeol and Stigmasterol (Table 4 and Fig. 6).

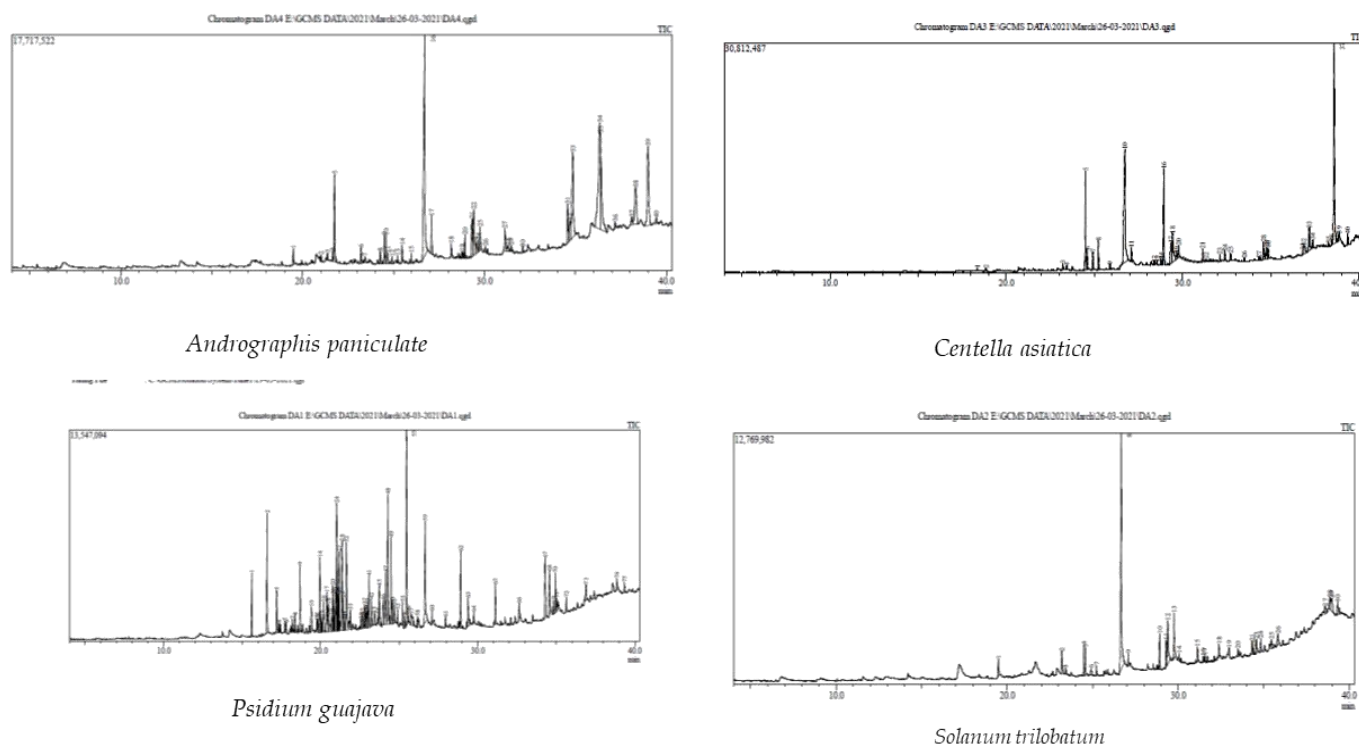


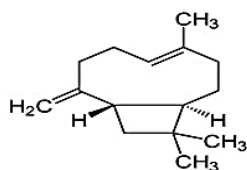
Fig. 2: GC-MS Chromatogram of Ethanolic Extracts

Table 2: Phytocompounds identified in the plant extract of *Psidium Guajava* by using GC-MS analysis

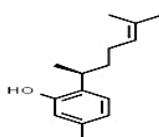
Retention Time	Peak Area%	Name of the Compound	Molecular formula	Molecular Weight	Name of the phytocompounds
16.585	3.85	Caryophyllene	C ₁₅ H ₂₄	204	sesquiterpene
19.782	0.39	Curcuphenol	C ₁₅ H ₂₂ O	218	sesquiterpene
19.949	2.51	Caryophyllene Oxide	C ₁₅ H ₂₄ O	220	Sesquiterpene
25.222	0.7	Phytol	C ₂₀ H ₄₀	296	Diterpene
26.65	4.9	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Saturated fatty acid
29.751	0.34	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284	Saturated fatty acid

Table 3: Phytocompounds identified in the plant extract of *Solanum Trilobatum* by using GC-MS analysis

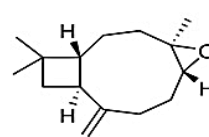
Retention Time	Peak Area%	Name of the Compound	Molecular formula	Molecular Weight	Name of the phytocompounds
19.48	2.47	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	Saturated fatty acid
23.2	2.92	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	Saturated fatty acid
23.442	0.77	Loliolide	C ₁₁ H ₁₆ O ₃	196	Monoterpene
24.905	1.09	Phytol	C ₂₀ H ₄₀ O	296	Diterpene
26.673	40.44	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Fatty acid
27.09	1.11	Stearic acid	C ₁₈ H ₃₆ O ₂	284	Saturated fatty acid
29.298	3.52	Linoleic acid	C ₁₈ H ₃₂ O ₂	280	Fatty acid
29.392	701	Oleic acid	C ₁₈ H ₃₄ O ₂	282	Unsaturated fatty acid
32.974	1.17	Cynaropicrin	C ₁₉ H ₂₂ O ₆	346	Sesquiterpene lactone
34.328	1.43	dl-alpha-Tocopherol	C ₂₉ H ₅₀ O ₂	430	Alpha tocopherol



caryophyllene



Curcuphenol



Caryophyllene oxide



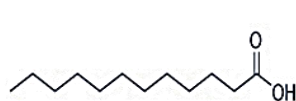
Phytol



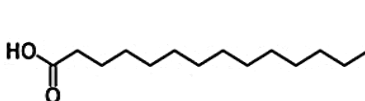
Hexadecanoic acid



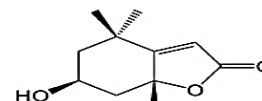
Octadecanoic acid

Fig. 3: Anticancer phytocompounds identified in the plant extract of *Psidium Guajava* by using GC-MS analysis

Dodecanoic acid



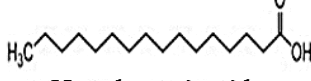
Tetradecanoic acid



Loliolide



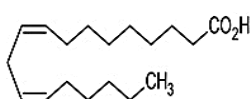
Phytol



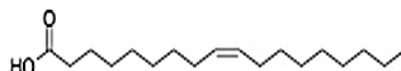
n-Hexadecanoic acid



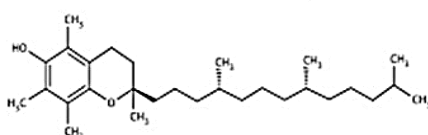
Stearic acid



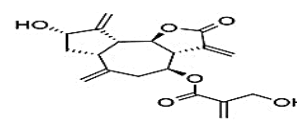
Linoleic acid



Oleic acid



dl-alpha-Tocopherol



Cynaropicrin

Fig. 4: Anticancer phytocompounds identified in the plant extract of *Solanum trilobatum* by using GC-MS analysis

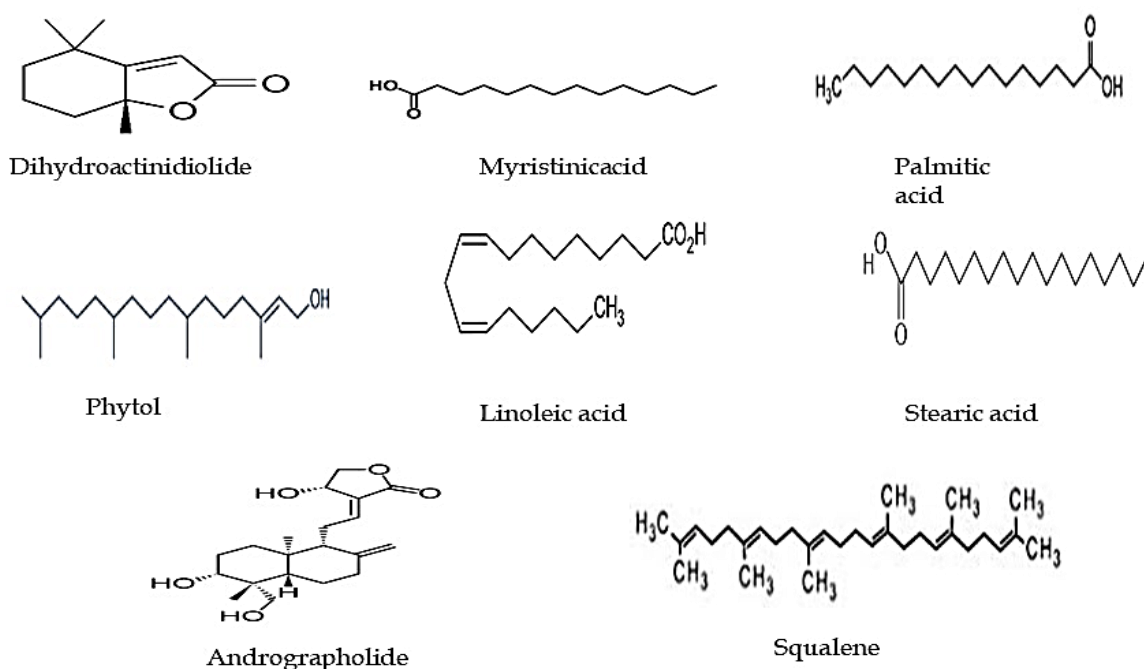


Fig. 5: Anticancer phytocompounds identified in the plant extract of *Centella Asiatica* by using GC-MS analysis

Table 4: Phytocompounds identified in the plant extract of *Centella Asiatica* by using GC-MS analysis

Retention Time	Peak Area%	Name of the Compound	Molecular formula	Molecular Weight	Name of the phytocompounds
18.849	0.21	Dihydroactinidiolide	C ₁₁ H ₁₆ O ₂	180	Volatile Terpene
23.203	0.62	Myristinic acid	C ₁₄ H ₂₈ O ₂	228	Saturated fatty acid
25.885	0.39	Palmitic acid	C ₁₆ H ₃₂ O ₂	256	Saturated fatty acid
28.393	0.2	Phytol	C ₂₀ H ₄₀ O	296	Diterpene
28.926	2.76	Linoleic acid	C ₁₈ H ₃₂ O ₂	280	Fatty acid
29.769	1.21	Stearic acid	C ₁₈ H ₃₆ O ₂	284	Saturated fatty acid
32.705	0.77	Andrographolide	C ₂₀ H ₃₀ O ₅	350	Labdane Diterpenoid
38.259	0.34	Squalene	C ₃₀ H ₅₀	410	Triterpene

Table 5: phytocompounds identified in the plant extract of *Andrographis paniculata* by using GC-MS analysis

Retention Time	Peak Area%	Name of the Compound	Molecular formula	Molecular Weight	Name of the phytocompounds
19.48	0.85	Dodecanoic acid	C ₁₂ H ₂₄ O ₂	200	Saturated fatty acid
21.356	0.27	Caryophyllene oxide	C ₁₅ H ₂₄ O	220	Sesquiterpene
23.201	0.81	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228	Saturated fatty acid
23.423	0.36	Loliolide	C ₁₁ H ₁₆ O ₃	196	Monoterpene
24.267	0.68	Globulol	C ₁₅ H ₂₆ O	222	Sesquiterpene
24.908	0.4	Phytol	C ₂₀ H ₄₀ O	296	Diterpene
27.091	1.62	Stearic acid	C ₁₈ H ₃₆ O ₂	284	Saturated fatty acid
26.704	19.75	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	Fatty acid
29.313	2.36	Linoleic acid	C ₁₈ H ₃₂ O ₂	280	Fatty acid
36.39	8.64	Andrographolide	C ₂₀ H ₃₀ O ₅	350	Labdane Diterpenoid
38.11	0.68	Lupeol	C ₃₀ H ₅₀ O	426	Diterpene
38.989	9.38	Stigmasterol	C ₂₉ H ₄₈ O	412	Phytosterol

3.4. *In vitro* anticancer activity

Andrographis paniculata, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* ethanolic extracts were initially screened at a single concentration of two-fold dilution using the colorimetric MTT to test their *in vitro* cytotoxicity against HeLa (cervical cancer cells) and HCT116 (colon cancer cells). Doxorubicin was used as the reference drug in this study. The cytotoxicity of the tested compounds was estimated in terms of percent growth inhibition compared to untreated control cells. All the compounds effected >70% inhibition and were retested by a twofold dilution from 6.25 to 100 μ M. The results are expressed as IC₅₀ (inhibitory concentration 50%), the concentration of the

compound which inhibits the tumor cell growth by 50% and the data are presented in Table 6 and Fig.7.

Table 7: Anticancer activity of the Plant extracts

Compounds	Antitumor activity	
	IC ₅₀ (μ M) a	
	HeLa cell	HCT116
<i>Andrographis paniculata</i>	27.54	30.19
<i>Centella asiatica</i>	89.12	97.72
<i>Psidium guajava</i>	239.88	269.15
<i>Solanum trilobatum</i>	32.36	35.48
Doxorubicin ^b	20.89	22.91

^aThe IC₅₀ value was defined as the concentration at which 50% survival of cells was observed. The results are listed in the table.
^bUsed as a positive control

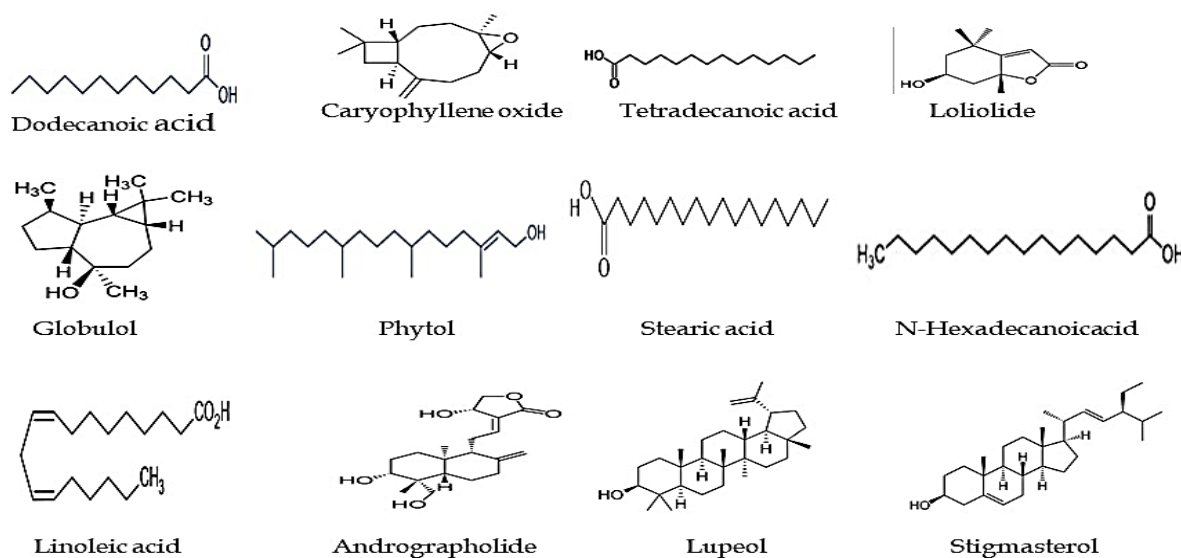


Fig. 6: Anticancer phytocompounds identified in the plant extract of *Andrographis paniculata* by using GC-MS analysis

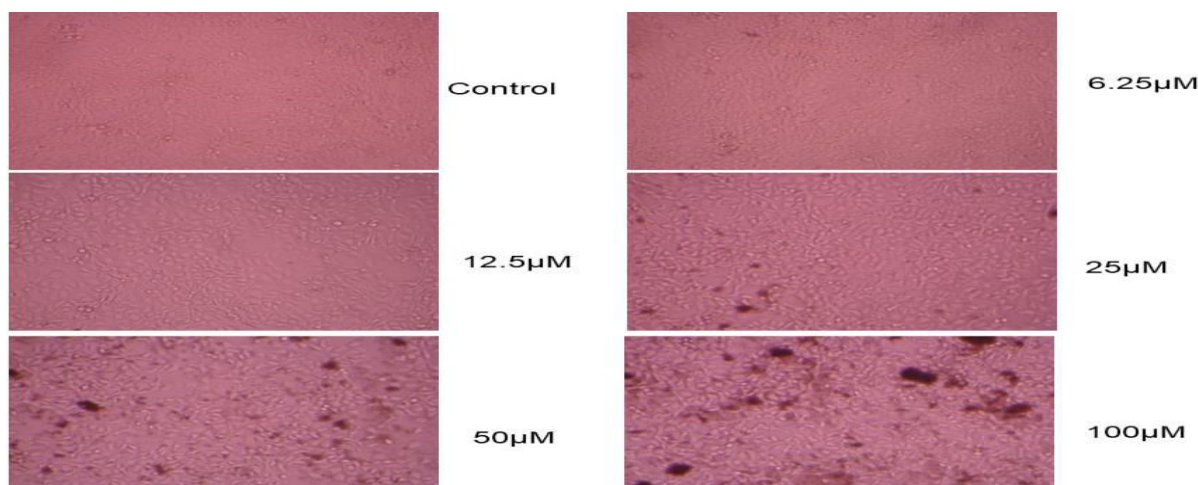


Fig. 7: Anticancer activity of the *Andrographis paniculata* extracts in HeLa Cell Lane

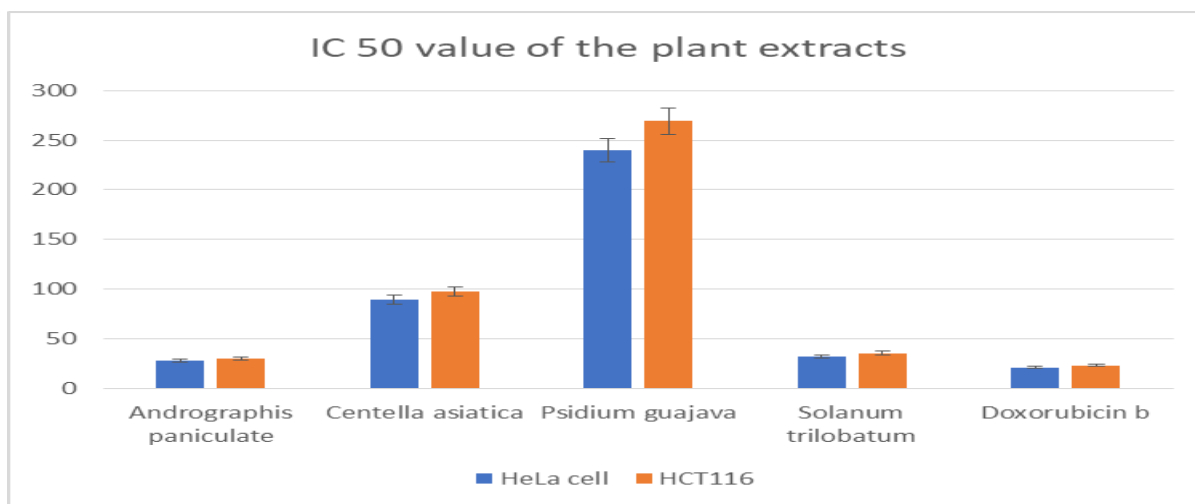


Fig. 8: IC₅₀ value of the plant extracts in HeLa and HCT116 Cell Lane

Cell growth inhibition was analysed by MTT assay and the results show that the extracts have an inhibitory effect on the proliferation of HeLa and HCT116 cells in a dose-dependent manner. Despite the four medicinal plants extracts having comparable anticancer activity, the ethanolic extracts of *Andrographis paniculata* and *Solanum trilobatum* show higher anticancer activity than *Centella asiatica* and *Psidium guajava*. The *Andrographis paniculata* (LC₅₀=27.54 μ M HeLa & 30.19 μ M HCT116) and *Solanum trilobatum* (LC₅₀=32.36 μ M HeLa & 35.48 μ M HCT116) show equipotent activity against respective cell line than Doxorubicin (LC₅₀=20.89 μ M HeLa & 22.91 HCT116 μ M). In general, many of the IC₅₀ values for HCT116 cells are lower than those for the corresponding HeLa cells.

Plants have always played an important role in medicine both traditionally and in modern times in many countries, since they produce bioactive molecules that are the source of many potential and powerful drugs. As a result of their phytochemical content, medicinal plants are useful for healing and curing human diseases. A phytochemical is a naturally occurring chemical found in medicinal plants, leaves, vegetables, and roots that possesses defense mechanisms and protects against various diseases [36]. A lot of research has been conducted on the development of natural-product-based cancer treatment as an alternative or a complementary treatment due to fewer side effects and easy accessibility [37].

Herbs contain phytosterols, phenolic acids, triterpenes, flavonoids, anthocyanins, saponins and carotenoids, which have been shown to have cancer chemopreventive and antioxidant effects [38]. In today's conventional medicine, 60% of the ingredients are

natural products. Out of these, 60% have been reported to be anticancer [39, 40]. Discoveries of anticancer cancer agents have specificity for cancer cells and induce cell death and inhibit tumor growth. Cancer is one of the most common diseases and many cancer treatments have been developed. Additionally, chemically-derived drugs have been developed to treat cancer. Current methods, however, have their limitations because they are toxic to non-targeted tissues and further aggravate human health [41].

4. CONCLUSION

In our study, we have identified phytochemicals present in leaf extracts of *Andrographis paniculata*, *Centella asiatica*, *Psidium guajava* and *Solanum trilobatum* using IR and GC-MS spectroscopy. The extracts were tested for anticancer activity on two human cancer cell lines, a colon cancer cell line (HCT116) and a cervical cancer cell line (HeLa). It appears that even though the four medicinal plant extracts show comparable anticancer activity, the ethanolic extracts of *Andrographis paniculata* and *Solanum trilobatum* have higher anticancer activity than the extracts from *Centella asiatica* and *Psidium guajava*. While the results are promising, further study is necessary, especially to isolate and identify the active principal component of the product, and to assess the cost-benefit ratio as well as its ability to control anticancer agents.

Conflict of interest

None declared

Source of funding

None declared

5. REFERENCES

1. Siegel RL, Miller KD, Jemal A, *CA Cancer J Clin*, 2020; **70**:7-30.
2. Pucci C, Martinelli C, Ciofani G, *Ecancermedical-science*, 2019; **13**:961.
3. Chikara S, Nagaprashantha LD, Singhal J, Horne D, Awasthi S, Singhal SS, *Cancer Lett*, 2018; **413**:122-134.
4. Singh S, Sharma B, Kanwar SS, Kumar A, *Front. Plant Sci*, 2016; **7**:8973.
5. Sak K, *Chemother. Res. Pract*, 2012; **2012**:1-11.
6. Baskar R, Dai J, Wenlong N, Yeo R, Yeoh K, *Front. Mol. Biosci*, 2014; **1**:24.
7. Wood DM, Athwal S, Panahloo A, *Diabet. Med*, 2004; **21**:625-627.
8. Yin SY, Wei WC, Jian FY, Yang NS, *Altern. Med*, 2013; **2013**:302426.
9. Nguyen NH, Nguyen TT, Ma PC, Ta QTH, Duong TH, Van Giau V, *Molecules*, 2020; **25**:1996.
10. Duong TH, Beniddir MA, Trung NT, Phan CD, Vo VG, Nguyen VK, Le QL, Nguyen HD, Le Pogam P, *Molecules*, 2020; **25**:1830.
11. Rao YK, Vimalamma G, Rao CV, Tzeng Y, *Phytochemistry*, 2004; **65**:2317-2321.
12. Xu C, Chou GX, Wang ZT, *Fitoterapia*, 2010; **81**:610-61
13. Cheung HY, Cheung CS, Kong CK, *J. Chromatogr. A*, 2001; **930 (1-2)**:171-176.
14. Kishore PH, Reddy MV, Reddy MK, Gunasekar D, Caux C, Bodo B, *Phytochemistry*, 2003; **63(4)**:457-461.
15. Suguna L, Sivakumar P, Chandrakasan G, *J. Experimental Biology*, 1996; **34**:1208-1211.
16. Ncube EN, Steenkamp PA, Madala NE, Dubery IA. *Applied Biochemistry and Biotechnology*, 2016; **179**:685-696.
17. Jamil SS, Nizami Q, Salam M, *Natural products radiance*, 2007; **6(2)**:158-170.
18. Edeoga HO, Okwu DE, Mbaebie BO, *Afr. J. Biotech*, 2005; **4**:685-688.
19. Anushika S, Wannee J. *Pharmacognosy Review*, 2017; **11(21)**:35-38.
20. Kiritkar KR, Basu BD, *Indian Medicinal Plants*, 2nd ed.; Bishen Singh Mahendra Pal Singh: Dehradun, India, 1999; **3**:1762.
21. Mohanan PV, Rao JM, Kutty MAS, Devi KS, *Biomedicine*, 1998; **18**:106-111.
22. Nagarajan SM, Kandasamy S, Chinnappa R, *Anc. Sci. Life*, 2009; **28**:3-5.
23. Pandurangan A, Lal Khosa R, Hemalatha S, *Iran. J. Pharm. Res*, 2008; **7**:217-221.
24. Manosroi J, Dhumtanom P, Manosroi A, *Cancer Letter*, 2006; **235**:114-120.
25. Cito AM, Souza AA, Lopes JA, Chaves MH, Costa FB, Souza AS, Amaral MP. *Anais da Academia Brasileira de Ciências*, 2003; **52**:74-76.
26. Fernandes KP, Bussadori SK, Marques MM. Bach E, Wadt N, Santos EM, Pavesi VC, Martins MD, *Revista de Sa'ude P'ublica*, 1995; **29**:457-466.
27. Seo N, Ito T, Wang N. Yao X, Tokura Y, Furukawa F, Takigawa M, Kinataka S. *Anticancer Research*, 2005; **25**:3763-3770.
28. Radha R, Sermakkani M, Thangapandian V, *International Journal of Pharmacy and Life Sciences*, 2011; **2(2)**:562-567.
29. Kumoro A C, Hasan M, Singh H, *Science Asia*, 2009; **35**:306-309.
30. Yadav, RNS, Agarwala M, *Journal of Phytology*, 2011; **3(12)**:10-14.
31. Merlin NJ, Parthasarathy V, Manavalan R, Kumaravel S. *Pharmacognosy Res*, 2009; **1(3)**:152-156.
32. Stephen S, *Anal. Chem.*, 2012; **84**:7274-7282.
33. Hubschmann HJ, *Handbook of GC-MS: Fundamentals and Applications*. Third ed. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA, 2015.
34. Mosmann T, *J. Immunol. Methods*, 1983; **65**: 55-63.
35. Praveen SG, Shivani P, *Future Journal of Pharmaceutical Sciences*, 2020; **6**:115.
36. Dineshkumar G, Rajakumar R, *Innovare Journal of Science*, 2016; **4(4)**:9-12.
37. Graidist P, Martla M, Sukpondma Y, *Nutrients*, 2015; **7**:2707-2718.
38. Naidu JR, Ismail RB, Yeng C, Sasidharan S, Kumar P. *Journal of Phytology*, 2012; **4(1)**:13-18.
39. Wei SY, Tang SA, Sun W, Xu B, Cui JR, Lin WH. *Cancer Letters*, 2008; **262**:114-122.
40. Senthilraja P, Kathiresan K, *Journal of Applied Pharmaceutical Science*, 2015; **5(3)**:80-84.
41. Ochwang I, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, Kiama SG, *Journal of Ethnopharmacology*, 2014; **151**:1040-1055.